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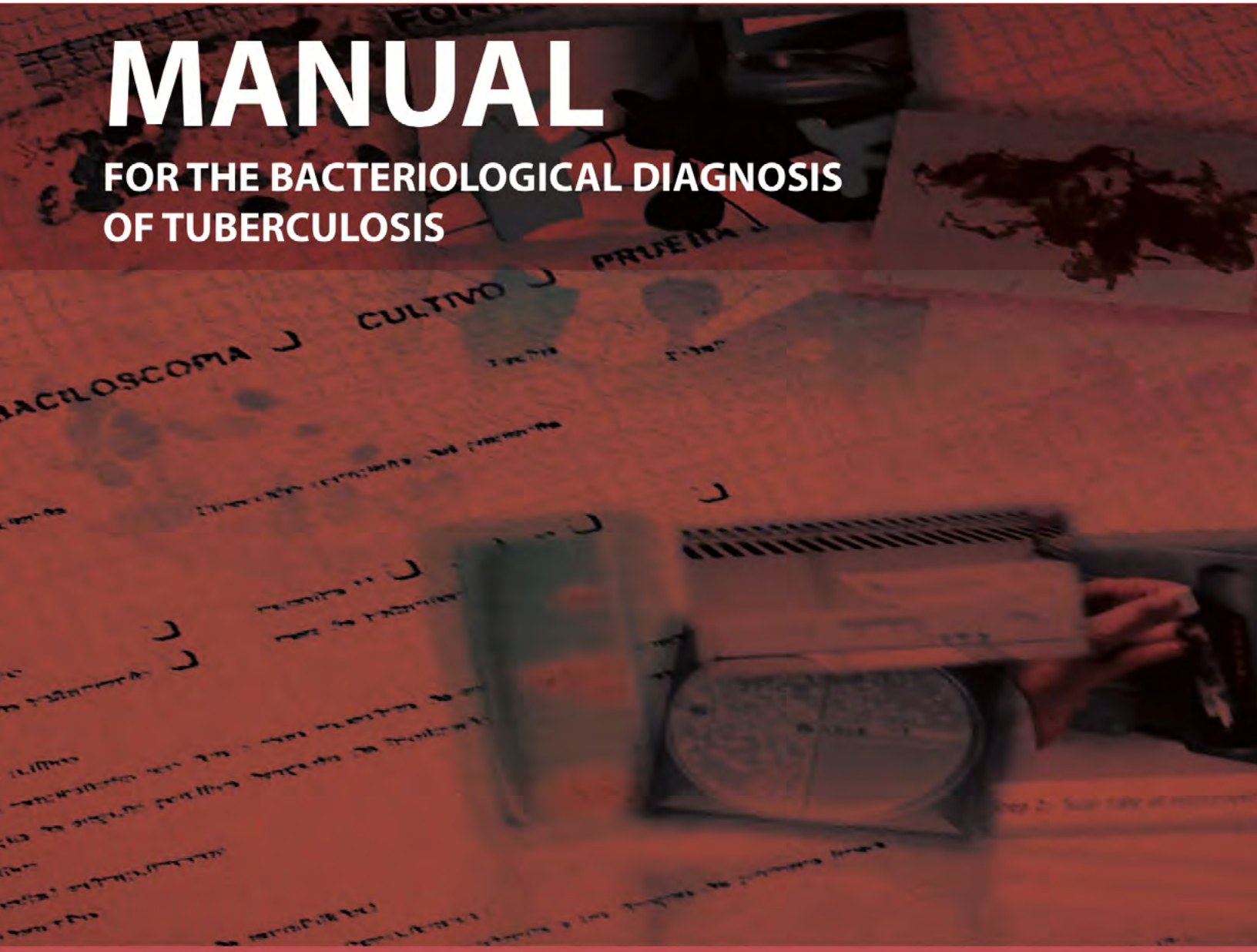
PROGRAMA "FORTALECIMIENTO DE LA RED DE LABORATORIOS DE TUBERCULOSIS EN LA REGIÓN DE LAS AMÉRICAS"

MANUAL

FOR THE BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOSIS



PART 4: Manual of external quality assessment procedures for bacteriological methods applied to the diagnosis and treatment monitoring of tuberculosis

Organización Panamericana de la Salud
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MANUAL

FOR THE BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOSIS

Andean Health Organization - Hipólito Unanue Agreement Unanue

MANUAL FOR THE BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOSIS. PART 4: MANUAL OF EXTERNAL QUALITY ASSESSMENT PROCEDURES OF BACTERIOLOGICAL METHODS APPLIED TO DIAGNOSIS AND TREATMENT MONITORING OF TUBERCULOSIS / Program "Strengthening the Tuberculosis Laboratories Networks in the Region of the Americas" - Lima: ORAS - CONHU; 2019. xx p .; ilus, tab.

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This manual is intended as a guideline to establish or improve the external quality assessment of the bacteriological methods applied to the diagnosis and treatment monitoring of tuberculosis as part of the overall efforts to improve the quality in the laboratories of tuberculosis

ANDEAN HEALTH ORGANIZATION - HIPOLITO UNANUE AGREEMENT, 2019

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Manual of external quality assessment procedures for bacteriological methods applied to the diagnosis and treatment monitoring of TB.

ABBREVIATIONS and ACRONYMS

DNA	Deoxyribonucleic acid
Ak	Amikacin
AFB	Acid fast bacilli
EQA	External quality assessment
SM	Smear microscopy
CLSI	Clinical and Laboratory Standards Institute
Cm	Capreomycin
BSC II	Biosafety cabinet type II
QE	Quantification error
E	Ethambutol
FL-LPA	Line Probe Assay for First-Line drugs. (H and R)
HFN	High false negative
HFP	High false positive
LFN	Low false negative
LFP	Low false positive
Xpert	Xpert MTB/Rif
H	Isoniazid
Km	Kanamycin
LPA	Line probe assays
NRL	National Reference Laboratory
RL	Reference Laboratory
SRL	Supranational Reference Laboratory
NTM	Non- tuberculosis mycobacterium
NTP	National Tuberculosis Program
SOP	Standard operating procedure
R	Rifampicin
FQN	Fluoroquinolone
SR	Symptomatic respiratory patients
SL-LPA	Line Probe Assay for Second Line drugs
TB	Tuberculosis
CFU	Colony forming units
HIV	Human immunodeficiency virus
ZN	Ziehl Neelsen

QUALITY ASSURANCE

All activities of the National Tuberculosis Program (NTP) must have three characteristics: **coverage, permanence and technical quality** and these are achieved only **if the activities are integrated** into the general health services in an **organized** manner. The success is based on the acceptance of the members of the health team of their responsibility towards the community and on the sustainability of this motivation over time.

The quality in the laboratory is not only the result of technical factors (suitability, good reagents, precise methods and equipment, standardization, good criteria for the application of methods and interpretation of results), **but also of administrative factors** (organization, systematization of procedures, availability of supplies, equipment maintenance, records and appropriate environment, among others). **The quality management system directly or indirectly observes all factors that affect the quality of each process in its preanalytical, analytical and post-analytical phase.**

The establishment of a quality management system requires a sequence of activities: **planning, evaluation and identification of the failures to be corrected as a priority, establishment of a retraining program and technical and / or administrative support designed to correct these shortcomings and, finally, measuring the impact of the system.** The results of each sequence of activities will guide the design of a new cycle. It is, therefore, an **interactive process.**

"Quality assurance is part of the management system intended to provide confidence that an organization meets the quality requirements"
[CLSI GP26-A4]

"It encompasses a series of activities that allow laboratories to achieve and maintain high levels of accuracy and competence despite changes in test methods and the volume of samples tested".
[www.cdc.gov/labstandards]

Note: CLSI is a nonprofit organization that promotes the voluntary development and use of guidelines and standards in the area of health care. Its members belong to internationally recognized organizations. Among its many actions, it's a member of the international standardization organization, responsible for ISO quality standards.

In other words, **quality assurance is the set of activities designed to evaluate the work for the measurement of the quality of a product (in our case called diagnosis)**, in order to detect the presence of errors in the development of the product and establish corrective measures where and when they are necessary to maintain, in this case, a diagnosis of certainty that improves or contributes to optimize the clinical management of the patient and indirectly increase the effectiveness of epidemiological surveillance. It is part of the **QUALITY MANAGEMENT SYSTEM**, which is responsible for observing the activities as a whole, in order to ensure the quality of all laboratory processes.

The result of a punctual evaluation says, in general, very little. It can be only a reflection of a success or a fortuitous error, of a moment of organization or disorganization of the laboratory, of the good functioning or temporary failure of an equipment. Expressed as the result of a test of a student; much more significant are the average and evolution of results throughout his career. For this reason, the control or evaluation of technical quality **should produce periodic results, which allow evaluating the trend over time of the parameters that qualify the quality of work.**

The process **must be open and comprehensible to all** laboratories of all levels that compose the network. The system must be permeable to feedback, so that all participants can contribute to its usefulness, proposing improvements or modifications that should be considered by the working group.

Quality assurance

It is, basically, an educational and motivating process

It is intended to maintain and optimize the technical and operational quality of the diagnosis itself.

It results, finally, in greater efficiency to assist the patient with TB and to control the pathology

It tends to strengthen knowledge, develop technical capacity and stimulate a responsible attitude towards work. In no way should be confused with examination or inspection.

Among the objectives of all NTPs, under the "End of Tuberculosis" Strategy, is to achieve universal access to a high quality care for all TB patients with the goal of ending the TB epidemic by 2035. Ensuring early detection of cases through guaranteed quality bacteriological tests is still a necessary step for the TB cure. Therefore, TB laboratories network that provides diagnoses with a high degree of quality is fundamental for the elimination of the disease.

The need to ensure good quality is increasingly recognized, and **programs have more and more voluntary adherents, especially because there is an increase in the degree of awareness that errors still occur in laboratories with excellent performance.**

The control leads to:

- **Identify the most frequent errors**
- **Describe procedures and controls that minimize the likelihood of producing false results or suboptimal performance of bacteriological methods**
- **Raise diagnostic quality**

Different strategies have been proposed for the control of methods, they can be combined or used separately in different moments or areas, according to the possibilities that exist and the information that you want to obtain.

COMPONENTS OF THE QUALITY ASSURANCE PROGRAM

The elements considered key to a quality assurance program are:

a) Internal quality control
b) External quality assessment
c) Performance indicators monitoring
d) Continuous improvement

a) Internal quality control:

It is the responsibility of each of the laboratories that execute techniques. In particular, the head of the laboratory should establish a system of regular controls and the recording of the controls results in the work routine, reserving for him the periodic review of the critical points and the results monitoring. Protocols on developing specific internal quality control materials for each test or procedure are incorporated into each SOP and includes

- The control of
  - materials, supplies, equipment
  - Procedures
  - Records and traceability
  - The preparation and delivery of reports
- The results monitoring
- The corrective measures to be applied when the imprecision of the result exceeds the limits considered acceptable

Its objective is to immediately avoid and / or correct individual errors committed with patient samples.

b) External quality assessment (EQA)

It is the responsibility of the, district, national and international reference laboratories depending on the structure and organization of the laboratory network in each country and each one laboratory to be evaluated.

The EQA constitutes an essential support for the accreditation and / or certification of a laboratory or at least it should be considered an important point to comply with the minimum standards that guarantee that the tests carried out in a laboratory are accurate and reliable.

"The term EQA is used to describe a method that allows to compare the analysis of a laboratory using an external source. This comparison can be made with respect to the performance of a group of external laboratories or the performance of a reference laboratory. "(Source: Quality management system in the laboratory: manual I. World Health Organization 2016)

Its objective is the identification of laboratories with technical or operational failures and the detection of the causes of these failures. This should be followed by a technical support plan designed in particular for each of those laboratories, The implementation of that plan may include retraining / training, supply of supplies, repair or replacement of equipment, derivation of the samples or isolations received by that laboratory with unacceptable performance to another unit, until problems were solved.

Supervisory laboratories must have solid and up-to-date knowledge, not only on bacteriology of TB, but also on the technical and operational aspects of the NTP, and experience in the specialty and field. The supervisory laboratory must participate in an EQA program for the techniques it evaluates, be recognized as a reference laboratory and have demonstrated the capacity to support other laboratories in the resolution of technical problems, in personnel training and in the supply of supplies.

There are companies or organizations that provide materials for the EQA of microscopy, culture, species identification and drug susceptibility testing techniques. In the case of using these materials, it is important to ensure that the company or organization that provides them has competence in the performance of proficiency testings and in the type of test that is being evaluated.

The EQA can be done in the form
Direct
On-site supervision
Indirect
Proficiency testing
Blinded rechecking

On-site supervision:

It consists of the visit by personnel of national, regional or district level to the laboratories of the network to observe, in the place, the working conditions and the technical and operative procedures. The activity must be planned at regular intervals and includes the proposal of corrections, if necessary, in accordance with the characteristics of each service. A guide of items to verify, questions to answer in situ and interviews with authorities of different levels was used during the visit. The preparation of a report of the visited laboratory (s) with strengths and weaknesses to overcome is a fundamental instrument to stimulate behavioral and / or infrastructure / biosafety conditions changes in those services in which deficiencies were detected.

These visits allow the diagnosis of situations in the early stages, provide training, motivation and support to staff, especially in peripheral environments and establish strong relationships with people, which encourage early notification of any problem, allowing a quick solution of them. They are especially critical during the early stages of implementing a new technology.

Proficiency testing:

It consists in the objective and retrospective comparison of the results obtained in different laboratories, through an experience coordinated by an external entity, such as a regional, national or international reference laboratory. Is defined as:

> **"A program in which multiple samples are sent periodically to the members of a group of laboratories for analysis and / or identification, in which the results of each laboratory are compared with those of other laboratories of the group and / or with an assigned value "[CLSI GP27-A2]**

This methodology allows checking preanalytical, analytical and post-analytic key processes. It does not measure the routine performance of a laboratory, but it can identify laboratories with the greatest deficiencies. It is recommended at least once a year.

There is a certain degree of uncertainty as to whether the analysis of a panel of samples / smears / strains such as those used for this type of quality control is a tool that allows us to know if the "most accurate result" is occurring. For this reason, the panels must be designed so that they can identify frequent and serious errors that compromise the accuracy of the results. The result of some specimen of the panel may not be as expected, the bacilli of a sample can be grouped despite the effort invested to homogenize them, they can discolor in a smear, or they can lose viability, and the strains can mutate or lose some clones with peals successive. It is increasingly understood that "the best result" of a study conducted on biological material is the result "consensus" of several laboratories that have experience and have shown good quality of work over time.

The proficiency testing requires the joint work of all the members of the network, and it is the joint contribution that allows evaluating the quality of work. The results are monitored to analyze trends over time. The feedback, regarding the results of the aptitude tests, as well as the proposed recommendations, must be communicated in a fast way to the supervised personnel of the laboratories for the immediate taking of corrective actions.

Blinded rechecking

The blinded rechecking is usually done by sending materials from the periphery to be re-tested by the reference level (regional or national laboratory) and comparing the results of the evaluated laboratory with those of the controller. It is applied as part of the EQA of smear microscopy (SM) with the submission of smears to be reread and will be seen in detail in the Section of external equality assessment of this technique.

In addition, this component can be implemented for other diagnostic methodologies such as species identification or phenotypic or molecular drug susceptibility testing by sending some of the isolates from the peripherical service to the reference laboratory to be blindly investigated. In general, this type of evaluation serves to complement or supplant, only in exceptional cases, the proficiency testing.

c) Performance indicators monitoring

All laboratories must ensure that all tests they perform are submitted to EQA by their participation in formal national or international programs. In addition, performance monitoring using laboratory quality indicators, also known as performance indicators, is an effective way to know the quality of laboratory results and identify areas for improvement. They are useful for internal and external evaluation, depending on whether the evaluation is carried out by the laboratory itself, comparing its results with those expected (internal quality control), or by an external laboratory, comparing the results in the context of the rest of the laboratories of the network (EQA). To implement this type of control, all laboratories must collect and analyze test data on a regular basis, using a standardized format for documenting them. Expected values should be set for all monitored indicators and any unexplained change in them should be investigated, such as an increase in error rates, a change in the positivity rate of *Mycobacterium tuberculosis* detection or in the resistance rate to R, or a significant change in the amount of tests performed. They should be reviewed by the laboratory manager and should always be linked to corrective actions if unexpected results or trends are observed.

The proficiency testing, together with the monitoring of the quality indicators, complement the evaluation carried out through a regular program of on-site supervision. And they allow the evaluation of quality, at least partially, when adequate human or financial resources are not available to implement this program with enough coverage and frequency.

d) Continuous improvement

The quality assurance cycle includes four steps: plan, do, check and act. These steps must be repeated regularly in order to ensure the continuous improvement of each process generated in the laboratory.

This Involves the continuous monitoring of the results thrown by the different methods used for the evaluation of the quality in order to identify aspects that should be improved, the identification of nonconformities by the staff members or audits that arise from the analysis of the data and the development of creative solutions to the identified problems.

Continuous improvement is the most difficult process to implement routinely and systematically, but it is an essential part of the implementation of the service quality.

DIRECT EXTERNAL QUALITY ASSESSMENT

On-site supervision

It is the best method to observe the conditions of a laboratory and the practices that are carried out in it. It is the essential component of External Quality Assessment. The NTP norms established for each of the operational activities and each country must be considered.

The characteristics of the supervisor must be taken into account when selecting and training the personnel responsible for this task. A supervisor should have:

- Solid and updated knowledge of techniques and operations of the laboratory, objectives and strategies of the NTP, the epidemiological and operative situation of the region, the characteristics of the population and the organization of the health system;
- Field experience: knowing the conditions in which the persons perform the work in the Health Services and with capability of identifying anomalous situations;
- Interest in the work that will be done;
- Good interpersonal relationships;
- Flexibility to analyze the problems and to propose appropriate corrective measures for each situation, practical and feasible to apply;
- Available time and financial resources that allow the payment of mobility and travel (in the case that it is necessary to eat and / or to spend the night away from home) to travel to the local levels.

Supervision may arise from discrepancies or repeated technical deficiencies in a laboratory, important and repeated errors or omissions, detected by proficiency testing or blinded rechecking; they can also be programmed for laboratories that have recently implemented a specific technique or when newly trained people are incorporated or, simply, as part of a regular and periodic activity of the laboratory network and the NTP.

In order to optimize the human and material resources necessary to carry out this activity, and not to unexpectedly alter the work routine of the laboratory to be visited, it is essential that the visits are scheduled in advance, elaborating an annual agenda and as far as possible in coincidence with activities of on-site supervision of the NTP team or other health teams or general laboratory supervision.

Procedures

Preparation of visits

• Conveniently announce the visit and its objectives to avoid suspicions or absences of staff. Surprise visits are always confused with inspection and it may happen that, if not notified, people they should interview have not gone to work.

• Analyze the epidemiological and operational information of the area to be visited.

• Analyze the available information of the laboratories to be visited in relation to biosafety, equipment, resources, and workload and performance indicators, identifying the critical points that must be specially observed.

In health centers, it is necessary to

• Before initiating the visit, state the objectives of the on-site supervision by making interviews with the director or authorities of the establishment and, when appropriate, with the head of the general laboratory.

• Contact with doctors, nurses and other health care workers of the service that are usually related to the laboratory for diagnosis or treatment monitoring, in order to know the laboratory strengths and weaknesses perceived by the health team and strengthen relationships between the groups; these relationships may not be fluid and the supervisor can help to improve them.

• Obtain information about the organization, records and available resources for obtaining specimens and / or isolations (as appropriate) and for sending them to the laboratory, both in the visited institution and in the entire catch area in case the laboratory receives samples or isolations from other health centers or laboratories,.

• Visit the laboratory and verify the items included in the corresponding technical guide, prioritizing those that are cause for concern and those that have not been verified in previous visits. Resolve doubts or questions that appear during the visit. Record unmet demands or proposals that exist in relation to the reference laboratory. Make a brief return on the observed to the laboratory team involved in TB, noting the strengths and the necessary and possible improvements that depend exclusively on the laboratory. Motivate the staff highlighting their importance and the significance of their daily work.

• Carry out, at the end of the visit, a brief informative meeting with the same people interviewed at the beginning to let them know what was observed, especially the achievements that the laboratory may have obtained during the previous period and the difficulties encountered that can be solved by the authorities or by the health team. Advocate and document the commitment of the authorities to manage what is necessary to achieve the improvement of the bacteriologic diagnosis.

• Make a written, succinct report, which mainly mentions the activities or changes to which the laboratory workers and the authorities had committed during the visit and leave a copy in the visited laboratory. A copy should also be sent to the head of the NTP and / or summarize the status of an area or the entire network, identifying the services that require interventions by the NTP. In addition of being a reminder of what has been observed and recommended, it serves to coordinate activities by the laboratory network and the NTP, in order to plan future actions, to train health care workers, to provide supplies, to plan upcoming supervision or other activities.

If, due to special circumstances, the supervisor cannot provide a written report at the end of the visit, you must define how and when to provide the report to the visited laboratory.

Aspects to observe:

In the institution

• Compliance with the goals of localization of symptomatic respiratory patients (SR) and cases and possible reasons for the success or deficiency;

• Infection control in relation to the collection and transport of samples;

• Traceability of samples and laboratory results;

• Delay in the availability of laboratory results.

In the laboratory

• Human resources, adequacy and training;

• Working environment, biosafety, existence and maintenance of equipment (microscopes, automated PCR equipment, centrifuges, stoves, refrigerators, etc.);

• Supply of inputs and their quality;

• Execution of technical procedures and compliance with operating rules;

• Technical and operational aspects of the procedures performed for the diagnosis of TB;

• Transport of samples and strains to the reference lab.

Annex A shows examples of guides developed for laboratories with different technical complexity, which may be useful to support the activity of supervisors during the visit. These guides, which include an exhaustive list of all operational and technical elements that should be observed, can be sent in advance so that the laboratory completes at least some of the required information. The supervisor can then verify the reality of what is reported by the laboratory, which speeds up the procedure. This is particularly useful when there is a very limited time available for each visit.

At the same time, they can be used as documentation of the visit, in order to record

the current conditions and the necessary actions to strengthen the operation of the laboratory.

This list is indicative and should be adapted according to the specific needs of each country. Those aspects that are considered adequate in each item are clarified inside the text so that the supervisor can keep them in mind, but the questions must be open, without previously disclosing what is considered acceptable so as not to induce the answers.

It is advisable not to qualify the technicians and / or professionals by points; this methodology generates rejections towards the supervisor and supervision. However, the management of the TB laboratory network must have a tool that allows it to quickly visualize the evaluations made in each laboratory of the network, the general result (acceptable or not) and the critical points that must be solved in each service.

INDIRECT EXTERNAL QUALITY ASSESSMENT OF SMEAR MICROSCOPY

Basic definitions

Acceptance number (d): the maximum number of false negative errors allowed in the sample above which the NTP / NRL can no longer be sure that the supervised laboratory has achieved the expected quality.

Controller: Term used to describe the supervisory laboratory or technician responsible for rechecking slides.

High false positive (HFP): a negative smear that is mistakenly considered positive (1+, 2+ or 3+) by the supervised laboratory.

High false negative (HFN): a positive smear (1+, 2+ or 3+) that is mistakenly considered negative by the supervised laboratory.

Low false positive (LFP): a negative smear that is mistakenly considered low positive (countable) by the supervised laboratory.

Low false negative (LFN): a low positive (countable) smear that is mistakenly considered negative by the supervised laboratory.

Major error: includes high false positive and high false negative errors.

Minor error: includes low false positive and low false negative errors.

Panel testing set: set of slides of different degrees of positivity and negatives that has been prepared by the NRL to be used in the proficiency testing in order to evaluate the ability of the microscopists to examine, inform and eventually stain smears.

Positivity rate: proportion of positive smears between all slides examined in the participant laboratory (prepared from both diagnostic and treatment monitoring samples) for a certain period of time.

Quantification errors (QE): difference of more than one degree of positivity in the reading of a positive smear between the controller and the participant laboratory (scanty versus 2+ and 3+ or 1+ versus 3+)

Sensitivity (relative to controllers): it represents the level of expected capacity of the readers of the participant laboratory to detect the positive smears, compared with that of the controllers.

Specificity (relative to controllers): represents the level of expected capacity of the readers of the participant laboratory to detect the negative smears, compared with that of the controllers.

Total number of negative slides: annual number of slides minus the number of positive slides processed in the participant laboratory.

Slides Batch: set of slides (usually between 50 and 100) prepared in the reference laboratory that contain a similar predefined amount of AFB and from which the panel testing sets for the proficiency testing are prepared.

Smear with adequate quality of sample (established microscopically): that smear that has been classified microscopically as coming from a mucopurulent or mucosal sample.
Smear with inadequate quality of sample (established microscopically): that smear from a sputum sample in which epithelial cells (saliva) are mostly observed.

General considerations

The EQA for AFB smear microscopy can be done by two methods:

- Rechecking of routine smears

By this method the supervisory laboratory rechecks a sample of the smears made in the work routine by the participants laboratories and evaluates the quality not only of the microscopic reading but also of other technical aspects such as the type of the specimens processed, the characteristics with which the smears and staining technique were made. The observation of these technical aspects aims to prevent possible errors by avoiding situations in which microscopic reading is difficult. Since this method allows analyzing the competence of a laboratory in its work routinely and indirectly, the performance of the health system to achieve the collection and remission of samples of good quality, is considered the best alternative for the EQA for the SM. However, it is also the one that requires greater investment of human and logistic resources for the supervisory laboratory, so its implementation constitutes a real challenge for the laboratory network.

- Sending panel testing sets from the NRL to the participant laboratories

In general, it evaluates only the quality of the reading. When incorporated without staining, it is also possible to evaluate the quality of the staining. It is not useful to assess the quality of routine laboratory work.

Each method has different advantages and disadvantages, as well as different resource requirements that will be developed in the following sections of this manual. The choice of how to implement the EQA in each country depends on the characteristics of the laboratories that make up the network, the available resources, as well as the ability to obtain additional resources to support the activities of EQA. It is probable that, in principle, not all the necessary resources for the implementation of the methods recommended in this manual are available in all the laboratories of the network. However, it is advisable to develop an expansion plan of the EQA program with a "step by step" approach, so that, in principle, a network can use only those methods that the resources permit; in this way, it will be possible to show that there are problems in the quality of the implementation of the SM and justify the allocation of additional resources necessary to expand the activities and introduce the improvement processes.

The use of one of these modalities or the combination of the two in all the laboratories or only in some laboratories with certain characteristics must be decided by NRL.

Rechecking of routine smears sent from peripheral laboratories (Periphery -Center Supervision)

Ideally this method should allow the evaluation of the individual work of each technician during a defined period, so the sample to be read should be large enough to achieve statistical significance of the results for each of the technicians of the laboratory. However, in practice this is not possible, because it would cause a great overload in the work of the controllers. Therefore, the sampling of the smears to be reread is generally done by laboratory in relation to the work of a long period (e.g. one year), and using a statistical method for the selection of the sample (the smallest possible) that will allow the identification of services that "could" be operating below the minimum level set by the NTP. Given that the sample of smears is relatively small, when it is detected that a laboratory has poor quality, it must be considered that this finding should preferably be validated by other actions, in most cases, a technical visit, which will identify possible sources of errors that require corrective action.

The rechecking does not intend to confirm the diagnosis of the patients and it does not replace the internal quality control and the regular visits.

The rechecking is a task that requires an important investment of human and logistic resources. There must be enough staff at the intermediate and central levels of the network because, if the controllers are overloaded with the rechecking of a large number of slides to reread and the slides of the work routine are added, they are likely to make more mistakes in reading that the laboratory workers that are being evaluated. Therefore, when the network is composed of a high number of laboratories, the EQA through the rereading technique should be decentralized, in such a way that a first controller had only about 10-20 laboratories in charge; clearly, the number of laboratories in their charge will depend on the size of the sample per service / year to be rechecked and on whether these controllers have full or partial dedication to the rechecking activity. Thus, in order to implement the rechecking method in an organized and efficient manner, a structured laboratory network with defined functions and capabilities is required for each level, following a scheme similar to that described below:

Peripheral laboratories located in primary care health centers or hospitals. The staff has technical competence to perform SM, generally using the Ziehl-Neelsen (ZN) staining. They must have the capability to keep the smears properly and send them to the supervisory laboratory in a convenient way and according to locally established standards.

Intermediate laboratories in hospitals or larger cities. The staff has the technical competence to perform microscopy by ZN, and may have the capacity to perform fluorescence microscopy if the workload is high. The intermediate laboratories must be able to plan and execute the rechecking of smears of the laboratories of their regional network,

recommend corrective measures when necessary and assist in their implementation.

Reference laboratory. The reference laboratory plays an essential role in the organization and maintenance of the smear rechecking method and, it must have the capability to provide training in this method, execute the rechecking of microscope slides to the intermediate laboratories, evaluate the performance of the intermediate laboratories in their role as controllers, recommend corrective measures when necessary and assist in their implementation.

The following are essential aspects for the evaluation to be accurate:

- The supervisory laboratory must have experience in the realization of SM in the usual working conditions and in accordance with technical and operational norms of the NTP.
- The sample of the smears to be reread must be **representative:** the number must be sufficient and the random selection must be made by the controller.
- Rereading must be done blindly: the first controller who rechecks should not know the results of the participant laboratory.
- The discordant results **should be re-read by a second controller** working in the same supervisory laboratory or central laboratory.

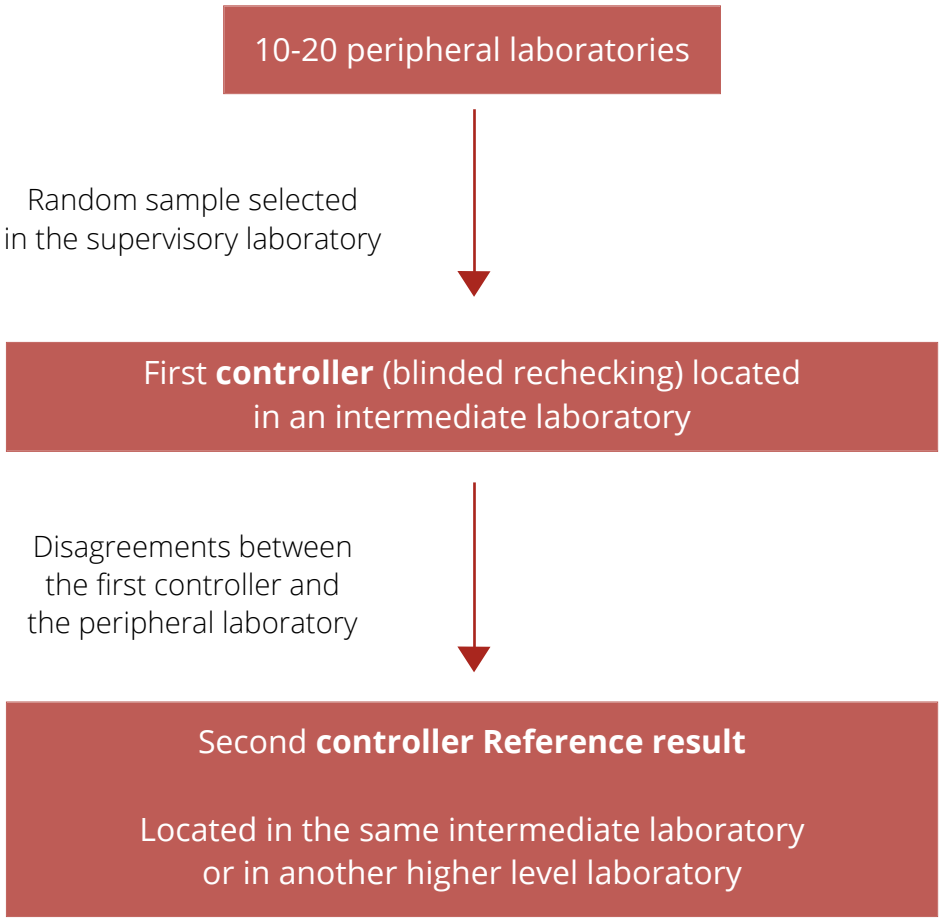
With respect to the selection of the representative sample of smears to be reread, it was customary to request and read all of the positive sheets and 10% of the negatives of a period. In this way it was ensured that there were no false positive results. However, the number of smears to be reread to control the quality of the laboratories that made more than 2000 slides per year was unnecessarily high. On the other hand, it is not necessary to recheck all the positive smears because the EQA is intended to evaluate the quality of the laboratories, not the individual results. In 2002, a group of international experts proposed a sampling methodology more representative of the laboratory work "lot quality assurance sampling", based on statistical methods, which contemplated rechecking the lowest number of smears with an assured level of confidence (APHL / CDC / IUATLD / KNCV / RIT / WHO *External quality assessment for AFB Smear microscopy*, Washington, DC: APHL, 2002).

On the other hand, there is no absolute certainty that the results of the supervising technician are the "true" results, even though he has more experience and, unfortunately, there is no absolute reference or "gold standard" result. Therefore, **the discordant slides between the first controller and the supervised laboratory should be read by another technician** from the same laboratory or from a higher level (we will call second **controller**). The result of this last reader must be considered definitive. Even in intermediate or peripheral laboratories

that present good reading efficiency, it is reasonable that a percentage of the smears must be reexamined by a second controller in order to resolve discrepancies. The total absence of disagreements in several centers with positive slides would suggest that it has not really been read blindly.

The following figure shows a decentralized model of organization of the rechecking method:

Figure: Example of organization of the EQA by the rechecking method



Requirements and necessary resources

- Structured laboratory network.
- Supervisory laboratory with experience in conducting SM with adequate number of human resources for analysis, monitoring of information, visits and implementation of corrective measures.
- Sufficient number of trained supervising microscopists . Although in general it is recommended that each controller re-read 10-20 services, the number of needed controllers will depend on the sample size per service / year and the dedication to the re-reading activity of each controller.
- The performance of controllers must be continually evaluated in the routine of rereading.
- Sufficient microscopes for EQA and retraining, under a regular maintenance program.
- System established to determine the appropriate number of smears to be reread.
- Standards of rereading slides that include the analysis of the results and the resolution of the discrepancies.
- System of smear collection including availability of economic resources for shipments of the slides to the supervisory laboratory.
- Standardized forms for records and reports of results
- Fluid communication systems.
- The reference laboratory must have the necessary mediums to implement corrective measures, including retraining.
- System established to collect the information of the rechecking activities carried out by the intermediate laboratories towards the NRL.

Criteria to establish the number of slides to be reread

The calculation of the slides to be reread using the "lot quality assurance sampling" depends on several factors: the annual positivity rate, the total number of negative microscope slide read in a year in each participating laboratory and the expected sensitivity to be demonstrated by the laboratory technicians in reading SM. This sample size allows detecting those laboratories with a number of errors that exceeds the acceptable level previously established by the NRL.

This sampling is very useful in the evaluation of laboratories with positivity rates higher than 5% and that perform more than 1000 slides per year. However, when the levels of positivity or the workload are lower than these values, the proportion of smears to be reread can be extremely large and the use of the rechecking method for the SMEQA could be inapplicable.

Thus, for each one of the possible situations, in this manual, different options will be presented that allow designing an accessible plan of SM's EQA according to the available resources and the workload of each laboratory.

1-Option A. sampling of all microscope slide processed in the laboratory

To perform the calculation using this methodology, proceed as follows:

• Collect the information of **the total number of smears with negative and positive results recorded during the last year** (consider the slides prepared from diagnostic and treatment monitoring) of all laboratories that perform SM.

• Calculate **the positivity rate** of each laboratory using the following formula:

$$\frac{\text{Number of positive smears}}{\text{(Number of negative smears + number of positive smears)}}$$

• Record the positivity rate of all the laboratories to be monitored in a certain area / region and calculate the average of the positivity rate of the area / region.

• Consider the use of the average positivity calculated for the area / region to which the laboratories to be monitored correspond for the calculation of a single sample size for all laboratories, if more than 80% of them have a similar positivity rate and analyze a number of negative smears exceeding 1000 slides/ year.

• Calculate individual sample sizes for each laboratory if the average cannot be applied as stated in the previous point.

• Determine the sample size of slides to be re-read annually based on the information in Table 1, which has been prepared considering a relative sensitivity reached by readers of 80% in relation to controllers.

For doing this:

- Identify the sample size at the intersection of the line of the annual number of negative microscope slide and the column of positivity rate. Search for both parameters the values closest to those calculated for each laboratory or for the average, as appropriate, since the calculation of an exact sample size would yield a result very close to that calculated with the approximation.

  For example, if the number of negative smears is close to 1000 and the positivity rate is close to 10%, the annual sample for rereading corresponds to 96 smears / year (Table 1)

- Add to the selected microscope slide all those reported as positive by the laboratory to be monitored during the study period, when a laboratory is supervised for the first time or false positives have been observed in previous controls.

Table 1. Periphery-Center. Representative number of sheets to be reread

Number of negative smears/ per year	No. of slides to be reread				
	Positivity rate 5%	Positivity rate 7,5%	Positivity rate 10%	Positivity rate 13%	Positivity rate 15%
200	107	86	72	61	54
500	154	114	89	71	62
1000	180	128	96	76	66
2000	197	135	100	79	68
5000	208	141	103	80	69
50000	216	144	104	82	69

Table prepared based on the lot quality assurance sampling for a sensitivity of 80%, specificity of 100%, number of acceptable errors (d) = 0, and 95% confidence interval. The sample size decreases proportionally with the increase in positive smears rates. Generates a final sample size that includes positive and negative smears.

Note: the acceptable number of errors (d) has a direct impact on the sample size - the larger the number, the larger the required size. To obtain the smallest and most efficient sample size, an acceptable number of errors of zero is recommended, **but this mediums that a simple error should be considered as a warning of possible problems that should be investigated.** The increase in the number of errors acceptable to 1 will allow an error, but, as a consequence, the size of the sample will increase significantly. In relation to the sensitivity, this can be set between 75-80% since this reduces the size of the sample significantly, which will contribute to making the implementation of the rereading method more feasible. Even with this sensitivity of 75-80%, errors can be detected in many laboratories. The level of sensitivity used to calculate the number of smears to be selected (sample size) should be set by those responsible for the EQA program at the national / regional level, and in no case should be entrusted to the controller in charge of selecting the smears nor to the staff that performs the rereading.

- Calculate the number of smears to be reread for each participant laboratory and each supervising technician

- Determine the number of smears that can be reread annually by controllers without overload. Keep in mind that, for each technician, the maximum number of smears that are recommended to be examined daily cannot be higher than 20-25 slides/ day for smears stained by ZN and 80-100 smears/ day for those stained by auramine. If among the activities of the controller's daily work routine is the realization of SM of samples processed in his service, to this maximum number of daily slides the number of extensions read by each supervising technician in his work routine must be subtracted.

- If the maximum number of smears that the supervisory laboratory can reread is

less than that obtained by sampling, reduce the sensitivity value to 75% and recalculate the sample size using Table 2.

Table 2. Periphery-Center. Representative number of smears to be reread

Number of negative smears/ per year	No. of smears to be reread				
	Positivity rate 5%	Positivity rate 7,5%	Positivity rate 10%	Positivity rate 13%	Positivity rate 15%
200	91	71	59	48	42
500	121	89	69	54	47
1000	136	96	73	56	49
2000	145	102	77	59	51
5000	152	104	78	59	51
50000	156	106	79	60	52

Table prepared based on the lot quality assurance sampling method for a sensitivity of 75%, specificity of 100%, number of acceptable errors d = 0, and 95% confidence interval. The sample size decreases proportionally with the increase in positive smear rates. Generates a final sample size that includes positive and negative smears

- If the number of slides obtained using a sensitivity of 75% is still greater than the controllers can process and new readers / controllers cannot be incorporated, use another EQA methodology (stratified sampling or sending of panel testing sets) until resources needed for rechecking slides by the lot quality assurance sampling method can be obtained.

2. Other options to establish the number of smears to be reread

The lot sampling of all the smears processed in the laboratory is plausible to be applied in the evaluation of laboratories with positivity rates higher than 4-5% and with a work load that is at least equal to or greater than 1000 SM per year. In areas where the prevalence of positivity of routine smears is moderate-low or with very decentralized service networks (generally with a predominance of laboratories with a low workload), the required sample size could be extremely large, since that a significant number of services would require the rereading of a significant proportion of the volume of slides processed in the work routine, and therefore the use of this methodology for the selection of the sample of smears to be read may not be applicable. Thus, for these situations, this manual will present two options that allow selecting a sample of smears for rereading in laboratories with <4% positivity and / or <1000 slides per year, in order to design an accessible plan of EQA for SM according to the available resources:

Option B: Stratified sampling of the smears examined for treatment monitoring

Option C: Combination of rechecking and panel testing sets submission methods

Option B: Stratified sampling of the smears examined for treatment control

This sampling is especially recommended for laboratories with positivity rates lower than 4% but whose workload is moderate / high (generally greater than 1000 smears/ year), since the annual collection of at least 40 treatment monitoring samples is required for its rereading.

Through this methodology, the treatment monitoring smears are sampled, adding a small number of diagnostic sample smears. The rationality of the method is based on the fact that the positivity rate of the treatment control samples is generally higher than 10%, depending on the prevalence of multidrug-resistant TB, the frequency with which the treatment monitoring controls are performed (monthly or bi-monthly) and other factors such as, for example, the prevalence of HIV-TB. Considering that the reproducibility of the samples for treatment monitoring (most of which are positive (1+) and with few bacilli) is lower than that of the diagnostic samples, it is considered appropriate to assume that the technicians of the supervised laboratory can reach a relative sensitivity of 65% (as opposed to the 75-80% established for the "sampling of all the smears p rocessed in the laboratory" developed in the previous point). Taking into account the aforementioned sensitivity and a positivity rate greater than or equal to 10%, the total number of treatment monitoring slides to be re-read annually (calculated using the lot quality assurance sampling methodology) results only in about 30-40, independently of the number of processed negative smears(Table 3).

Table 3. Periphery-Center. Representative number of treatment monitoring smears

Number of negative smears/ per year	No. of smears to be reread			
	Positivity rate 10%	Positivity rate 13%	Positivity rate 15%	Positivity rate 18%
100	33	28	25	21
200	40	31	27	23
500	44	33	29	24
1000	46	34	29	24
2000	47	36	31	26
5000	48	36	31	26
50000	48	36	31	26

Table prepared based on the lot quality assurance sampling method for a sensitivity of 65%, specificity of 100%, number of acceptable errors d = 0, and 95% confidence interval.

Using this methodology, it is recommended to read annually about 50/60 slides by laboratory. This comprises around 40 treatment control smears randomly selected during the year. About 10 to 20 diagnostic smears, randomly selected during the year are added to this number of smears in order to avoid biases in routine reading.
In practice, proceed as follows:

- Collect the sample of control monitoring smears following the rules of sample collection that will be developed in the section "Selection of the slides in the supervisory laboratory" (Procedures Section) and only counting slides for treatment monitoring..

- Repeat the same procedure, once the previous step has been carried out, on the smears corresponding to the diagnostic samples until the established number of smears is collected (for example 10).

Option C. Combination of methodologies

For centers with a positivity rate <4% that process less than 40 treatment monitoring smears/ year (usually laboratories with a low workload) or in which, for operational reasons, it is not feasible to apply the stratified methodology (for example, it is not logistically possible to sample all the slides made in a year), the combination of methodologies is recommended, through:

- The rereading of the smears corresponding to one month of each semester of work for laboratories with less than 500 smears/ year or one month of work per year for laboratories with a workload of ≥500 smears/ year.

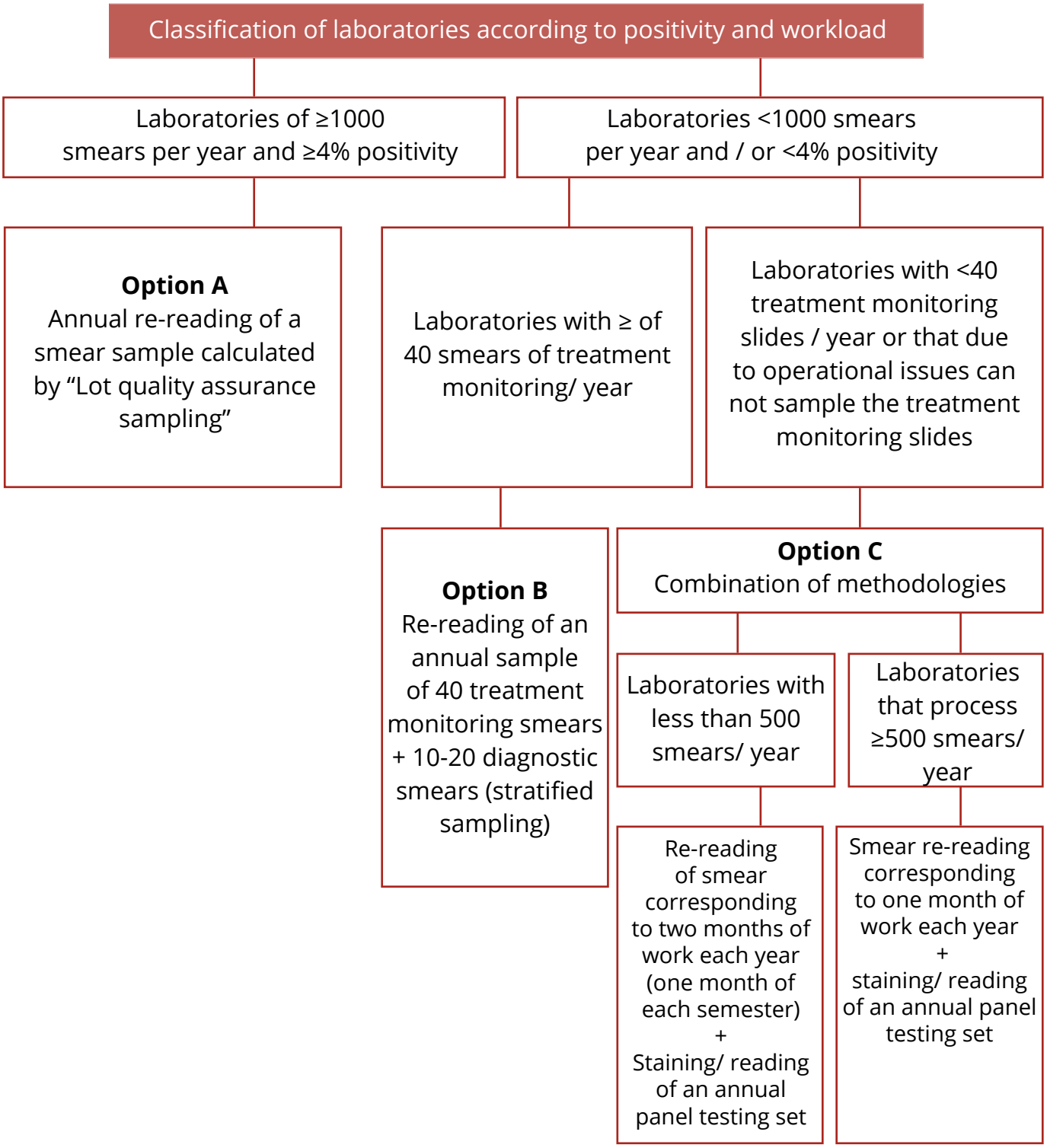
- Sending panel testing set per year for the proficiency testing.

In this way the quality of the reading will be based especially on the results obtained from the reading of the panel testing sets, while the rereading of routine smears will allow the qualification of the samples, smears and stains, fundamental aspects to achieve the prevention of errors.

In relation to the laboratories that process less than 500 smears/ year, which have been included in this EQA modality, it is necessary to point out that international experience shows that this low workload (less than 10 smears per week) may not be the optimum for maintaining skill in reading slides. Therefore, the managers of the laboratory networks should evaluate the feasibility of holding SM services whose workload is less than 500 smears/ year or concentrate the realization of the SM in nearby laboratories, as long as a transport system can be secured.

In scheme 1, the different applicable options for the calculation of No. of slides to be reread for the EQA are summarized.

Scheme 1. Suggested options for EQA according to levels of positivity and workload



Procedures

The methodological steps that have to be followed for the EQA to be accurate and applicable in field conditions are detailed below. The development of the procedure includes the preparation of a set of forms, corresponding to Annexes B.2 to B.6, which have been designed to guide the supervisory laboratory in the procedure of blind rereading of the smears, the evaluation of the performance of the controllers, the preparation of the results report and the consolidation of the results of the re-reading of the supervised laboratories belonging to a certain area.

1. Periodicity of sending the slides to the supervised laboratory for the selection of the sample

The smears will be selected in the supervisory service, by a coordinator who works in supervisory laboratory, once the smears corresponding to specific period of time have arrived to the supervisory laboratory. When the calculation of the number of the smears to be reread is made by sampling options A and B (See diagram 1), the selection of the sample can be made using two alternatives:

- Sampling of all the smears processed during a year:

The sampling of the smears processed during the whole year of work is considered the most recommended for the collection of the sample of slides to be reread. For this, the service will be required to carry out partial shipments (e.g. monthly, quarterly or quarterly) of all the smears made in the year to the supervising laboratory. In this way the supervisory laboratory will take a sample of the smears of each period of the year, following the following indications:

  - Divide the calculated annual sample size of each laboratory by 3 if the control frequency is quarterly or 4 if it is trimestral (e.g. if the annual number of smears to be reread is 96 and the control is performed quarterly, the number of sheets to be collected in each period it will be equal to 96/4 that is 24 smears per quarter).

  - With the information calculated in the previous point, make a list of the laboratories to be monitored and the N ° of slides to be selected during each period.

- Sampling of the slides processed during a period of the year.

When the previous alternative, for operational reasons, was not possible, it is acceptable that the sampling is carried out only during a given period of the year. For this, the remission of the total number of slides made in only a specific period of the year, which must be established by the Reference Laboratory (e.g. one month), will be required. This is feasible as long as the workload of the laboratory during this period (in this case, one month) is enough to take the number of slides calculated by the chosen sampling methodology (batch sampling of all the processed slides- option A- or stratified sampling of the treatment

monitoring smears -option B). In this case, the participant laboratory will send the corresponding smears of that period upon their request by the supervising laboratory. Where possible, each laboratory should be supervised at least twice a year. Newly integrated laboratories or those in which significant discrepancies are found should be monitored more frequently, e.g., once every two months, until adequate and stable quality is assured.

To carry out this procedure, the supervising laboratory must follow the following steps:

- Prepare, at the beginning of the year, a schedule with the laboratories of the network to be supervised from January to June and from July to December (or from January to December in the case that it has been decided that only the one-month smears can be collected every year). Laboratories should not know in advance in which months they will be supervised.

- Inform, at the end of each month, (by e-mail, by telephone, for example) to the laboratories that were selected to be supervised that month, that they must submit all of their slides corresponding to the current month.

This last form of remission of slides from the participant laboratory is also the one that should be used when the calculation of the number of smears to be reread has been established through option C (See Scheme 1), since it establishes the re-reading of the totality of the smears made in the participant laboratory in a month of each year or each semester, according to the workload of the service to be evaluated. Therefore, the laboratories in which it has been decided to use option C should be included in the schedule of laboratories to be monitored during a given period.

An example of the Registry used to document the schedule of laboratories to be monitored during a semester is shown in Annex B.1. The same record can be used to record the date of application of the smears to the service to be supervised, the receipt of the acknowledgment of receipt (in case the notice has been given by e-mail, for example) by the participant laboratory and the date of receipt of the smears in the supervisory laboratory. Observe that the date of application for the smears corresponds to the last days of the month or the first days of the month following the one requesting the slides, since this ensures that the laboratory to be monitored does not know in advance that will be evaluated during that month..

Conservation of the slides in laboratories

All the laboratories of the network that carry out SM must adequately conserve all the processed smears, including the smears used in the internal quality control of the staining.

To do this, proceed as follows:

- Remove the immersion oil, after examining the smears, leaving the slide in an upright position on an absorbent paper until the next morning. Then gently support the face of the

slide that has the smear of the samples on another strip of absorbent paper. Never try to remove the remaining oil by rubbing the smear.

- Save the smears in histological boxes in the same order in which they were processed, without separating the positives from the negatives. The result of its reading should not be labeled in the smear. If special boxes are not available, the slides can be stored in cardboard boxes individually wrapped in paper, in bundles that group together those of a day or a week, labeled with the date, in the order in which they were made. Do not put on this label the result of reading.

- Keep them in a cool and dry place to avoid the effects of heat and humidity on the staining.

Note: For those laboratories in which the sampling of the smears is carried out over a period of one year (for example, one month of each semester), the laboratory must keep the smears at least during the 15 days after each month, for the case that the supervising laboratory requests the slides for its rereading. The laboratories that have been selected must continue to keep the smears of each month within the corresponding semester or year, given that, if their performance is not acceptable, the smears processed during another month of the same year/ semester may be requested.

To be sent to the supervisory laboratory, prepare all of them in a shipping box by adding a **copy of the corresponding Laboratory Record** for that period. The record should include information about whether the microscope slides correspond to a diagnostic or treatment monitoring.

Selection of microscope slide in the supervisory laboratory.

Once the smears from the laboratory to be monitored have reached the supervising laboratory, the coordinator responsible for the selection of smears should proceed as follows:

- Mark the period covered by the sampling in the laboratory record and record the total number of smears made during this period.

- Divide the number of smears made during the period by the sample size.

- Round the result to the next whole number; this allows to calculate the "sampling interval". For example, suppose 230 smears were made in the quarter and you must take 24 slides, so divide 230/24 = 9.6. That is, you must take 1 slide in 10.

- Record in the sampling form (Annex B.2) the name of the laboratory to be evaluated and the period covered by the collection of the sample.

- Select the first smear of the sample, using a random number from 1 to 9, counting from the first record included in the sample. For ex. if the random number is 4, the smear number 4 counting from the first record of the period to be evaluated will be the first slide. Slides must always be selected using the laboratory record and not from the box with the slides.

- Register only the identification of the first slide in the Form of Annex B.2, without recording the result.

- Begin counting, starting from the first smear selected in the record, using the number corresponding to the "sampling interval", until reaching the second smear and registering it in the form. For ex. following the previous example, if the first smear was No. 4 of the record (smear 239) and the sampling interval is 10, the second smear will be No. 14 (4 + 10, smear249) (See example).

Example of selection of smears to be reread in the supervisory laboratory

Order	Date	Name & Last name	ID	Inmuno-comp	Previous treatment	Derivative Service	Type of sample	Results						Observations
								Xpert	Diagnostic			Control Treatment	culture	
									1	2	Other	Month	Result	
236	02-May		21457890			Hospital	Sputum			Neg				
237	02-May		15789456			Hospital	Sputum		2+					
238	03-May		01935678			Hospital	Sputum		Neg					
239	03-May		15789456			Hospital	Sputum			3+				
240	04-May		04123456			Hospital	Sputum			Neg				
241	05-May		14789001			Hospital	Sputum		Neg					
242	05-May		16732456			Hospital	Sputum		Neg					
243	05-May		29523778			Hospital	Sputum		3+					
244	06-May		18900023			Hospital	Sputum		Neg					
245	09-May		29523778			Hospital	Sputum			2+				
246	09-May		01000897			Hospital	Sputum		Neg					
247	10-May		12456723			Hospital	Sputum		Neg					
248	11-May		01342356			Hospital	Sputum		Neg					
249	12-May		14777890			Hospital	Sputum		Neg					
250	13-May		23412333			Hospital	Sputum		Neg					

- Continue in this way until reaching the required sample size. In case the end of the period is reached before collecting the whole sample, return to the beginning of the record and continue counting there.

- Collect the selected smears in a box and check the list again.

- If there is a missing smear, register its absence on the form and pick up the next slide of the record, placing its identification at the end of the form.

• If at the end of the selection of the sample, by chance, no positive smear has been included, add one or two from the register data, searching from the beginning.

• Send the sample of smears with the form of Annex B.2 to the controller.

• Save the copy of the laboratory record with the results of the selected smears in a physical or electronic folder that cannot be accessed by the supervising technicians.

Comparative reading of smears

Once the slides have been selected, they should be examined by the first controller. The controller should only receive the slides and a copy of the form with the slides identification numbers without the results.

Blinded rereading procedure

The first controller should:

• If the smears have traces of immersion oil, immerse them in a mixture of 80/20 ethyl ether / ethanol and let them air dry

• f the participant laboratory performs fluorescence microscopy, restain all microscope slides with auramine before its rechecking. For doing this, it is very useful to employ staining baskets, since they allows to stain several slides at the same time with less effort than using the classic staining on support. Staining quality will not be evaluated when these smears are being rechecked.

• Perform the reading of the microscope slides according to the technical standards established for the reading of the smears during the daily diagnosis work (check the same number of fields as established for routine reading in the Guidelines for the bacteriological diagnosis of tuberculosis Part 1. Updating the smear microscopy (2018)).

• Restain all the smears stained by ZN in case discoloration is suspected. This could happen when after the rereading of the smears it is observed that the first controller systematically detects quantification less than the one reported by the supervised laboratories.

• For the restaining, use the same procedure as for routine staining. It is not necessary to bleach the smears before restaining them. When staining, record on the form that this procedure has been done indicating the date on which it was made.

• Record the results of the microscopic reading in the Form of Annex B.2.

• Register in the form of Annex B.2 as "excluded / problems of identification" or "excluded / damaged" the smears that are not clearly identified or that are severely damaged and do not reread them.

• Carry out, during the reading, a technical analysis of each smear re-read in relation to the quality of samples and the characteristics of the smear made from sputum samples (size, thickness, homogeneity). For smears that have not been restained, the colour intensity of the bacilli, the presence of artifacts and the background staining will also be recorded. The evaluation of these topics will also be recorded in the Form of Annex B.2.For this analysis it is suggested to use the guidelines described in Table 4.

Table 4. Classification of the slides according to the specimen, smear and staining characteristics

Specimen quality	(only for sputum smears stained by ZN)
Mucopurulent	Most fields have leukocytes, in addition to mucus.
Mucosa	Most fields have mucus and very isolated leukocytes.
Saliva	In most of the fields epithelial cells, scarce mucus and very few leukocytes are observed.
Smear quality	
Good	To the naked eye of the controller, the smear is 2-3 cm long and 1-2 cm wide. It is homogeneously distributed and the contrast color is not intense. Microscopically, most of the fields present a sufficient amount of material, so that when moving the micrometric focus to an amplification of 800-1000x, between 1 and 3 levels are observed.
Fine	Most microscopic fields have little material.
Thick	To the naked eye of the controller, the appearance of the smear is blue or dark brown (depending on the contrast dye used). Microscopically, most of the fields have abundant material and when moving the micrometric focus to an amplification of 800-1000x, more than 3 levels are observed.
Not homogeneous	Presents fine and thick areas.
Short	It is less than 2-3 cm long.

Quality of staining (only for smears stained by ZN that have not been restained before rereading)	
Good	You can read 100 microscopic fields with good staining throughout the smear. It is considered that the microscopic fields have good staining when the background color does not present fuchsia red artifacts (precipitates or fuchsin crystals) and the contrast is light blue. In some cases, it is accepted that the background color has a slight pink hue. If bacilli are observed, they should appear intense fuchsia red.
Good (with crystals / precipitates fuchsine)	You can read 100 good microscopic fields, despite the fact that in the rest of the smears there are fields with crystals or fuchsin precipitates.
Good (with lack of discoloration)	You can read 100 good microscopic fields, despite the fact that in the rest of the smears are fields with insufficient discoloration (intense pink background color).
Deficient	The presence of crystals / precipitates or the lack of discoloration do not allow reading correctly at least 100 microscopic fields.

- After rereading, allow the immersion oil to drain by placing the smear in an upright position on an absorbent paper, and then rest the face of the slide that has the smear gently on another strip of absorbent paper (without rubbing). Place the slides back in their original box.

- Keep all the slides protected from the light and in a dry and cool place, until the disagreements have been resolved (if there is any).

Evaluation of the results

Evaluation of the specimen/ smear quality

• Calculate the percentage of mucopurulent, mucous and salivary sputum samples.

• From these calculations, calculate the percentage of sputum samples with adequate quality that is obtained by adding the percentages of mucopurulent and mucous samples.

• Observe in the report that a laboratory has an unacceptable level of adequate specimens, only when a significant amount of smears are thin without leukocytes (usually> 30%). This recommendation is due to the fact that the smears sample to be reread that results from the application of sampling options A and C (see Scheme 1) includes diagnostic and treatment monitoring samples, and it is well recognized that the samples of treatment monitoring are usually presented as fine and with very few leukocytes. This analysis, therefore, is not valid when stratified sampling is used, given that, in this case, most of the smears will come from treatment monitoring.

• Also note in the results report that the quality of the smears is not good when, among sputum samples considered as having adequate quality, there is a tendency to perform smears with some defect (short, non-homogeneous, thick, fine).

Evaluation of the staining quality

• Calculate the percentages of each one of the staining grades of all the smears stained by ZN in the participant laboratory, both belonging to pulmonary and extrapulmonary specimens.

• Consider that a laboratory has an acceptable level of technical quality of staining, when the proportion of good staining plus good staining with objections is higher than 95%.

• Even when this percentage is reached, observe in the results report that the quality of the staining is not optimal, when there is a tendency to perform smears with some defect (precipitates / fuchsin crystals, lack of discoloration, very intense contrasting staining) even when 100 microscopic fields with good staining can be observed.

Assessment of the reading quality

For the evaluation of the reading quality, a series of activities must be carried out, in which the local coordinator and possibly a second controller participate following a chronological sequence of actions, as described below:

Activities of the local coordinator

• Copy the results obtained originally by the participant laboratory corresponding to the smears read by the first controller in the Form of Annex B.2.

• Identify the discordant results between the first controller and the supervised laboratory; these can be positive vs. negative, or quantification errors (See the section on "Basic definitions").

• List the smears with discordant results in the form of Annex B.3. Record the name of the laboratory, the identification number of each smear and the two discordant results such as "result 1" and "result 2". To ensure that the second controller does not know the identity of each result, alternately use each column to place the results of the supervised laboratory and the controller, that is, for example, for some laboratories use the column "Result 1" for the result of the supervised laboratory while for other laboratories use the column "Result 2".

• Ask the first controller to separate the discordant smears to be read by a second controller.

• Do not provide, at this time, any feedback to the supervised laboratory, since the errors have not yet been validated.

Activities of the second supervisor

• Restain all the smears with discordant results, unless they have already been stained during the first control; in the latter case it will only be restained when the smears have been poorly preserved after the first restaining.

• Recheck these slides. Use the results of the supervised laboratory and the first controller (Result 1 and Result 2) to determine the number of fields to be read. Thus

- For disagreements, positive (1+, 2+, 3+) / negative, read two lengths

- For countable positive / negative mismatches, examine five lengths;

- For differences in quantification, examine as many fields as necessary to yield a confident result. To determine the number of fields to be examined according to the number of bacilli observed, use the indications found in the Manual for the Bacteriological Diagnosis of Tuberculosis Part I: Smear Microscopy of PAHO (2008).

• Record the results in the column corresponding to the second controller and send the coordinator along with the smears.

Activities of the local coordinator

• Copy the results of the second controller in the appropriate column of the form in Annex B.2.

• Using the results of the second controller as a reference, determine who was responsible for the error, by using Table 5. Remember that mistakes can be made by the technician in the supervised laboratory or by the first controller.

Table 5. Comparison of smear readings

Participant laboratory reading	**Final result at the supervisory laboratory**				
	Negative	Countable	+	++	+++
Negative	**Concordance**	Low False negative	High false negative	High false negative	High false negative
Countable	Low false positive	**Concordance**	**Concordance**	Quantification error	Quantification error
+	High false positive	**Concordance**	**Concordance**	**Concordance**	Quantification error
++	High false positive	Quantification error	**Concordance**	**Concordance**	**Concordance**
+++	High false positive	Quantification error	Quantification error	**Concordance**	**Concordance**

• Complete in the table located in the lower part of the form in Annex B.2 with the numbers of the smears examined and the errors committed by both the first controller and the participant laboratory.

Results interpretation and report

When it has been decided to sample the processed smears throughout the year of work, the final interpretation of EQA results is only possible after all the slides corresponding to the full sample size of that year have been processed. However, when errors are important (more than one HFP or HFN or several LFN and LFP) after the first period / s controlled, this should be reported immediately and a technical visit to the laboratory should be made in order to identify the causes of errors. Also, even if the laboratories do not present errors or the errors identified are not serious, you can choose to make partial reports of feedback in order to motivate the laboratories to continue with the collection and storage of the smears and improve their performance.

Prior to sending the report to the supervised laboratory, validate the results of the first controller's re-reading.

For this you must:

- Analyze the N° of FN committed by the first controller. If the FN number is very high or, it systematically detects a quantification less than that reported by the supervised laboratory, it is possible to suspect that the AFB may have faded prior to the reading of the first controller.

- Discard the results of the rereading if AFB discoloration is suspected since the results obtained lack reliability, and restain all the slides before rereading them by the first controller

Results analysis

When for the calculation of the sample size by the "Lot quality assurance sampling" method, a value of 0 is taken as an acceptable number of errors (d), in principle, the performance of any laboratory in which an error was identified would not be acceptable. However, the interpretation of results should consider the limitations of the system. As already mentioned, for the method to be applicable in field conditions, the sample must be small in order to avoid overloading the controllers, but because of this small sample size, the occurrence of some incidental findings is expected. Additionally, the absence of a gold standard value for the rereading procedure and the limitations inherent in the SM technique, associated with the inhomogeneous distribution of AFB in sputum (which causes limited agreement among readers for smears with scarce AFB) or the possible discoloration of the bacilli under inadequate storage conditions, make that the results of the rereading must be interpreted with caution. Therefore, **the finding of a single error does not prove that there is a real problem in the supervised laboratory** and the investigation could indicate that this finding was a random detection of a casual error or false alarm. **This is a fundamental concept that must be taken into account by the NRL when performing the interpretation and report of the EQA by the rechecking method.** For this reason, laboratories that clearly exceed the performance limits established by the NTP will have priority for corrective action: more than one HFP or HFN, or several LFN or

LFP. In the other cases, a new rereading of smears will make it clear if there is a real problem in the laboratory.

The identification of errors, therefore, does not automatically show that the laboratory has inadequate quality, but that the presence of such errors must be interpreted taking into account the type and frequency of identified errors. The following table presents possible causes, interpretation and recommendations in relation with the most frequent findings in the rechecking process.

Table 6. Possible causes, interpretation and recommendations for the most frequent findings in the rechecking of sputum smear examination.

Most frequent findings	**Possible causes**	**Interpretation of the finding / recommendations to be made in the report**
An isolated HFP error	- Errors in the results transcription in the list of slides sent to the supervisory lab. - Indeterminate causes.	- Request that the result of the smears be reviewed in the original register
Some LFP	- Limitation of rereading technique (may be due to false negative results of controllers).	- Low relevance finding.
Frequent presence of HFP (usually accompanied by several LFP)	- Microscope in poor condition that hinders the differentiation of artifacts and AFB. - Serious problems with the laboratory record. - Lack of proper training. - Problems with staining. Was AFB discolored before rereading? It is not recoloured before the second controller performs the reading of the discordant smears.	- Additional research is required. - A technical assistance visit may be necessary to elucidate the real causes of the identified errors. Note: To review the actions to follow to establish the causes of the disagreements during the visit, refer to the Table of Annex B.7.
Some LFN or an HFN	- Limitation of the rereading technique. - Errors in the results transcription in the list of results sent to the supervisory lab. - Indeterminate causes.	- Finding of low relevance if it is a LFN. - Request that the result of the smear be reviewed in the original record if it is an HFN.

Presence of an excessive number of FN (more than one HFN or several LFN)	- Superficial reading (in some cases related to work overload). - Problems with the preparation of staining solutions (inadequate concentrations of the primary dye / decolorizer / contrast dye, use of staining solutions after the expiration date). - Problems in the staining technique (deficient heating of fuchsin, insufficient time of exposure to the primary dye, excessive time with the contrast dye). - Problems in the preparation of the smear(very thick smear). - Microscope in inadequate conditions (usually insufficient light).	- Additional research is required. - A technical assistance visit may be necessary to elucidate the real causes of the identified errors. Note: To review the actions to follow to establish the causes of the disagreements during the visit, refer to the Table of Annex B.7.
Various quantification errors	- Problems with staining solutions. - Problems with the staining technique. - Problems with the microscope.	- Request to check the preparation of the dyes and the staining technique - Send dyes and smears prepared in the supervisory laboratory in order to evaluate the staining/ microscope.

Report

• Prepare the supervision report using a form similar to that presented to record the results of the re-reading (Form of Annex B.2).

• Include a summary of the observations made about the specimens, smears and staining characteristics of the slides and the reading quality. Table 6 can be used as a guide to perform the interpretation of results and the recommendations to include in the report according the most frequent errors found in the rechecking method.

• If minimum values required for considered a laboratory with adequate quality for samples / smears or staining characteristics have not been reached, or there is a "tendency" ¿ to make smears with some technical defect (both in the smear preparation and / or in the staining) (See Table 4), this observation should be noted in the report, indicating the **risks** of altering the quality of the SM, **the probable reasons for the error** and how to **fix** it. For example: Excessive fuchsin crystals constitute a risk of being confused with AFB; probable cause: fuchsin concentration higher than normal or unfiltered; solution: filter the fuchsin solution daily, check if the amount of basic fuchsin is the norm. The "Manual for the bacteriological

diagnosis of tuberculosis. Part 1. Smear microscopy Update (2018) "provides a guide that describes the most frequent problems associated with the staining and preparation of the smears, the causes of these findings, their consequences in the occurrence of false positive and / or false negatives errors and the corrective measures to be applied to solve the problems.

• If a "trend" to quantify results systematically lower than those of the supervisory laboratory is observed, it should be noted in the report, because this can lead to false negatives in specimens with low bacillary richness.

Note: periodically, the reference laboratory may request the copies of the reports sent to the supervised laboratories in order to analyze their structure and characteristics.

Annex B.8 presents some results reports examples.

Actions to be taken in case of disagreements

• Together with the results report, send the discordant smear(s) to the person in charge of the participant laboratory, asking him / her to read it again and / or consult his / her records, because sometimes the disagreements may be due to transcription errors of results in the list sent to the supervisory laboratory.

• Visit those centers with more than one HFP or HFN or several LFP or LFN in the annual sample in order to find out the causes of the errors. The total number of false negatives (LFN + HFN) and the proportion of HFN are used as a basis to prioritize visits to laboratories in which the occurrence of FN is a problem that requires research.

• During the technical visits, analyze all the possible sources of error found in the rereading of the smears and solve the identified errors. The suggested steps to investigate the reasons for the errors during the technical visit are presented in Annex B.7.

• In the case that it is determined that the microscopist is unable to correctly identify the bacilli, plan his retraining.

• Follow up periodic EQA by rechecking. This follow-up procedure will clarify whether the problem is fortuitous or permanent. If you have doubts about whether there is a problem in the laboratory or if the findings are simply by chance, it is better not to judge negatively the performance of the laboratory, but simply to show the results and encourage them to solve the deficiencies found. In these cases, it is also recommended to reinforce quality assessment by sending, reading and interpreting a panel testing set of slides prepared by the NRL.

Evaluation of controllers' competence

To facilitate the analysis of the competence of a controller it is useful to evaluate their performance in the rereading of several laboratories. The local coordinators of the re-reading, located in the intermediate and reference laboratories, are in charge of ensuring that the performance of the controllers is analyzed, since the validity of the results of the re-reading depends on this activity. The filling of a form with characteristics similar to the Form in Annex B.4, can be helpful for this activity. This form can be sent from the intermediate laboratory to the reference laboratory, in order to globally evaluate the performance of the controllers, and indirectly the role of the intermediate laboratories.

• Suspect inadequate performance of the first controller in the following situations:

- No discrepancies are detected in the readings of several laboratories in which there are regularly positive results. This is considered an unexpected result and may be associated with the lack of reading blinding by the supervisor.
- The first supervisor commits more FN than those incurred by the laboratories he oversees. Because the reproducibility of smears with 1 to 9 AFB in a length is approximately 50%, the number of LFP and LFN must be similar between the results of the participant laboratories and those of the supervisory services. An unequal distribution could indicate a problem for the controllers. Therefore, if the first supervisor commits more FNs than those incurred by the laboratories he oversees; this may be associated with a lax reading of the controller. In extreme cases, this may cause that false negatives have not been detected, which would invalidate the results.

However, this analysis based on the comparison of the number of errors between the controllers and the supervised laboratory is not valid in the following cases:

- When positive results are added to the rereading sample of smears. This results in a proportion of positivity of the smears sample re-read by the controller greater than that of the supervised laboratory. In this case the controller is at a disadvantage with respect to the supervised laboratory and it is expected that he can naturally commit more FN. However, as a general rule, the percentage of true positives (TP) identified by the first controller (VP identified by the controller x 100 / (VP identified by the controller+ FN committed by the controller)) should be considered to be greater than 90%.
- When, due to the conditions in which the smears are stored, it could happen that the AFB have become discolored. If the first controller does not restain before rereading, then he will simply commit FN errors because the bacilli are discolored and are not visible under the microscope. In extreme cases, when the number of FN committed by the supervisor is very high (much greater than those committed by the laboratories that it supervises) and, because it cannot be asserted

that the occurrence of these FN by the supervisor is due to an inaccurate reading or discoloration of the bacilli, the reading would be invalidated and it would be necessary to restain all the smears before re-reading by the first controller.

Also suspect inadequate performance of the second supervisor in the following situation:

- When a high percentage of FP confirmed by the second supervisor is identified. When due to a lax reading, the first controller identifies some probable FP, if the second controller performs adequately, it will detect the probable FP of the participant laboratory as FN of the first controller and correct the error committed by the first controller. But if the reading of the second controller is also not very rigorous, this error of the first controller will not be corrected and therefore a high number of FP will be detected, while the detection of FN both for the first controller and for the supervised laboratories will be very rare. Whenever several FPs are detected in many centers, this should alert the performance of the controllers. However, this may also be a consequence of carrying out the rereading without restaining.

In summary, to consider competent a first controller must have registered among their results:

- Discordance with the readings of several laboratories in which there are regularly positive;
- Less FN and less LFP than the supervised laboratories (this analysis has relative validity when the proportion of positivity of the sample to be read is greater than that of the supervised laboratories or when there is no restaining of the smears by the first controller before rereading);
- Absence of HFP errors.

Global rechecking results record

• Prepare, at the end of each year, a report of the performance of the participant's services, including a list of the errors identified in each laboratory, by type of error. Optionally, this list can be made for each series of samples (trimesterly, quarterly, semiannually). The form in Annex B.5 presents a model of this type of report.

• Perform, in addition, a report with the consolidated results of the main performance indicators calculated for all the laboratories corresponding to an area. A model of this report can be found in Annex B.6. The calculation of these global indicators and their comparison with the parameters determined in previous years, allows us to analyze the trend of coverage indicators (number and percentage of laboratories evaluated) and overall performance of laboratories (percentages of errors FN and FP, percentages of laboratories with more than one HFN or HFP, etc.), in order to be able to identify if the corrective actions applied have been effective in maintaining and / or improving the quality of the SM.

Note: or the realization of the reports it is not recommended to present indicators in which all the errors are added together (FN and FP), since a single value of concordance or discordance is not informative, particularly since the occurrence of these errors are usually associated with different qualitative problems related to the quality of the smear and the staining. This type of analysis (percentage of agreement) will cause the loss of valuable information; it is important to bear in mind that the objective of the EQA is not to score laboratories but to detect performance problems. It is also recommended not to calculate error frequencies for each laboratory, since the result of this calculation is inaccurate (wide confidence limits) due to the small sample size. On the other hand, error rates can be calculated for several laboratories of a specific area.

Calculation of percentage of false positives and negatives for a group of laboratories in an area

Positive informed films by laboratories

$$\text{\% false Positives} = \frac{\text{Slides considered positive by the participant laboratories and negative by the controller}}{\text{Total smears reported as positive by the participant laboratories}} \times 100$$

$$\text{\% false Negatives} = \frac{\text{Slides considered negative by the participant laboratories and positive by the controller}}{\text{Total smears reported as negative by the participant laboratories}} \times 100$$

Monitoring the quality of each laboratory

• Monitor the performance of each partipating laboratory by examining some EQA indicators (N° of identified HFN, LFN, HFP, LFP) over a period of time. This will allow to temporarily monitor the quality of them (identify their systematic errors, progress achieved, place it in the category of laboratory with consistent good quality) and facilitate the planning of technical visits, training or acquisition of resources, in accordance with the performance they have shown. In Annex B.9. a standard form is presented to record this information. A simple way to quickly analyze the data discharged on it, is through the use of spreadsheets that allow the application of "conditional formats" to some columns, so that the cells will be automatically labeled by color, according to the laboratories that remain classified as below or above a predefined performance level. Thus, for example, a spreadsheet (with the characteristics of the Form in Annex B.9) could be designed in such a way that the cells corresponding to the N° of HFN or HFP would be colored red when the N° incorporated on it was ≥ 2, of yellow when

the value included was 1 and of green when it was 0. Similarly, the cells corresponding to the N° of LFN and LFP could be formatted so that they are colored red when the values are greater than or equal to 3, yellow for a value of 2, and green for values less than 2. An example of a form with these characteristics, in which it is visually identified that the corrective measures applied were effective to reverse the errors committed by the laboratory, can be found in the Annex B.10.

Proficiency testing (Supervision Center-Periphery)

The modality "Center-Periphery", consists of sending a panel testing sets of slides prepared at the Reference Laboratory for reading in the laboratory to be monitored and compare results. This method evaluates only the ability to read and report results, but not the integral procedure of the SM. Some unstained slides can be added to evaluate the quality of the staining solutions/ staining technique. This method does not evaluate the performance of the entire laboratory, but that of each technician individually.

Some of the advantages of this method are:

- Offers the possibility of carrying out a quality assessment in a large number of laboratories at the same time,
- Allows having consensus results,
- Generates little work overload for the participant laboratories.

Its disadvantages are that it does not evaluate routine practice and implies a work overload for the supervisory laboratory.

This type of evaluation is useful:

- To have data on the quality of the reading, especially in terms of recognition of bacilli.
- To supplement the EQA by routine SM rechecking method when the number of smears performed by the laboratory and / or its positivity are very low (Option C of the EQA method; Combined Methodology).
- To evaluate technicians after training
- To maintain the ability of recognizing the AFB in laboratories that, because they process few samples, have little opportunity to observe positive SM.

Necessary resources

- Reference laboratory with technical and operational capacity for the preparation and validation of batches of smears.
- Panel distribution mechanisms and economic resources for shipping.
- Supervisory laboratory staff with sufficient time to analyze the results.
- Forms and fluid communication systems.
- Capacity of the reference laboratory to implement the necessary corrective measures including retraining.

Preparation of panel testing sets

Some years ago, the panel testing sets were prepared using the smears made in daily practice of the supervisory laboratories. Another option that was used was the realization of a large number of smears from the same sputum sample. However, since this procedure did not guarantee homogeneity in the number of AFB to be read in all the prepared smears, it was necessary to stain and read each of the smears before preparing the panel testing sets. In addition, these smear procedures had consistency problems, which made difficult to compare yields among the different technicians.

Currently, it is recommended to prepare panel testing sets from smears specially developed for this purpose, which we will call slide batches, (See Section of Basic Definitions) following a uniform procedure described in Annex C.1 of this manual. This procedure also includes registers and forms to collect information on the quality of the slide batches that make up the panel testing sets (Annex C.2 and C.3).

These specially made slides have the following characteristics:

- they can be stained or unstained
- they have a known amount of AFB
- present minimal variations in the amount of bacilli present in each slide with the same semiquantitative result, which minimizes the variation of the expected results
- allow to provide a uniform test for the participating technicians

The preparation of panel testing sets requires time, practice and experience. It is recommended that they are carried out and validated in the NRL so that they have uniformity throughout the country.

Number and type of slides that make up the panel testing set

The number of slides of each panel testing set should be enough to validate the test, but do not overload the work of the participating technician or the NRL that prepares the panels. In practice, it is considered impossible to prepare a panel large enough to perform an evaluation of the technician's performance that has statistical significance. Panel testing sets of 10 slides are considered appropriate (representing about half the maximum number of ZN-stained slides that a technician could examine per day without loss of quality). There must be negatives and positives of different degrees including

countable positive slides. Exactly identical panels should be prepared for all laboratories that will participate in each round. The degree of difficulty increases as the proportion of slides with low positivity increases. NRLs that start in the use of panels usually focus on major errors, so it is common to use panels with low degree of difficulty. As EQA programs become established, it is recommended to monitor minor errors as well; the use of panels of higher degree of difficulty can be useful for such purpose.

Some examples of panel testing sets are presented below:

	Degree of Difficulty		
Slides	**1st**	**2nd**	**3rd**
3+	1	1	-
2 or 3+	-	-	1
2+	1	1	-
1+	1	2	2
Countable	2	3	3
Negative	5	3	4
Total	**10**	**10**	**10**

The sets can be composed of stained and unstained slides. This will depend on the objectives of the panel evaluation and the available resources. The following table summarizes the advantages and disadvantages of stained vs. unstained slides.

Table 7. Advantages and usefulness of stained and unstained slides to be used in proficiency testings

Utility and advantages	Disadvantages
Stained sets	
- They allow evaluating the quality of the technicians in the reading and results report. - They ca help to identify problems with the quality and operation of microscopes. - They can be used several times, depending on the conditions of conservation of the slides.	- They do not allow getting information about the ability of the technicians to stain the slides nor in the quality of the staining solutions.
Unstained sets	
- They allow evaluating the quality of the technicians in the reading and report of results. - They also allow to evaluate the quality of the staining solutions/ staining technique - Their use reduces the workload and the consumption of staining solutions at the NRL	- They can only be used once.

In the case of unstained sets, when a laboratory has more than one technician, one should choose to send a single panel that will be stained by a technician and read by all the others, or to sending of a panel for each technician; in the latter case, the workload for the NRL will increases significantly.

The composition of the panel testing sets sent to each laboratory must be registered in a form like the one shown in Annex C.4.

General considerations and recommendations for the organization, reading and evaluation

The most important aspects to consider are the following:

- **Frequency of evaluations.** It is recommended that the frequency of sending panels be at least once a year when using the "Combination of rechecking method supplemented by panels (Alternative C, see Scheme 1). If the EQA system for SM is only carried out through the use of panels, it is advisable to increase the frequency to twice a year.

- **Cooperation of intermediate laboratories.**
It is very convenient that the rounds of panels are carried out in close collaboration with the laboratories of intermediate level; thus, the NRL will send the panels to the intermediate laboratories and these will be in charge of distributing them to the peripheral laboratories; in the same way, they will be responsible for requesting the results and sending them to the NRL; they will also be in charge of making technical visits to the laboratories with low performance technicians and of retraining the staff if necessary.

- **Instructions for participating laboratories:**
The panel testing sets must be sent together with a results report form that includes a description of the purpose of the test and basic instructions for the development of the activity, e.g. the need or not to stain the slides, the time established to send the results. An example of this form can be found in Annex C.5.

- **Distribution of the panel testing sets.** Distribution by mail or other delivery system is advisable when visits to laboratories by the intermediate laboratory cannot be done frequently or when simultaneous shipment to all laboratories is planned.
If they are sent by mail, it is advisable to have a suitable container that prevents breakages of the slides. The delivery of results from the peripheral services can also be done by postal mail or by electronic mediums, as long as the forms are available in this format.

- **Time set for the return of panel results.** Based on the conditions of each country, the NRL must determine the established time for the returning of sets results. A period of 15 days to a month is considered reasonable.

Registration and results evaluation

Annex C presents models of forms and records for sending panel testing sets to the laboratories and for evaluating and reporting the results. These include:

- form to record the results obtained in the reading of panels by each of the evaluated technicians (Annex C.5)
- instructions for carrying out the test (included in Annex C.5)
- the registration in the reference laboratory of all the results obtained by the technicians participating in the test (Annex C.4)
- the report form of results of the evaluated laboratory (Annex C.6)

For the results evaluation is necessary to consider the following points:

Resolution of disagreements

No system of panels preparation and distribution is free of problems, so the reference result of each slide of a panel set must be validated before scoring participants in order to establish whether the found discordances are the responsibility of the technician and / or due to problems in the preparation of the panel sets.
For doing this:

- If the logistics of the test allows the return of the panels together with the results report, reread in the NRL all the discordant slides in order to establish the responsibility of the disagreements.
- If it is not possible to return the panel sets to the NRL, proceed to post-validation of the panels after receiving the results of the network laboratories that have been evaluated. If 20% or more of the participants fail to correctly report a slide, this may be indicative of problems in the NRL for the preparation of the slides. In this case:

  - Request all participating laboratories to return the panels for re-reading. If after the rereading, the problems with the preparation of a slide are confirmed, this slide must be discarded for the results report of all the participants of the test.
  - If the return of the panels is not possible, take the decision to eliminate a slide of the results analysis if more than 20% of the participants fail in the result of this slide, unless the discordant results are concentrated in technicians of a laboratory or in microscopists newly incorporated into the network.

Assigning scores

- Assign scores, considering the number and type of error, following the classification of errors presented in Table 5 of this manual. The evaluation should consider major and minor errors. Quantification errors are considered minor errors.

For a panel composed of 10 slides (maximum score; 100), technicians will receive

- 10 points for correctly identifying a negative slide (0 AFB / 100 fields);
- 10 points for correctly identifying and quantifying a slides positive even when

there is a low number of bacilli (positive countable);
• 5 points for each low false negative or low false positive error;
• 5 points for each positive slide with quantification error;
• 0 points for each high false positive or high false negative.

• Consider that a microscopist has sufficient efficiency when he obtains a minimum of between 80 and 90 points (as initially established by the NRL) and does not present any FPH or FNH result. As the network improves in quality, the requirements should be increased, as well as the degree of difficulty of the panels (for example, by including more smears with few bacilli).

• If the laboratory has received stained and unstained panel testing sets, the supervising laboratory will analyze each panel (stained and unstained) individually, compare the results and interpret the set deciding if the errors are due to problems in the staining and / or the reading. If the positives of the stained slides are correctly reported, while the errors are only in the positives of the unstained slides, the problem is in the staining solutions or the staining technique. This can be verified by restaining and reading the returned slides. If the errors are identified in stained and unstained slides, the most plausible reason may be the lack of ability to recognize the AFB or the use of a microscope in poor conditions.

• In case the microscopist has obtained the minimum score established by the NRL as acceptable, but presents an HFN or HFP result, it is recommended to send a new panel in order to determine if there is a staining / reading problem or if the finding is simply because of an administrative error (error when recording the result).

Proficiency test report

• Inform the results of each slide, the score obtained and the number of errors committed by type of error.

• Indicate, in addition, the probable causes of error, the suggestions and recommendations to solve the causes of the identified errors, considering for the analysis, the occurrence of false positive and negative errors separately. A model of such a report can be found in Annex C.6.

Table 8 shows the most frequent types of errors, the possible causes of these errors, the interpretations and suggested recommendations to incorporate in the report of the proficiency testing.

Table 8. Possible causes of errors, interpretation and recommendations for the most frequent findings in the proficiency testing.

Type of panel testing set	Most frequent findings	Possible causes	Interpretation of the finding / recommendations to be made
Stained panel	An HFP error or an isolated HFN	- Errors in the transcription of results in the results form. - The same causes listed for the finding of more than one FP (see below).	- Send a new panel. - If the problem persists after reading the second panel, see recommendations to be made in case of the finding more than one FP (see below).
	Some LFP or LFN	- Limitation of the rereading technique.	- Low relevance finding.
	Presence of one or more HFP together with one or more LFPs	- Microscope in poor condition that hinders the differentiation of artifacts and AFB. - Lack of proper training.	- Additional research is required. - A technical assistance visit may be necessary to elucidate the real causes of the identified errors. Note: To review actions to follow to establish the causes of the errors during the visit, refer to Annex B.7.
	Various quantification errors	- Problems with the microscope. - Lack of proper training.	- Request to revise normatized quantification scale - A visit may be necessary to analyze the conditions of the microscope.
Unstained panel	An HFP error or an isolated HFN	- The same as those listed for the stained panel.	- The same as those listed for the stained panel.
	Some LFP or LFN	- Limitation of the rereading technique.	- Low relevance finding.
	Presence of one or more HFP together with one or more LFPs	- The same as those listed for the stained panel. - Problems with staining solurions/ staining (presence of primary dye crystals due to lack of filtering or heating thereof, lack of discoloration).	- Additional research is required. - A technical assistance visit may be necessary to elucidate the real causes of the identified errors. Note: To review actions to follow to establish the causes of the errors during the visit, refer to Annex B.7.

	Presence of one or more HFN together with one or more LFN	- Little rigorous reading. -Microscope in inadequate conditions (usually insufficient light). - Lack of appropriate training to identify AFB. - Problems with the preparation of staining solutions (inadequate concentrations of primary dye / decolorizer / contrast dye, use of dye solutions outside the expiration date). - Problems in the staining technique (deficient heating of fuchsin, insufficient time of exposure to the primary dye, excessive time with the contrast dye).	- Additional research is required. - A technical assistance visit may be necessary to elucidate the real causes of the identified errors. Note: To review actions to follow to establish the causes of the disagreements during the visit, refer to Annex B.7.
	Various quantification errors	- The same as those listed with the unstained panel sets. - Problems with staining solutions / staining technique.	- Request to revise the normatized quantification scale. - A visit may be necessary to analyze the conditions of the microscope / staining solutions preparation / staining technique.

• Send the result to the participating technicians in a confidential and timely manner. Sample reports are shown in Annex C.8.

• Record the test data of each technician or laboratory, in order to be able to plan the technical visits, training or acquisition of resources, according to the performance demonstrated by the technicians / laboratories.

• It is highly recommended to make visits to those centers where low scores technicians have been identified, in order to recognize the problems and to be able to make the corresponding recommendations; Cooperation with intermediate laboratories is essential for the fulfillment of these field activities. During technical visits, all possible sources of error should be investigated. The possible causes of the errors identified and the suggested steps to identify the reasons for the findings during the technical visit are presented in Annex B.7.

• Conduct an annual summary report with the results obtained by the technicians participating in panel testings. An example of such a report can be found in Annex C.7.

INDIRECT EXTERNAL ASSESSMENT FOR CULTURE

General considerations

There is a gap in international standards regarding the methods to be applied for this type of evaluation. Thus, even if they are not universally applicable, this guide proposes two methodologies for the external evaluation of the technical quality of cultures:

1. Analysis of culture quality indicators
2. Evaluation of the quality of culture medium based on eggs and liquid medium

1. Analysis of culture quality indicators

Periodic quality monitoring through the performance indicators calculated by the laboratories in the process of internal quality control, allows to detect technical procedures that deviate from the norm, which may affect the quality of the culture and therefore the diagnostic quality in the network. It is a fundamental input for the planning of technical visits, training or acquisition of resources.

Procedure

Data collection:

In Annex D.1, a model form is presented to request the information laboratory of the network that cultivates.. The minimum collection frequency must be at least yearly in order to be able to carry out an individual and general follow-up of the laboratories in the network. In cases where a special situation needs to be analyzed (introduction of new methodology, abrupt and unexpected increase or decrease in the number of cases detected by culture), the request could be adapted to the aspect that one wants to focus (by laboratory, geographical area, prevalence or national level) and the frequency of data collection could be increased.

Analysis of the information

Following the model of the indicators of internal quality control proposed in the "Manual for the bacteriological diagnosis of tuberculosis part II: culture of OPS (2008)" and updated according to the "Manual of Mycobacteriology Laboratory of the GLI (2014)" is recommended perform the analysis of the following parameters:

a) Contamination percentage
b) Relationship between smear and cultures results
c) Contribution of the culture to diagnosis
d) Analysis of the delay in the production of the results report

a) Contamination percentage

When the samples are not properly decontaminated, either because there is a fault during the analytical phase of the culture or because the samples have been inadequately conserved during transport, which increases their no mycobacterial load, the percentage of contaminated tubes increases.

The indicator to analyze (which is calculated on the cultures made to **the samples containing normal flora, mostly respiratory samples) is as follows:**

N° of contaminated tubes * 100 / Total number of tubes planted

The value should not exceed the average value estimated as normal for the percentage of contamination of tubes in solid medium (3-5%) or liquid medium (8-10%).

This indicator can be analyzed taking into account the coverage and route area of each sample transport system, the type of transport used (private-owned), (motorcycle-car), (with or without cooling), time of travel and temperature per area, and the internal delays of each laboratory in the processing of samples.

b) Relationship between smear results and cultures:

Diagnostic samples with positive sputum smear or positive Xpert result should normally result from positive culture. The degree of positivity of the culture (on the scale of crosses) should normally be equal to or greater than that of smear microscopy (also on the scale of crosses).

This parameter allows to evaluate the culture procedure in its entirety, beginning with the conservation of the sample, since a smear positive or Xpert positive sample may result with negative culture, because the bacilli may have lost viability because of the bad conditions of conservation and transport. Additionally, it evaluates the decontamination of the sample and concentration of the bacilli by centrifugation and or the quality of the culture medium.

It is advisable to analyze this parameter by separating the samples grown in solid or liquid medium.

The indicator that is recommended to monitor is the proportion of smear positive or Xpert positive samples with positive culture. For calculation, count the number of smear-positive diagnostic samples that resulted in a positive culture and the total number of smear-positive diagnostic samples processed per culture and apply the following formula

N° of positive diagnostic samples by bacilloscopy or Xpert with positive culture * 100 / N° of diagnostic smears positive or Xpert processed by culture

The expected values are> 95-98%. The requirement may increase within this range as an improvement in quality is managed.

c) Contribution of the culture to diagnosis

Culture is a fundamental tool in the elimination of TB since it allows the detection of paucibacillary cases. However, its value depends on the epidemiological situation, the population studied (the sensitivity is decreasing for children or immunosuppressed with respect to non-immunosuppressed adults) and the location of the disease (sensitivity is lower for extrapulmonary TB than for pulmonary TB).

There are more uniform guidelines in relation to adult pulmonary TB. For this reason, adult patients (not samples) are selected for the calculation of this parameter with bacteriologically confirmed pulmonary TB from diagnostic respiratory samples.

They are classified into the following categories using the information collected with the form presented in Annex 1, based on the items:

-B2a when smear (SM) is used as the first diagnosis:

a. SM (+) and culture (+)
b. SM (+) and unrealized culture
c. SM (-) and culture (+)
d. SM (+) and culture (-)
e. SM (+) and contaminated culture
f. SM not performed and culture (+)

Contribution of the culture to diagnosis $\frac{c}{a + b + c + d + e} \times 100$

-B2b when the closed amplification system Xpert MTB/RIF is used as the first diagnostic test in replacement of the SM

a. Xpert MTB/R(+) and culture (+)
b. Xpert MTB/R (+) and unrealized culture

c. Xpert MTB/R (-) and culture (+)
d. Xpert MTB/R (+) and culture (-)
e. Xpert MTB/R (+) and contaminated culture
f. Xpert MTB/Rnot performed and culture (+)

Contribution of the culture to diagnosis $\frac{c}{a + b + c + d + e} \times 100$

In the case that both methodologies are used, the comparison between both can be made. As with other population-based indicators, the network must establish the "normal" values for each methodology, establishment, jurisdiction or nation. Since the culture is more sensitive than sputum smear and closed amplification methods, it is expected that its contribution to the diagnosis of adult lung forms is at least:

- 15-20% of cases with bacteriological confirmation when compared to smear microscopy.

- 10% when the closed molecular method is used considering that both tests (culture and closed molecular method) are applied to a population whose proportion of smear negative cases with positive culture is 20%. However, applied to populations where smear microscopy has a lower yield, such as HIV positive patients, the contribution of the culture should be between 15-25% (considering that the proportion of cases smear negative with positive culture is 50%). It is important to bear in mind that, with the use of the Ultra cartridge, the contribution of the culture will be less than the one described.

Summary of the characteristics of the quality indicators of the culture technique:

Quality indicators	characteristics
Contamination percentage of contamination (per tube) It is calculated on the cultures made to the respiratory samples that contain normal flora.	The acceptable range in solid medium is 3-4% and in liquid medium it is 8-10%. **Numerator:** Number of inoculated tubes that are contaminated in a certain period of time **Denominator:** Total number of tubes that were inoculated for culture in the same period Multiply by 100 to express as a percentage
Proportion of smear positive or Xpert positive samples with positive culture It is calculated taking into account diagnostic samples that contain normal respiratory flora.	The acceptable range of recovery of positive cultures between respiratory specimens with positive sputum smear or positive Xpert is> 95-98%. **Numerator:** Number of diagnostic samples with smear positive or Xpert positive that were reported as positive culture in a period of time **Denominator:** Number of smear positive or Xpert positive samples cultured during the same period Multiply by 100 to express as a percentage
Contribution of the culture to the diagnosis of cases of pulmonary TB (in relation to smear microscopy) It is calculated taking into account adult patients (not samples) with pulmonary TB confirmed bacteriologically from respiratory samples.	The expected range is that 15-20% of the cases with bacteriological confirmation are cases with negative sputum smear and positive culture. **Numerator:** Number of patients with negative smear-negative pulmonary TB who were reported as a positive culture in a period of time. **Denominator:** Number of patients reported with pulmonary TB by bacilloscopy and / or culture in the same period of time.

Contribution of the culture to the diagnosis of cases of pulmonary TB (in relation to the Xpert MTB / RIF or Xpert Ultra MTB / RIF test) It is calculated taking into account adult patients (not samples) with pulmonary TB confirmed bacteriologically from respiratory samples.	The expected range is that about 10% of cases with bacteriological confirmation are cases with Xpert MTB / RIF negative and positive culture (for a population whose proportion of smear negative cases with positive culture is 20%). In populations for which the proportion of cases with smear negative and positive culture reaches 50% (HIV positive), the contribution of the culture should be between 15-25%. With the use of the Ultra cartridge, the contribution of the culture will be less than described. **Numerator:** Number of patients with pulmonary TB Xpert MTB / RIF or Xpert Ultra MTB / RIF negative that were reported as positive culture in a period of time. **Denominator:** Number of patients with pulmonary TB diagnosed by Xpert MTB / RIF or Xpert Ultra MTB / RIF and / or culture in the same time period.

The different indicators can be analyzed by performing the following disaggregations: by laboratory, type of population studied, origin of the samples that have been derived, method of culture and culture medium used.

The causes of higher or lower values of each of the three indicators expressed are summarized in the following table:

Indicators of culture and possible causes of failures

	Alarm signs		
	Average value %	**If it is much greater to investigate**	**If it is much less to investigate**
Contribution of the culture to the bacteriological diagnosis	15 - 20% over sputum smear 10% over the closed molecular method	A	B, C, D, E, F, G, H
Percentage of contaminated tubes	3-5 % (solid medium) 8-10% (liquid medium)	C, D, E	F
Proportion of smear positive samples with positive culture	95-98%	No problem	C, D, E, F, G, H, I

A	• Smear-reading errors: "false negatives" • False negative errors of the Xpert MTB / RIF test associated with problems with the conservation of the cartridges, malfunction of the modules, or equipment • A high percentage of cases of advanced pulmonary TB is being investigated, including pediatric patients or people living with HIV (does not indicate a technical laboratory problem)
B	• Poor culture request (respiratory symptomatic subjects (RS) are not being investigated), patients who are not RS are being investigated)
C	• Excessive delay between the taking and processing of samples due to inconveniences in transport logistics (excessive transport path areas, inadequate vehicles) • samples preserved without refrigeration
D	• Decontaminant concentration lower than normalized • Short contact time of the sample with the decontaminant
E	• Defects in the sterilization process • Carelessness in procedures that require sterile (misuse of bunsen burner or lighter, improper use or damage to the biological safety cabinet) • Excessive movement of personnel in the work area, generation of air currents by fans or air conditioning equipment, etc.

F	• Very energetic decontamination of the samples by - bconcentration of the decontaminant higher than the norm and / or - excessive contact time with the decontaminant • Excess malachite green in the culture medium
G	• Low medium sensitivity (lack of homogeneity, overheating when coagulating, excessively acid pH) • Incubation of cultures at very high or oscillating temperatures
H	• Low speed or overheating in the centrifuge
I	• Smear reading errors: “false positives” • The samples do not correspond to patients studied for diagnosis but are patients in control of treatment.

The data of each laboratory are referred to Annex D.2: Culture yield form. It is convenient that the incorporation of those laboratories that carry out culture but that do not produce their own mediums, is done using the laboratory code that provides them with a subscript in order to show a problem that relates to the sensitivity of the environment. Annex D.3 allows to collect and monitor the indicators of culture yield with the quality of the medium (evaluated as explained below) to show some type of relationship between them.

d) Analysis of the delay in the production of the results report

The timely notification of the results is fundamental for the clinical management of the patient. The monitoring of the time of emission of the results allows that some laboratory procedures can be optimized (for example, the appropriate use of rapid culture methods and the elimination of unjustifiable delays). On the other hand, it helps identify challenges with NTP algorithms and the workflow of each laboratory, information systems and reporting systems.

The recommended indicators are the following:

• Name: Time for the issuance of reports of samples processed by culture in solid medium

Formula: (Number of reports issued for term / Total number of reports issued when solid medium is used) * 100
Criteria of acceptability: At least 95% of the culture results must have been reported within 21 days (smear positive and / or positive Xpert) and within 63 days (smear negative and / or Xpert with trace detection or negative) of processed sample when using the conventional method with solid medium.

• Name: Time for the issuance of reports of samples processed by culture in liquid medium

Formula: (Number of reports issued for term / Total number of reports issued when using liquid medium) * 100
Acceptability criteria: At least 95% of the culture results must have been reported within 8 to 10 days (smear positive and / or positive Xpert) and of 43 days (smear negative and / or Xpert with trace detection or negatives) of processed sample with automated reading equipment.

Ideally, the time for the issuance of reports should be taken from the collection of the sample, but this is not always known. In the case of having this information, it is suggested to divide in delay from the reception until the processing (it must be between 24 to 72 hours in the case of weekend), and from the processing to the report of results.

On the other hand, the laboratory must also take into account the delay in transporting the sample, and try to measure it. If it is not possible to measure, at least it should be discussed with the health centers that the time between collection and arrival in the laboratory can not or should not be> 24 hours.

For both cases it should also be verified that a similar percentage of the positive cultures have been informed within 48 hours of the development detected, or since the alarm of the automated reading equipment has sounded. Solid medium should be inspected at least weekly or as more frequently if the standard indicates.

• Name: Times of sending culture reports once issued

Formula: (Number of reports delivered to term / Total number of reports) * 100
Acceptability criteria: At least 95% of the reports issued must be sent within 2 days and 100% up to 5 days after.

Request the follow-up of the response time indicator. In case of excessive delays, the possible causes should be investigated, and the laboratory should be guided with the possible technical, administrative or computer solutions.

There are several factors that can lead to delays, such as excessive workload in relation to operators and equipment available, inadequate scheduling of tasks or lack of resources for the rapid delivery of results. They may also be related to the procedures, from which the stage (s) in which this delay occurs must be identified: processing of samples, reading of cultures, record of results, writing of the report, delivery of the result. The identification of the causes should preferably be carried out during a technical assistance visit, to then reorganize the activity accordingly.

Report of the quality of the culture

A report template is presented in Annex D.4. This report arises from the analysis of culture indicators and their possible failures during the period evaluated. In the same one a paragraph of observations must be incorporated, expressing the situation of the laboratory of that year. In addition, it is important to analyze the previous year / s for the trend of the different parameters. It is advisable to emphasize the data / indicators / observations associated with an acceptable performance or those in which an advance has been demonstrated to stimulate the laboratory personnel. Annex D.4.1 presents an example of the evaluation of the quality of the culture and the observations made and Annex D.4.2 shows an example of the monitoring of these parameters over the years, in order to be able to detect visually any incidental or persistent deviations that may have been recorded for the laboratory.

2- Quality of solid and liquid culture medium

The sensitivity of the medium prepared in the laboratory network can have notable variations in relation to the experience, quality procedures of some critical inputs used (e.g. eggs). It is recommended that once a year or every two years, depending on the feasibility and the size of the network, the quality of the culture medium of all laboratories producing egg-based medium (Löwenstein Jensen and Stonebrink) or Middlebrook 7H11 / 7H10 neutral is monitored in a single experience, including firms that market some of those prepared medium or that market their dehydrated bases in the country. In cases where the general evaluation is impossible and values of the indicators of culture quality are not acceptable, among several users of the medium prepared by a network laboratory, or by a laboratory that elaborates mediums, an evaluation can be made with fewer batches of medium from all the laboratories involved or from a laboratory together with several batches of medium produced in the NRL.

The liquid medium is elaborated by the producing companies with standardized procedures and synthetic reagents. In general, they are used by several laboratories in the network. If they were eventually of poor quality, there would be evidence of unacceptable values for all the laboratories that use it.

Requirements and necessary resources

- Structured laboratory network.
- NRL that leads the experience.
- Transport system for the shipment of lots in a fast way.
- Sufficient equipment for the maintenance and incubation of the medium (refrigerators and incubator stoves at 37 ° C).
- Sufficient supplies for seeding the medium (micropipettes, 50 ml tubes for inoculum preparation, disposable 2 ml pipettes, trays for incubating tubes with inclination of 5-10°, plastic boxes to keep tubes upright, manual colony counter).
- Fluid communication systems.
- The reference laboratory must have the necessary mediums to implement corrective measures, including retraining.

Procedure

The reference laboratory asks the producing laboratories for 12 tubes taken at random from the lot in use or recently produced.
At the same time have must to collect , by mediums of the Annex D.5 Form Cultivator-Elaborator of mediums, the following information:

Brand of the reagents used for production
Procedure used for coagulation (equipment, temperature and time)
Output
Workload (number and type of cultivated samples)
Culture method used

If there are laboratories that produce to provide medium to other laboratories in the network, it is a good opportunity to gather information about this activity.

Preparation of the solution for inoculation in culture medium

• Use a reference strain of *M. tuberculosis* susceptible to all antituberculosis drugs (may be H37Rv) to assess the sensitivity of the lots of Löwenstein Jensen and Middelbrook 7H10 or 7H11 and one of *M. bovis* for Stonebrink. Given the importance of the inoculum in this type of evaluation it is convenient to have the reference strains in frozen aliquots following the protocol presented in Annex D.6. Preparation of aliquots for the inoculum.

• Perform a previous experience sowing different dilutions of both strains, following the protocol presented in Annex D.7. Inoculum preparation for previous experience. It is suggested to record the results in a form like to the one presented in Annex D.8. Inoculum test. Colony count registration. The inoculum size should allow to count colonies accurately and without difficulty and, at the same time, identify lots of sensitivity that escape normality, especially those of very low sensitivity. For this reason, it should be tried to have 20 to 50 colony forming units (CFU) in the volume sowed per tube.

• Analyze the results of the inoculum of the different controls carried out over the years in order to adjust it to determine what the optimal dilution is. For this purpose, a form can be drawn up, such as the one presented in Annex D.9. Quantification of the inoculum, monitoring of results.

Evaluation of the medium produced

• Create a SINGLE record. As an example, we describe how to design the record using an Excel file with all the experience data in different flaps. It is suggested to assign it the name "Control of average quality of culture (month and year)".

• Record the data, coding that was given to the laboratory and type of laboratory that sends the mediums, in an Excel as it is presented in Annex D.10. Data recording form, Part 1. The second part of Annex D.10 shows the information about the mediums received and the control's general results. At this point it is necessary to emphasize that each laboratory is identified with a code of letters or numbers to maintain confidentiality in the study. The rest of the data to complete in this form is at the discretion of each laboratory.

• Generate, in the second flap of Excel, a list of random numbers. Through the function RANDOM and HIERARCHY that allows to create random numbers between a specified range without having repetitions. In a cell write the word "values" and below = random () and press enter appearing the first value at random

Values
0,85067443

Extend that value down by randomly generating the number of numbers you need to have (for example 1000 cells)

Values
0,41741503
0,80127728
0,32358777
0,96986301
0,70439186
0,97580601
0,93658811
0,09306927
0,00871593
0,28147258

Place in the third column the word "numbers" and below = **hierarchy (.**

Select the first cell in the "values" column, leaving = **hierarchy (A2**

Mark the column "values" from cell A2 to the cell you want. Immediately press F4, close parentheses remaining = **hierarchy (A2; A2: A11),**
Press enter and the whole number appears randomly.

Values		Number
0,35625902		7
0,17939698		
0,876304		
0,35951495		
0,07473611		
0,37917889		
0,5208717		
0,05528894		
0,88751287		
0,93860532		

Drag from that number down and random numbers appear.

Values		Number
0,45184616		7
0,99401154		1
0,75667195		4
0,16828492		9
0,30486436		8
0,89548804		2
0,13944464		10
0,89045938		3
0,58526121		5
0,4637875		6

Since the numbers are changed instantly, to use them copy the numbers and paste on another sheet as special paste values. This list is the one that can be used to randomly assign numbers to received tubes.

• Assign the numbers to each of the received tubes.

Assign, to the first tube of the first received consignment, the first number of the list of random numbers generated, and so on until number 12 of that consignment is numbered. If a smaller number had been received, enter next to the vacant numbers "not received". The register that correlates each code with the batch of medium and the producer laboratory remains hidden for the operators who inoculate and read the plantings of the medium.

Identify the 12 numbers corresponding to the first batch of medium, heading the list (the laboratory code that prepared it, name of the medium and batch number), as shown

in Annex D.11. Form for assigning random numbers to the tubes received for quality control.

Condition the first ten of the 12 received tubes in a plastic box labeled with the name of the medium. In the same box condition all tubes of the same type of medium received. Store in the refrigerator (4 -8 ° C) all the boxes until the moment of beginning the experience to determine its sensitivity.

Use the remaining 2 tubes to control:

- aspect of the medium
- pH
- sterile

• Copy, in another flap, the list with all the numbers assigned to the same type of medium (Löwenstein Jensen, or Stonebrink, or Middlebrook 7H10, etc.), one below the other, without identifying the laboratory that prepared them.

• Order from least to greatest in order to know the total number of tubes of each medium to inoculate, as can be seen in the example presented in Annex D.12.

• Record the characteristics of the medium as shown in Annex D.13. Characteristics of the medium received from the participating laboratories, observing the tube of each batch that was in twelfth place and consigning

- Color

  Record the color intensity of the medium (light green: LG, green: G, dark green: DG)

- Homogeneity

  Note if there are lumps or bubbles, inhomogeneous color within the same tube or in different tubes of the batch

- Consistency

  Hit the base of the medium tube against the palm of the hand in a soft way.
  Note if it disintegrates easily and if you have water at the base of the tube.

- Packing

  Specify the diameter and length of the tube, if it has a suitable screw cap.
  Check if the flute peak occupies approximately ¾ of the length of the tube without touching the cap and note any anomaly.

• Record the ph and the result of the sterile test in another plan as shown in the example in Annex D.13 Registrar el ph y el resultado de la prueba de esterilidad en otra planila según se muestra en el ejemplo del Anexo D.13.

- ph

  Take the tube 11 intended for pH taking.
  Calibrate the peachimeter.
  Place the electrode on the surface of the medium, if it is surface.
  Break the medium, place it in a Petri dish and introduce the electrode between the medium if the electrode is common.
  Wait in both cases, that the value appears and register it.
  Wash the electrode with distilled water before proceeding again with the tube of the next batch.
  Discard the tube used.

- Sterile control

  Locate tube 12 of each batch in a box or tray.

Incubate at 37 ºC for 48 hours and for another 48 hours at room temperature.
Note in annex observations if there was any tube that has been contaminated at these temperatures in that period.

• Prepare the solution for planting following the protocol Annex D.14. Preparation of inoculum for control (the dilution to be prepared is the one chosen in the previous experience / s)

• Seed the tubes following the instructions below

- Take the box containing a certain type of medium (LJ or Stonebrink or Middlebrook agar)
- Order the tubes in racks by their code, from lowest to highest, thus guaranteeing a random location in the order in which they are planted.
- With a 2 ml pipette, seed with 0.1 ml inoculum.
- Discard the pipette once the total discharge is complete and take a new one. This generates less risk of contamination.
- Keep the tubes planted at an angle of approximately 15 ° to 37 ° C for one to two weeks.

Check that the seeding was absorbed and put in vertical position at 37 ° C until final reading.

• Count, with three different readers independently and blindly, the colonies developed in all the tubes, at 20 and 60 days of incubation.
Record the counting of each reader in separate spreadsheets as presented in Annex D.15. Form for colony counting.

Enter the values of each reader in the Excel spreadsheet whose design is shown in Annex D.16. Form for the transcription of the characteristics of the medium, colony counting and analysis of results.

Evaluation and interpretation of results

Characteristics of the medium

• Evaluate the different characteristics of the medium.

PH values lower than 6.5 for neutral medium, presence of bubbles that can indicate excess heat in the coagulation and / or lumps can affect the quality of the medium. The presence of the same batch with different intensity of green may be evidence of poor homogenization or the existence of residues in the tubes.

A very dark green may be marking an excess of malachite green or a very acid pH. Conversely, yellow medium may indicate a defect of malachite green or very alkaline pH. The easy disintegration of the medium may be due to the coagulation temperature has not been sufficient; although this does not usually affect the sensitivity of the medium, it may not be suitable for colony counting and subculture because the medium breaks easily and does not allow the correct distribution of material.

• Count the number of contaminated tubes by coloring the fields belonging to contaminated tubes of the different lots in Annex D.11.

Analyze if the contamination is random, in a lot or follows a numerical correlation.
Determine if it is due to a disadvantage of the environment or of planting.

Sensitivity of the medium

- Check the values of the counts and discard outliers (reading of a reader that escapes those recorded by the other two readers for the same tube).

- Average the readings of the 3 readers corresponding to the 10 tubes of each batch. You get the number of CFU / tube that developed on average in each controlled lot, at 20 and 60 days of incubation.

- Calculate the medium and standard deviation (SD) of the CFU / tube obtained for each batch evaluated and each type of medium (LJ, Stonebrink, Middlebrook agar) as presented in Annex D.16.

- Verify, for each batch in particular, that the percentage of the average number of colonies developed at 20 days in relation to the 60 days is at least 70%. This is done comparing the development of the 20 days in relation to the one obtained at the end of the incubation, at 60 days.

- Graph the distribution of CFU / tube averages obtained with all the controlled lots for each type of controlled medium (neutral LJ, neutral Stonebrink or 7H11). To do this, use the Medcal program or use the formula FREQUENCY of the Excel program.

- Evaluate that the distribution approaches the normal one, to continue with the statistical calculations. In case the distribution does not behave as Normal, it is necessary to apply a transformation to approximate the distribution to a normal curve. To do this, you can perform a quartile or percentile analysis or use a Q-Q plot. There are several statistical programs that facilitate the construction of this type of graph.

- Compare these parameters (medium and DS) with those calculated for each batch of medium and classify their sensitivity in the following way

  Good: the average of CFU / tube is within the average range +/- 1 DS

  Very Good: the average of CFU / tube is greater than average + 1 DS

  Not acceptable: the average of CFU / tube is less than average - 1 DS

All these calculations arise from the same form in Annex D.16.

- Verify, in the event, that the values are not acceptable, if this can be related to the detected characteristics of the mediums that could have affected the quality of the same, with the mark and expiration date of the inputs used in the elaboration of the medium or with the procedures applied for the elaboration or conservation of the medium.

Report

• Prepare a preliminary report by laboratory with the partial results of the control of the controlled mediums (see Annex D.17 Preliminary report of medium results). The objective of this report is to advance partial results of the evaluation, since, by the characteristics of the test and depending on the number of lots of different types of medium that have been included in the study, the final report, whose structure and complexity we will see next, usually occurs with some delay.

• Prepare a second report called "final" as shown in Annex D.18. Final report of medium results.

Present the results of all participating laboratories. The report must be prepared in such a way that each laboratory receives the comparative table of results of sensitivity of the experience that includes all the participating services with their corresponding codes.

Decode only the one corresponding to the laboratory to which the report is sent.

Include the characteristics, pH of the medium, the average development of CFU of the 60-day reading and the result of the sensitivity of the medium.

Check if there were problems in the development of the colonies between 20 and 60 days.
Incorporate, in consideration of each NRL, a graph that shows the frequency distribution of laboratories that recorded different averages of colony counts.

In Annex D.19. Data to be reported, along with the final report, a report model is shown, which includes the described fields, which can be easily obtained from the Excel spreadsheet shown in Annex D.16.

Conduct to follow against results of not acceptable quality

Invite to send a new batch of medium to those laboratories that had not acceptable quality in order to perform the control again and verify if the problem remains or was transient / incidental.

Monitoring the quality of each laboratory

• Monitor the trend of the results of each laboratory over time. When it is found that a laboratory produces medium that result in low sensitivity in two successive controls, it is requested that

- Refer to the internal controls of the last three lots prepared in order to analyze if there is information that allows to detect low sensitivity of the medium

- Do not use the type of medium that was repeatedly unacceptable, nor distribute them until it is proven that the laboratory has overcome the problems that caused the loss of sensitivity.

- Use culture medium from the NRL or another district so that you do not discontinue your diagnosis activities.

- Identify the cause If it does not arise from the information analyzed during the control, a technical assistance visit is necessary during which all the procedures related to the preparation of the medium are visualized.

- Retrain in the medium preparation method if necessary.

In Annex D.20. Monitoring of the quality of the culture medium, a model of electronic record of monitoring the quality of the culture medium is presented in which the average counts of the colonies obtained with each culture medium prepared by the laboratory are plotted in columns. Question in each of the checks carried out. Bar graphs are added, with a medium point that indicates the average CFU / tube corresponding to the total of batches evaluated in each experience, and two extremes that correspond to the interval of ± 1 standard deviation. In addition, it can also be useful to create a file in which the results of the sensitivity of each evaluated culture medium categorized as "Very good / Good / Not acceptable" in relation to culture yield are registered, using a model similar to the one presented. In Annex D.3.

In each experience, it is convenient to add to the final report of the evaluation of the quality of the culture medium, the monitoring of the quality of the medium produced by the laboratory during the whole period in which it has participated in the program. (Annex D.21. Report on monitoring the quality of the medium).

EXTERNAL EVALUATION OF THE INDIRECT QUALITY OF THE IDENTIFICATION OF *MYCOBACTERIUM TUBERCULOSIS* AND DRUGS SUSCEPTIBILITY TESTING

Proeficiency tests

Definitions

True resistant: resistant isolates that is classified as resistant by the laboratory that is being evaluated.

True sensitive: sensitive isolates that are classified as sensitive by the laboratory that is being evaluated.

False resistant: sensitive isolates that is classified as resistant by the laboratory that is being evaluated.

False sensitive: resistant isolates that are classified as sensitive by the laboratory that is being evaluated.

Sensitivity: assesses the success in the detection of drug resistance.

$$\text{Sensitivity:} \quad \frac{\text{Nº of real resistant} * 100}{\text{Nº of true resistant + No. of false sensitive}}$$

Specificity: evaluates the success in the determination of sensitivity to the drug.

$$\text{Specificity:} \quad \frac{\text{Nº of true susceptible} * 100}{\text{Nº of true susceptible + Nº of false resistant}}$$

Accuracy / Efficiency / Concordance: assesses the accuracy of the total results.

$$\text{Concordance:} \quad \frac{\text{Nº of correct results}}{\text{Nº of isolates evaluated}}$$

Reproducibility: evaluates the consistency of the results produced by the laboratory.

$$\text{Reproducibility:} \quad \frac{\text{Nº of strains with matching results of their duplicates}}{\text{Nº total of strains evaluated in duplicate}}$$

PROEFICIENCY TEST OF SUSCEPTIBILITY PHENOTYPIC

General considerations

IIn 1994, the WHO created the Supranational Laboratories Network, a network that normalizes and coordinates the indirect external supervision of the susceptibility testing throughout the world. The number of laboratories in this network grows to the extent that the need to establish a new laboratory in some region of the world is detected.

The quality of the SRL susceptibility testing is controlled once a year by the coordinating laboratory of the SRL Network (currently the Institute of Tropical Medicine of Belgium). The SRL repeats this control for the NRL of the countries to which they provide technical assistance. In turn, each NRL should do the same with the laboratories of the National Laboratory Network of their country that perform susceptibility tests.

The evaluation consists in carrying out the identification and susceptibility test to a panel of *M. tuberculosis* isolates that have selected resistance phenotypes in order to be able to evaluate, in each laboratory, the accuracy of the results of the susceptibility test against drugs. first line and second line in use: kanamycin, amikacin, capreomycin and quinolones (1). Isolation of an environmental mycobacterium is also usually included in order to evaluate the identification of the microorganism whose susceptibility test is being reported. Within the isolates, some strains are included that are sent in duplicate. The panel isolations receive a randomly assigned code, known only to the experience coordinator laboratory.

Once each SRL has completed the control and counts on the results, it prepares the same panel or panels with the same composition

(1) WHO has recently published a rapid communication on the key changes in the treatment of multidrug-resistant and RIF-resistant TB. These changes are based on the results of a meta-analysis aimed at estimating the association between the success of treatment with the use of individual drugs in patients with multidrug-resistant TB. In relation to the use of second-line injectable drugs, the results of this meta-analysis showed that kanamycin and capreomycin were associated with worse results than treatments without second-line injectables. Therefore, WHO no longer recommends the use of kanamycin and capreomycin in long regimens of multidrug-resistant TB because of the increased risk of failure and relapse. In addition, for NTP that use the shorter standardized regimen of multidrug-resistant TB, it is advisable to replace kanamycin with amikacin. Recognizing the fact that it will not be possible to immediately achieve the new WHO standards of care in each individual patient with multidrug-resistant TB, and that kanamycin and capreomycin are likely to be used during the transition phase, this manual includes the evaluation of DST for kanamycin and capreomycin with the purpose of providing a provisional guide, both for the EQA of phenotypic susceptibility tests and for the tests with strips probes for determination of susceptibility to second-line drugs.

as that received from the Belgian SRL to be distributed to the NRLs. To evaluate the intermediate laboratories of the networks of each of the countries, use a panel composed of 20 of the 30 WHO panel isolates selected in consensus by the three SRL of the region; the selection of these strains is aimed at evaluating especially the capacity of intermediate laboratories to detect H and R resistance with precision, avoiding the increase of biological risk as much as posible.

Procedure

Since the participating laboratories must handle multiresistant and pre-extended resistance strains of *M. tuberculosis*, it must be previously known if the laboratory meets the conditions to do so. In addition, to establish the number of panels to be sent to each laboratory, it is necessary to know the techniques used in each service to determine the sensitivity. For these reasons, request the participating laboratories to send (by mail or fax) a letter signed by the head of the laboratory that contains the answers to the queries presented in Annex E.1. Preliminary letter.

In those cases, in which the written declaration of the head of the laboratory does not specify that the BSC has the annual certification or it is verified that it has expired or not done in the last 12 months, **DO NOT send the isolation panel for the control**. In this case it may be useful to send a note to the authorities of the institution on which the laboratory depends, explaining the reason for the non-inclusion of the service in the control and the need to ensure BSC certification, not only for this particular but, especially, to protect the staff when they perform their work routine.

Once known the number of laboratories able to participate in the evaluation and the number of techniques that each laboratory performs to report a result and taking into account that a panel is prepared for each method used in each laboratory to report the susceptibility results to antituberculosis drugs, the total number of panels that must be prepared for the evaluation is counted. To this resulting number is added 3 panels, which are prepared as a reserve for the test, in case of any inconvenience with the distribution of the same to the participating laboratories or to carry out subsequent verifications in the coordinating laboratory.

Preparation of the panel

Each panel is made up of the same isolates as the panel received from the SRL. It consists of 30 strains of *M. tuberculosis* for the NRL and 20 strains of *M. tuberculosis* selected by consensus among the SRL to be sent to the laboratories of the network that perform only susceptibility test to R and H.

• Prepare Dubos or Middlebroook 7H9 medium to divide into 20/30 bottles with 40 ml each (depending on the number of panels to assemble).
• Label each bottle with the number of each isolate to aliquote.
• Incubate the bottles containing the culture medium for 48 hours in the stove for sterile control.
• Check that the medium is not contaminated before using.
• Take each isolate that is incorporated into the control and proceed as follows:

- Prepare a tube with glass beads for each isolate. The inoculum must be made from a culture in solid medium of approximately 15 days, with very good development. (Never use tubes that have a small number of colonies).

- With a bacteriological handle scrape the entire surface with bacterial development, avoiding taking culture medium.
- Download all the bacillary mass inside the tube with glass beads, making revolving movements of the handle on the pearls.
- Add 1 or 2 drops of sterile water, cover and vortex for 1 minute.
- Let stand 5 minutes
- Add approximately 1 ml of sterile water and stir again with vortex for 1 minute.
- Leave the suspension at rest for 15 minutes.
- Take the supernatant from the beads with a Pasteur pipette and discharge it into the bottles with Dubos or 7H9 labeled with each strain number.
- Aliquot 1ml of each strain, in the amount of cryotubes needed to supply all laboratories; these cryotubes must have external thread, o-ring and capacity of 2 ml. It is recommended to work in series of 6 to 8 strains per day to avoid mistakes. Separate in boxes the cryotubes belonging to the same strain to avoid confusion.
- Prepare Middlebrook 7H11 and Mueller Hinton medium plates and incubate on stove to sterile control for 48 hours.
- Take one tube of each distributed strain at random and sow 20 µl in two plates of medium Middlebrook 7H11 and in two plates of Mueller Hinton. You can plant 6 to 8 strains per plate in a timely manner and separate one seed from the other. A set of plates (one of Middlebrook 7H11 and Mueller Hinton) is incubated at room temperature and the other set at 37 ° C.
- Leave the cryotubes at 37 ° C until labeled.
- Control the plates at 48 hours to show evidence of growth of a contaminant. In case growth is observed, make an extension to corroborate contamination. If the tube is contaminated take another tube at random and re-perform the proceeding of sowing on the plates. In case of contamination is observed again, discard the cryotubes of that strain and start again from the fifth point.
- If no contamination is detected, leave another 48 hours in incubation to confirm that no late contamination appears. In case you appreciate proceed to discard the cryotubes of that strain and start again as in the previous point.
- Generate a list of random numbers that include a number between 1 to 2000 as specified in control of culture medium
- Label, once the presence of contamination has been ruled out, all the cryotubes containing 1 ml of the isolation following the list of random numbers.
- Place the tubes corresponding to each isolate in a box labeled with their number, until the packing of each panel.
- Record in a spreadsheet excel the numbers corresponding to each strain as presented in Annex E.2. Form with the coding of isolate per panel.
- Seal with parafilm each cryotube and begin to assemble the panels.
- Record in the same excel sheet the panel number and the tube number of each strain that integrates the panel.
- Place each tube in a separate plastic bag as the first containment container.
- Once conditioned the tubes of each strain per panel are frozen at -20oC or -70oC until distribution.

• Prepare the forms for the collection of the necessary information that is sent together with each panel as it is presented in Annex E.3. Information requested for Susceptibility Testing Control.

• Transport the panels with the biosafety measures required for this type of high biological risk material as recommended in the PAHO culture manual.

Conducting the susceptibility test in the supervised laboratory

• Make a smear contamination control of each vial received.

• Repeat each vial of the panel after confirming the purity.

• Perform blind susceptibility test. It must be done by the personnel who normally perform it, using the standard operating procedure that is applied in the laboratory work routine.

• Classify the result as sensitive or resistant and issue the result. It should be done by the laboratory technicians who do it in the work routine.

• Record all the results obtained, even the repetitions if any, in a form like to the one presented in Annex E.4, but adapted with the methodologies of each laboratory.

• Dump the results in Annex E.3, complete the same and send it to the organizing laboratory.

Analysis of results

Once the results have arrived at the laboratory coordinator of the test, it must:

• Repeat the susceptibility test only with those isolates whose results do not coincide with those expected after having received the spreadsheets from at least two laboratories evaluated.

• Incorporate the form presented as Annex E.5. General information of control participants, methodologies used, biosafety and dates of reception of isolations and referral of results.

• Generate an Excel spreadsheet with the fields presented in Annex E.6. Results of strains per drug and dump the results as follows:

- Complete the fields of the original numbers of the strains and the origin of the round to which the results belong.

- Copy that sheet as many times as drugs to evaluate and give each flap the name of the drug.

- Enter the "WHO consensus result" (the one agreed by the majority of the SNLs) in box 1 and the percentage of that consensus for each drug in the different flaps for the different drugs and mark the corresponding cells with a color to isolates whose percentages are less than 80%.

- Take the results form of one of the laboratories evaluated.
- Select the excel lapel corresponding to the first drug, for example: H.

- Incorporate the name of the laboratory evaluated and the methodology applied between parentheses, there are some laboratories that have the evaluation of several techniques.

- Incorporate the numbers of the isolate of the panel that are granted by the organizing laboratory.

- Compare the laboratory result, for each strain and drug, with the WHO consensus result.

- Enter, in column G, the number 1 if the results are coincident or add false sensitive (FS) or false resistant (FR) if it is discordant, according to the consensus result.

- Add the number 1 in the columns H "resistant hit (R)" or I "sensitive (S)", only in the case of concordant results.

- Add the number 1 in columns J and K "SC" (without considering by agreement less than 80%) right / wrong according to whether the result was concordant or discordant.

- Add in the column N "par OK", if both results coincide, the number 1 to one of the cells of the duplicate strains.

- Check if the calculations performed automatically are correct *. To do this, bear in mind the definitions expressed at the beginning of the section.

* A test can be performed by placing the results as if they were all correct and should give 100% sensitivity, specificity, efficiency / concordance and reproducibility. Then try a change of false positives or negatives and they should be expressed in changes in the different parameters.

• Evaluate the delay in the report of results, calculating the difference in days between the arrival of the panel to the laboratory to be evaluated and the arrival of the results to the coordinating laboratory.

• Evaluate the quality of the laboratory to differentiate *M. tuberculosis* from other mycobacteria, corroborating that the environmental mycobacterium is detected and that it has not carried out the susceptibility test in that strain.

• Classify the efficiency / concordance for each drug taking into account the experiences of the SNL in the region, as presented in the following table:

Quality	Concordance / Efficiency %	
	H/ R Km/Ak/FQN	E /Cm
Not acceptable	< 95,0	< 90,0
Acceptable	95,0-97,0	90,0-95,0
Good	97,1-99,9	95,1-99,9
Excellent	100,0	100,0

• Incorporate the precision results by drug and laboratory in Annex E.7.

• Then proceed to analyze the results and possible causes of each laboratory with unacceptable results

- Consider that an error can be made in the evaluation laboratory if, for a given isolate, the discordance between the results received from the controlled laboratories is greater than 80%.

- Verify the result of the repetition of the test performed upon receiving the first disagreements.

- Verify that there are no errors in the labeling of the isolates, in the conservation of the panels, or in the transcription of results.

- Discard from the analysis that isolate if the disagreements are not recorded only with the results of laboratories that have just begun to work or that have a history of unacceptable results.

- Analyze the predominance of false sensitive between discordances. If you find that most errors are false sensitive for one or more drugs, think that there may be an excess of drugs in the medium.

- Analyze the predominance of false resistant. If you find that most errors are false resistant to one or more drugs, think that there may be a drug defect in culture medium.

False sensitive (FS) and false resistant (FR) errors can be caused by imprecision in the procedures implemented for the preparation of drug culture medium, in dilutions, sowing, or reading and interpretation of results. They may also be due to transcription errors. These errors can be caused by the change of personnel.

All are serious errors because they may be occurring in the work routine. To explore these possibilities, the controlled laboratory can be asked for information about recent personnel changes, the SOPS that apply and the original records of the results of the susceptibility tests.

- Analyze the efficiency / concordance results for each drug in each laboratory over the years to interpret whether the identified errors are fortuitous or frequent.

- Analyze the results of the work routine to verify or elucidate if the error detected in the control is also manifested in the work routine or has only occurred in the quality control.

• Communicate the errors detected and the corrective measures to be implemented.

• Request the service to suspend the performance of the sensitivity test, if the event that it is confirmed that the error is repeated after the completion of several panels, and that the isolations of patients with risk of resistance are derived to be carried out in the reference laboratory until the quality of work is restored.

• Visiting the laboratory to verify in situ all the procedures related to the susceptibility test, with special attention to the weighing of the drugs, the dilutions carried out, the addition of the drug solutions to the culture medium, the measurement of the inoculum and the reading and interpretation of results.

Report:

• Check the results transcribed to the report of results between two people before sending the reports to the laboratories evaluated, in order to prevent errors in the preparation of the same.

• Issue a report with the results following the model presented in Annex E.8. Results report, trying to comment on the positive data, and highlighting the advances that are evidenced to stimulate the laboratory staff.

- Send the reports to the different laboratories as the results are received. Only in the case that discrepant results are found for the same strain for the two or three first results received, the reports should be retained to evaluate if there is an error in the conformation of the panel submitted. Once analyzed, the results are issued with the corresponding exception in the event that an error has been made by the coordinating laboratory.

- In addition, a report with the follow-up over the years of the efficiency / concordance percentages of each laboratory by method, as presented in Annex E.9. Monitoring the quality of the susceptibility test, a report model.

- Request the participating laboratory of the evaluation, analyze the results presented in the report and report any error that is detected or doubt that is generated.

PROFICIENCY TESTING FOR COMMERCIAL MOLECULAR DRUG SUSCEPTIBILITY TESTING

Automated closed system of extraction and amplification of DNA in real time (Xpert) for the detection of resistance to rifampicin of *M. tuberculosis* and open system of amplification and reverse hybridization (LPA) for the detection of resistance to rifampicin and isoniazid (FL-LPA) or second-line anti-tuberculosis drugs (SL-LPA)

General considerations

WHO recommend the use of two automated systems, one closed, of extraction and amplification of DNA in real time (Xpert MTB / RIF) and another open, of amplification and reverse hybridization (LPAs (FL-LPA and SL-LPA), an initial diagnostic test for the detection of *M. tuberculosis* bacteria and resistance to RIF (and INH for the case of LPAs), prioritizing, in the case of scenarios with limited resources, the symptomatic at risk of drug resistance to first or second line antituberculosis and those coinfected with HIV. In addition, the use of LPAs for the detection of resistance to second-line antituberculosis drugs among patients diagnosed with RR/TB or multidrug-resistant TB is recommended. This test (SL-LPA) allows detecting resistance to fluoroquinolones (such as levofloxacin or moxifloxacin) and second line injectable drugs (kanamycin, capreomycin, amikacin).

Before the implementation of these, it is necessary at the country level:

- Know the load of multidrug-resistance and extensively drug resistance tuberculosis in the area to apply the methodology,

- Develop work algorithms that guide its use complementing conventional methods, according to the epidemiological situation, needs and existing resources,

- Perform method verification,

- Implement a permanent system that allows knowing the quality of the technical procedures, result, and report of results of the diagnostic test.

An automated closed system of extraction and amplification of DNA in real time (Xpert) for the detection of M. tuberculosis and its resistance to rifampicin

1- Support for method verification

This evaluation is offered to the NRL that have implemented the Xpert system as a complement to the verification carried out with samples of the work routine in each of the countries, particularly for those scenarios where the incidence of multidrug resistance is low.

To support the verification of the method at the national level, the NRL will receive from the SRL a panel with approximately 19 isolates of *M. tuberculosis* susceptible and resistant to RIF (carrying the most frequent mutations that encode those resistances) and at least one isolation of an environmental *Mycobacterium*. Some of the isolates, to be incorporated, will be sent in duplicate. The panel will consist of inactivated bacillary suspensions containing around 5000 bacilli/ml (*). Together with the panel, the Annex F.2 "Information requested for susceptibility test control" will be sent, which contains instructions for the realization of the test, the list with the numbers of suspensions that correspond to each panel and a questionnaire about the characteristics of the local system validation experience, difficulties encountered in the implementation of the system and results obtained under routine conditions.

Once the panel is received, the NRL must:

- Perform the test following the standard operating procedure applied in the participating laboratory.

- Assign the execution of the aptitude test to the operator (s) responsible for carrying out the technique in the work routine.

- Assign the analysis and report of the results to the laboratory personnel who usually issue the reports.

- Fill the results in Annex F.2, classified as follows:

(*) Note: At present, there is no international recommendation about the methodology for the EQA of the Xpert MTB / RIF system. The composition of the panels proposed for this EQA consists of bacillary suspensions inactivated by heat, whose utility has been evaluated by the SRL of the region (see Annex F1). (Preparation of suspensions of inactivated mycobacteria). Internationally, different panels are under study, some based on bacillary suspensions inactivated by heat or chemically, others consisting of DNA samples. Some presentations are in liquid form and others have been lyophilized. International experiences will make it possible to prospectively evaluate the usefulness of these systems, allowing selecting those that demonstrate greater operational advantages (similarity to a clinical sample, ease of production, stability and transport safety). Thus, the SRL of the region, in accordance with the international recommendations for the development of the EQA of this methodology, may modify the characteristics of the confirmation of the panels, without affecting the general conditions of the execution and interpretation of the test.

Result	Interpretation	Report
MTB not detected	Negative. Repeat the test with a new sample	**N**
MTB detected, resistance to Rifampicin not detected	Positive for tuberculosis, without resistance to Rifampicin	**T**
MTB detected, resistance to Rifampicin detected	Positive for tuberculosis, with resistance to Rifampicin	**RR**
MTB detected, indeterminate resistance to Rifampicin	Indeterminate. Repeat the test with a new sample	**TI**
MTB detected (traces), indeterminate resistance to Rifampicin	Indeterminate. Repeat the test with a new sample	**TT***
Invalid / No result / Error	Invalid. Repeat the test with a new sample	**I**
***Only for use in the case of ULTRA cartridges**		

- Inform, before the invalidated result, the type of failure occurred http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=37760&Itemid=270&lang=es)

  - 5006/5007/5008 (Probe control failed, and the test was canceled before the amplification),
  - 5011 (Low signal in the amplification curve),
  - 2008 (Excessive pressure on the cartridge),
  - 2127 (Lack of communication of the module),
  - 2037 (Lack of integrity in the cartridge),
  - 2014/3074/3075/1011 (Disadvantages of temperature).

- Inform, classifying the reaction by the number of cycles required to reach the positive result, the amount of DNA in the sample (*)

  - High (less than 16 cycles),
  - Medium (from 16 to 22 cycles),
  - Low (23-28 cycles),
  - Very low (more than 28 cycles),
  - Traces (less than 37 cycles for the ULTRA cartridge)

(*) For result interpretation, consult module 7 at the following electronic address http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=37758&Itemid=270&lang=es

• Complete the rest of the questions in Annex F.2 and send it to the SRL, who will be responsible for making and sending a report (see the model in Annex F.3, Report of results) containing the following topics:

  • Results of sensitivity, specificity, accuracy, and reproducibility obtained in the aptitude test. For each isolation, the results reported by the SNL of the Institute of Tropical Medicine of Belgium in relation to i) the identification of M. tuberculosis; ii) presence and type of mutation in the rpoB gene region explored or absence of mutation. Based on the reported results, the possibility of cross-contamination will also be evaluated.

• Results of the local method validation experience in the country:

  - the accuracy of the method

  - analysis of the main errors / invalid results reported, possible causes and recommendations to avoid the occurrence of errors and invalid results identified.

  - estimation of the performance indicators: % of errors, % of invalid results and% of samples without results; considering the following proportions as reference values:

Indicator	Description	Acceptable value
Number and proportion of errors	Number of errors / Total samples tested	<3%
Number and proportion of invalid results	Number of invalid results / Total samples tested	<1%
Number and proportion of samples without results	Number of samples without result / Total samples tested	<1%

2- External quality assessment of the NRL and the laboratories of the Xpert user network

For this proficiency test, the NRL will receive from the SRL a panel composed of inactivated bacillary suspensions for the evaluation of the test in their own laboratory and in the laboratories of the network of each country that have implemented the system. In the case of the evaluation of the local laboratories of the network, the NRL will receive, together with the panels of inactivated bacillary suspensions, a list containing information about the presence / absence of mutations in the bacillary suspensions referred, as reported by the SRL Belgium (Annex F.4; Example of list of inactivated bacillary suspensions forwarded to the NRL of

country XX for the preparation of panels for proficiency tests); this information should be used to analyze the results obtained by local laboratories and calculate the diagnostic accuracy in each of the participating services.

In relation to the number of bacillary suspensions to be included in a panel, it must be considered that this should be large enough so that the exercise was statistically accurate for the evaluation of quality. However, the number of samples needed to complete this requirement would add a heavy workload to the routine of the service and would take a lot of economic resources difficult for the laboratory that is being evaluated. Most of the systems developed for the EQA by means of aptitude tests of molecular methods of resistance detection in the world (see Scott et al., J. Clin Microbiol., 2014. 52: 2493-2499, Nikolayevskyy et al, PLOS ONE 2016; DOI: 10.1371 / journal. pone.0152926) contain less than 5 samples / panel. In this manual, we propose the evaluation of panels with 5 samples only to detect serious errors. If resources allow, it is advisable, in order to increase the validity of the evaluation, to perform this exercise at least twice a year (using different samples in each of the panels), which increases confidence in the results.

Preparation of the panel

To prepare the bacillary suspension panels for EQA to the services of the laboratory network of each country, the NRL should proceed as follows:

- Calculate the number of laboratories in the network that will be evaluated by the NRL.

- Prepare the panels starting from the suspensions sent by the SRL whose bacillary concentration will be approximately 50 000 bacilli/ml. To do this, follow the instructions given in Annex F.5. Example for the preparation of panels for EQA. This example has been prepared for an NRL whose number of laboratories to evaluate is equal to or less than 15. The procedure should be adjusted, depending on the number of laboratories to be evaluated.

- Attach externally to each panel Annex F.6. Form for external quality control.

Methodology to be applied in the peripheral laboratories to be controlled

Once the local laboratories have received the panel and the form of Annex F.6, they should follow the following instructions:

• Perform the test following the standard operating procedure applied in the participating laboratory.

• Assign the execution of the proficiency test to the operator(s) responsible for carrying out the technique in the work routine.

• Assign the analysis of the results to the laboratory that performs the analysis of the results and usually issues the reports.

- Fill the results in a standardized form using Annex F.6, classified as follows:

Result	Interpretation	Report
MTB not detected	Negative. Repeat the test with a new sample	**N**
MTB detected, resistance to Rifampicin not detected	Positive for tuberculosis, without resistance to Rifampicin	**T**
MTB detected, resistance to Rifampicin detected	Positive for tuberculosis, with resistance to Rifampicin	**RR**
MTB detected, indeterminate resistance to Rifampicin	Indeterminate. Repeat the test with a new sample	**TI**
MTB detected (traces), indeterminate resistance to Rifampicin	Indeterminate. Repeat the test with a new sample	**TT***
Invalid / No result / Error	Invalid. Repeat the test with a new sample	**I**
***Only for use in the case of ULTRA cartridges**		

- Inform, before the invalidated result, the type of failure occurred http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=37760&Itemid=270&lang=es)

  - 5006/5007/5008 (Probe control failed and the test was canceled before the amplification),
  - 5011 (Low signal in the amplification curve),
  - 2008 (Excessive pressure on the cartridge),
  - 2127 (Lack of communication of the module),
  - 2037 (Lack of integrity in the cartridge),
  - 2014/3074/3075/1011 (Disadvantages of temperature).

- Inform, classifying the reaction by the number of cycles required to reach the positive result, the amount of DNA in the sample (*)

  - High (less than 16 cycles),
  - Medium (from 16 to 22 cycles),
  - Low (23-28 cycles),
  - Very low (more than 28 cycles),
  - Traces (less than 37 cycles for the ULTRA cartridge)

b) (*) For result interpretation, consult module 7 at the following electronic address http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=37758&Itemid=270&lang=es

- Complete the rest of the questions in Annex F.6 and send it to the organizing laboratory.

Interpretation and analysis of results in the coordinator (NRL)

- Analyze the quality of the laboratories, through a scoring system and a score above which the service is considered to have an acceptable performance. It is important to consider that each program must determine what is an acceptable performance, considering that the definition of an acceptable performance can be modified based on the experience with proficiency tests in each country and the maturity that the country develops in the implementation of the test.

- Consider as valid the results reported by the SRL Belgium in relation to i) the identification of *M. tuberculosis*; ii) presence and type of mutation in the region of the rpoB gene scanned or absence of mutation, which have been sent together with the panel of suspensions by the SRL.

- Interpret and analyze the results taking into account the following scoring system:
  - Assign a value of 20 points for each suspension correctly reported, which would give a general score of 100 points if the total results were correct. Those services that obtain a score of 80 or more without having a false resistant or false susceptible will be considered acceptable performance.
  - Determine the score considering the following criteria:

Table 9. Proposed evaluation system for determining the score obtained for each sample of bacilli sent in the proficiency test

Expected result: MTB detected Resistance to rifampicin not detected	
Reported result	**Score**
MTB detected Resistance to rifampicin not detected	20
MTB detected Resistance to rifampicin detected	0
MTB detected Resistance to indeterminate rifampicin	10
MTB no detected	0
Invalid / no result / error	0
Expected result: MTB detected Resistance to rifampicin detected	
Reported result	**Score**
MTB detected Resistance to rifampicin not detected	0
MTB detected Resistance to rifampicin detected	20
MTB detected Resistance to indeterminate rifampicin	10
MTB not detected	0
Invalid / no result / error	0

Expected result: MTB not detected	
Reported result	**Score**
MTB detected Resistance to rifampicin not detected	0
MTB detected Resistance to rifampicin detected	0
MTB detected Resistance to indeterminate rifampicin	0
MTB not detected	20
Invalid / no result / error	0

• Repeat the test by the coordinating laboratory (NRL) for that / suspension / in which more than 80% of the participants would have obtained an unsuccessful result.

• Generate an Excel spreadsheet with the fields presented in Annex F.7. and begin to dump the results as follows:

• Complete the fields of the original numbers of the strains and number of the round to which the results belong.

- Enter the presence or absence of a mutation in the strain according to the sequencing result reported by the SRL in box 1.

- Take the results form of one of the laboratories evaluated

- Enter the name of the evaluated laboratory.

- Incorporate the number of the strain that was granted by the organizing laboratory.

- Enter, in column G "2", the score obtained for each suspension sent according to the table "Proposed evaluation system" that gives scores from 0 to 20 according to the occurrence or non-coincidence of the results obtained with respect to the consensus results.

- Check if the calculations performed automatically are correct.
Evaluate the performance of the laboratory in this test.

• To evaluate the quality of the laboratory to differentiate *M. tuberculosis* from other mycobacteria corroborating that the environmental mycobacterium was not detected as *M. tuberculosis*.

• Analyze the invalid results generated by the following causes:

- the sample processing control (SPC) did not exceed the acceptance criteria,
- the sample was not well processed,
- the PCR was inhibited.

• Analyze the inconsistent or imprecise results of the routine arising from problems with the equipment and cartridges with errors as expressed in question 2 of Annex F.7 to try to guide possible technical or administrative failures.

• Based on the errors or mistakes with regard to the results of the work routine,

the laboratory's performance, possible cross-contamination and the performance of the equipment in each laboratory are evaluated. The main points to evaluate are the following:

Indicator	Description	Acceptable value
Number and proportion of errors	Number of errors / Total samples tested	<3%
Number and proportion of invalid results	Number of invalid results / Total samples tested	<1%
Number and proportion of samples without results	Number of samples without result / Total samples tested	<1%

- Consider items 5, 6, 7 and 8 presented in Annex F.6 and analyze their answers

Preparation of the results report

- Check the results transcribed to the report of results between two professionals or technicians before sending the report to the laboratories evaluated in order to prevent errors in the preparation of the same.

- Issuing a report with the results, trying to comment on the positive data, and highlighting the advances that are evidenced to stimulate the laboratory staff.

- Send the reports to the different laboratories as the results are received. Only in the case that discrepant results are found for the same strain for the first two or three results received, the reports should be retained to evaluate if there is an error in the confirmation of the remitted panel and to repeat the test using the suspension of the isolations that has been stored at -20 °C as protection of the panel.

- If after the repetition of the test, it is determined that the NRL made an error in the confirmation of the panel after having issued the first report with the results of the proficiency tests for some laboratories of the network, it will proceed to inform this finding in a second report.

- An issue, in addition, an observation of the questions asked in the questionnaire.

In case the quality is not acceptable, analyze with the laboratory the possible causes of the errors and make a technical visit to identify the reasons for the anomalous results and apply the corresponding corrective measures.

An open system of amplification and reverse hybridization (LPA) for the detection of resistance to rifampicin and isoniazid or to second-line antituberculosis drugs

1- Support for method verification

This evaluation will be offered to the NRLs that have implemented the LPA system (FL-LPA or SL-LPA) as a complement to the verification carried out with samples of the work routine, in particular for those countries where the incidence of multiresistant or extensively drug resistance tuberculosis is low.

Although the verification of this methodology can be done using the strains of the panel sent for the EQA of phenotypic tests, it is more appropriate, especially when using this methodology from sputum samples, follow the indications that are detailed below given that DNA concentrations of the panel prepared following this methodology resemble those found in sputum samples.

To support the verification of the method at the national level, the NRL will receive from the SRL a panel with approximately 19 inactivated M. tuberculosis isolates sensitive and resistant to the different drugs investigated by the system (carrying the most frequent mutations that encode these resistances) and less an isolation of an environmental Mycobacterium. Some of the isolates to be incorporated will be sent in duplicate. Said panel will consist of inactivated bacillary suspensions containing around 5000 bacilli/ml (*). Together with the panel, Annex G.1 "Information requested for susceptibility test control" will be sent, which contains instructions for the realization of the test, the list with the numbers of suspensions that correspond to each panel and a questionnaire about the characteristics of the local system validation experience, difficulties encountered in the implementation of the system and results obtained under routine conditions.

Once the panel is received, the NRL must:

- Perform the test following the standard operating procedure applied in the participating laboratory.

- Assign the performance of the test to the operator (s) responsible for executing the technique in the work routine.

(*) Note: At present, there is no international recommendation about the methodology for the EQA of the FL-LPA and SL-LPA system. The composition of the panels proposed for this EQA consists of bacillary suspensions inactivated by heat, whose utility has been evaluated by the SRL of the region (see Annex F.1). Internationally, different panels are under study, some based on bacillary suspensions inactivated by heat or chemically, others consisting of DNA samples. Some presentations are in liquid form and others have been lyophilized. International experiences will make it possible to prospectively evaluate the usefulness of these systems, allowing selecting those that demonstrate greater operational advantages (similarity to a clinical sample, ease of production, stability and transport safety). Thus, the SRL of the region, in accordance with the international recommendations for the development of the EQA of this methodology, may modify the characteristics/ procedures for the confirmation of the panels, without affecting the general conditions of the execution and interpretation of the proof.

• Assign the analysis and report of the results to the laboratories that emit the reports habitually, who will have to read and interpret the results aligning the strips with the instrument provided by the manufacturer, considering the following criteria:

- Validate the results of the test by observing the presence of the CC (conjugate control) and AC (amplification control) bands for each sample (when DNA is detected in the tested sample (positive result), the AC signal can be weak and even disappear, due to the competition with the DNA of the sample during the amplification, without invalidating the test).

- Consider that the absence of the AC band in the case of a negative test indicates errors during the amplification or the presence of inhibitors in the sample, which indicates that the test is not valid and the test must be repeated with the corresponding sample.

- Corroborate the presence of the TUB, locus *rpoB*, *kat*G and *inhA* bands for the FL-LPA system or of the *gyrA*, *gyrB*, *rrs*, and *eis loci*, for the SL-LPA system (indicates that the system has detected *M tuberculosis* complex and a region of each gene).

- Corroborate the presence of a mutation in the gene (*) (evidenced by the absence of wild type band with or without the presence of a mutant band for each group of genes).

- Corroborate that in the negative control only the presence of CC and AC band is observed, but that no other band is visible.
- Consider as an invalid result when the CC and AC bands do not appear in a negative sample or when in the negative control, other bands appear than those corresponding to AC and CC.
- Consider as an undetermined result when for a specific drug or a group of drugs, the corresponding locus control is absent while the test is valid (ie the conjugate control and the TUB band are visible with or without the amplification control).

• Analyze the results of each sample considering the presence/absence of the following bands:

FL-LPA		
Band	Present	Absent
CC		
AC		
TUB		
wild rpoB		
mutated rpoB		
Wild katG		
mutated katG		
wild inhA		
Mutated inhA		

(*) For the results to be valid, the bands must be of intensity equal to or greater than the intensity of the AC band. As an exception, for the FL-LPA system, it has been recommended that the rpoB WT8 band should be considered positive even when it is weaker than the AC if, simultaneously, the band corresponding to the mutation rpoB MUT3 does not appear.

SL-LPA		
Band	Present	Absent
CC		
AC		
TUB		
Wild rrs		
Mutated rrs		
Wild eis		
Mutated eis		
Wild gyrA		
Mutated gyrA		
Wild gyrB		
Mutated gyrB		

• Dump the final results in a standardized form using the form presented in Annex G.1.

• Consult, if necessary, for the interpretation of results, the following electronic address

http://www.stoptb.org/wg/gli/TrainingPackage_LPA o
http://www.who.int/tb/publications/lpa-mdr-diagnostics/en/

• Complete the rest of the questions in Appendix G.1. and send it to the SRL, who will be responsible for making and sending a report (see a model in Annex G.2.Report of results) containing the following topics:

• results of sensitivity, specificity, accuracy, and reproducibility obtained in the aptitude test. For each isolation, the results reported by the SRL of the Institute of Tropical Medicine of Belgium in relation to i) the identification of *M. tuberculosis* will be considered valid; ii) presence and type of mutation in the region of the *rpoB* gene explored or absence of mutation. Based on the reported results, the possibility of cross-contamination will also be evaluated.

• Results of the local method validation experience in the country:

- the accuracy of the method
- estimation of the proportion of non-interpretable results (invalid and undetermined results) considering a percentage lower than 5% as an acceptable reference value. For its calculation the following formula will be used:

Indicator	Description	Acceptable value
Number and proportion of non-interpretable results	Number of non-interpretable results / Total samples tested	<5%

The results are not interpretable when they correspond to invalid or undetermined results. This is when the test is invalid because the CC and AC controls did not appear, in a negative sample or in the case of the negative control because bands other than AC and CC appeared or when the test is indeterminate because the controls indicate that the test is valid (ie the CC and TUB bands appear, with or without the presence of the AC band in a positive sample), but bands indicating the presence or absence of mutations cannot be interpreted, because control of the locus is absent for a drug or group of drugs.

2- External quality assessment of NRLs and LPA user network laboratories for the detection of resistance to first- or second-line anti-tuberculosis drugs

For this proficiency test, the NRL will receive from SRL a panel composed of inactivated bacillary suspensions for the evaluation of the test in their own laboratory and in the laboratories of the network of each country that have implemented the system. In the case of the evaluation of the local laboratories of the network, the NRL will receive, together with the panels of inactivated bacillary suspensions, a list containing information about the presence / absence of mutations in the bacillary suspensions submitted, as reported by the SRL Belgium (Annex G.3; Example of list of inactivated bacillary suspensions forwarded to the NRL of country XX for the preparation of panels for aptitude tests); this information should be used to analyze the results obtained by local laboratories and calculate their diagnostic accuracy.

In relation to the number of bacillary suspensions to be included in a panel, it must be considered that this should be large enough so that the exercise was statistically accurate for the evaluation of quality. However, the number of samples needed to complete this requirement would add a heavy workload to the routine of the service and would take a lot of economic resources difficult for the laboratory that is being evaluated. Most of the systems developed for the EQA by means of aptitude tests of molecular methods of resistance detection in the world (see Scott et al., J. Clin Microbiol., 2014. 52: 2493-2499, Nikolayevskyy et al, PLOS ONE 2016; DOI: 10.1371 / journal. pone.0152926) contain less than 5 samples / panel. In this manual, we propose the evaluation of panels with 5 samples only to detect serious errors. If resources allow, it is advisable, in order to increase the validity of the evaluation, to perform this exercise at least twice a year (using different samples in each of the panels), which increases confidence in the results.

Preparation of the panel

- Calculate the number of laboratories in the network that will be evaluated by the NRL.

- Prepare the panels starting from the suspensions sent by the SRL whose bacillary concentration will be approximately 50 000 bacilli/ml. To do this, follow the instructions given in Annex F.5. Example for the preparation of panels for quality control. This example has been prepared for an NRL whose number of laboratories to evaluate is equal to or less than 15. The procedure should be adjusted, depending on the number of laboratories to be evaluated.

- Attach externally to each panel Annex G.4. Form for external quality control.

Methodology to be applied in the peripheral laboratories to be controlled

Once the local laboratories have received the panel and the form of Annex G.4, they should follow the following instructions:

- Perform the test following the standard operating procedure applied in the participating laboratory.

- Assign the execution of the aptitude test to the operator (s) responsible for carrying out the technique in the work routine.

- Assign the analysis of the results to the laboratory that performs the analysis of the results and usually issues the reports, using the instrument provided by the manufacturer to align the strips and using the criteria described above in the section "Carrying out the susceptibility test in the NRL "(support for the validation of the trial)

- Analyze the results of each sample considering the presence/absence of the following bands:

FL-LPA		
Band	Present	Absent
CC		
AC		
TUB		
Wild rpoB		
Mutated rpoB		
Wild katG		
Mutated katG		
Wild inhA		
Mutated inhA		

SL-LPA		
Band	Present	Absent
CC		
AC		
TUB		
Wild rrs		
Mutated rrs		
Wild eis		
Mutated eis		
Wild gyrA		
Mutated gyrA		
Wild gyrB		
Mutated gyrB		

- Fill the final results in a standardized form using Annex G.6.

- Consult, if necessary, for the interpretation of results, the following electronic address http://www.stoptb.org/wg/gli/TrainingPackage_LPA o http://www.who.int/tb/publications/lpa-mdr-diagnostics/en/

- Complete the rest of the questions in Annex G.4. and send it to the organizing laboratory.

Interpretation and analysis of results in the coordinator (NRL)

To analyze the quality of the laboratories, this manual proposes a scoring system and a score above which the service is considered to have acceptable performance. However, it is important to consider that each program must determine what is acceptable performance, considering that the determination of an acceptable performance can be modified based on the experience with proficiency tests in each country and the maturity that the country develops in the implementation of the test.

For each suspension sent, consider the results reported by the SRL of the Institute of Tropical Medicine of Belgium as valid in relation to i) the identification of M. tuberculosis; ii) presence and type of mutation in the region of the scanned gene or absence of mutation.

- Assign a score for each of the drug/drug families investigated by the systems, that is, assign two scores, one attributed to the ability to detect resistance to INH and another to RIF, corresponding to the LPA for first-line drugs. Similarly, for second-line LPA systems, calculate two scores corresponding to the accuracy to detect resistance to fluoroquinolones and second-line injectables.

- Consider a value of 20 points for each suspension correctly reported, which would give a total score of 100 points if the total results are correct. Those services that obtain a score of 80 or more without having a false susceptible or false resistant will be considered acceptable performance.

- Determine the score for each drug studied in the FL-LPA or SL-LPA system considering the following table:

Table 10. Proposed evaluation system for the determination of the score obtained for each sample of bacilli sent in the proficiency test

Expected result: MTB detected Resistance to each drug not detected	
Reported result	**Score**
MTB detected Resistance to the drug not detected	20
MTB detected Resistance to the drug detected	0
MTB detected Indeterminate drug resistance	10
MTB not detected	0
Not valid	0
Expected result: MTB detected Resistance to the drug detected	
Reported result	**Score**
MTB detected Resistance to the drug not detected	0
MTB detected Resistance to the drug detected	20
MTB detected Indeterminate drug resistance	10
MTB not detected	0
Not valid	0

Expected result: MTB not detected	
Reported result	**Score**
MTB detected Resistance to the drug not detected	0
MTB detected Resistance to the drug detected	0
MTB detected Indeterminate drug resistance	0
MTB not detected	20
Not valid	0

• Repeat the LPA test by the coordinating laboratory for the suspension(s) in which more than 80% of the participants would have obtained an unsuccessful result. If it is determined that the error occurred in the NRL after issuing a first report with the results of the proficiency testing for some laboratories in the network, we will proceed to report this finding in a second report.

• Generate an Excel spreadsheet with the fields presented in Annex G.5 Worksheet for analysis of results, and begin to dump the results as follows:

- Complete the fields of the original numbers of the strains and number of the round to which the results belong.

- Enter the presence or not of mutation in the strain as reported by the SRL in box 1 and the result of the sequencing.

- Take the results to form of one of the laboratories evaluated.

- Enter the name of the evaluated laboratory.

- Incorporate the number of the strain that was granted by the organizing laboratory.

- Enter, in column G "2", the score obtained for each suspension sent according to the table "Proposed evaluation system." That gives scores from 0 to 20 according to whether or not the results obtained with respect to the consensus results.

- Check if the calculations performed automatically are correct.

• Evaluate the performance of the laboratory in this test.

• To evaluate the quality of the laboratory to differentiate M. *tuberculosis* from other mycobacteria corroborating that the environmental Mycobacterium was not detected as *M. tuberculosis.*

• Analyze the inconsistent or imprecise results of the work routine arising from the equipment problem or due to possible technical or administrative failures. Based on errors or mistakes regarding the results of the work routine, the performance of each laboratory, the possible cross-contamination and the performance of the equipment will be evaluated. Among the main parameters to be evaluated is the proportion of non-interpretable results

(invalid and undetermined results) considering a percentage lower than 5% as an acceptable reference value. For its calculation the following formula will be used:

Indicator	Description	Acceptable value
Number and proportion of non-interpretable results	Number of non-interpretable results / Total samples tested	<5%

This is when the test is invalid because the CC and AC controls did not appear, in a negative sample or in the case of the negative control because bands other than AC and CC appeared or when the test is indeterminate because the controls indicate that the test is valid (ie the CC and TUB bands appear, with or without the presence of the AC band in a positive sample), but bands indicating the presence or absence of mutations cannot be interpreted, because control of the locus is absent for a drug or group of drugs.

- Consider the other responses issued in Annex G.4 and analyze their responses.

Report

- Check the results transcribed to the report of results between two professionals or technicians before sending the report in order to prevent errors in the preparation of the same.

- Issue a report with the results following the model presented in Annex G.2. Results report, trying to comment on the positive data, and highlighting the advances that are evidenced in order to stimulate the laboratory staff.

- Inform, before the invalidated result if it was due to amplification failures (absence of AC band in a negative sample) or conjugate failures (absence of CC band)

- Send the reports to the different laboratories as the results are received. Only in the case that discrepant results are found for the same strain for the first two or three results received, the reports should be retained to evaluate if there is an error in the confirmation of the remitted panel.

- When the discrepant results have been obtained after the issuance of the first report, and after the repetition of the test, it is identified that the cause of the disagreement is due to an error in the confirmation of the panel in the NRL, a second must be sent report, notifying about this finding.

- An issue, in addition, an observation of the questions asked in the questionnaire.

- Request the strips obtained after the processing of each sample in case the quality of the service has turned out not acceptable.

- Analyze the interpretation of results once the strips are received.

- Evaluate the possible causes of the errors and the possibility of making a technical assistance visit, to investigate the possible reasons for the errors found and apply corrective measures.

EXTERNAL ASSESSMENT OF INDIRECT QUALITY

Blind verification

It is highly recommended that the intermediate laboratories of the network that perform phenotypic and genotypic susceptibility tests from isolates, send to the NRL those multirresistant isolates or monorresistant to RIF or INH, in order to confirm the susceptibility pattern of said isolates and perform susceptibility to second-line drugs (if it had not been performed at the intermediate level); this procedure will make it possible to monitor, in real time, the quality of the laboratories of each network.

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ANNEXES

This section presents all the suggested forms (spreadsheets and reports) useful for EQA of the different diagnostic techniques that will be addressed in this manual.

It is important that these instruments can be available in electronic format, through the use of basic computer programs that can facilitate the filling and analysis of information (for example through the inclusion of formulas added to the databases for the calculation of indicators or parameters of sensitivity, specificity and efficiency, among others). The use of this type of forms in electronic format also facilitates the sending of results and reports to the different levels of the network and to the NTP.

Annex A.1: Guide for the technical visit to laboratories performing SM and / or molecular methods with closed systems (Xpert MTB / Rif or Xpert MTB Ultra / Rif)

This guide has been developed to support the work of the supervisors during the technical visit to the laboratories that perform these methods. If the laboratory only performs smear microscopy, please omit the questions related to molecular methods with closed system (Xpert MTB / Rif or Xpert MTB Ultra / Rif). The contents of the questions cover some points of quality management in such a way that the laboratories incorporate these practices in their work routine.

Table of Contents

Summary of the visited laboratory

Visited laboratory: .....

Location: .....

Province / State / Department: .....

Responsible of the lab: .....

Date of the visit: ..... / ..... / .....

Date of previous visit: ..... / ..... / .....

Staff dedicated to the tuberculosis diagnosis

	Number	Daily hours / person dedicated to the diagnosis of tuberculosis
Professionals		
Technicians		
Technical assistants		
Administrative		

Name of professionals / technicians interviewed: .....
.....
.....
Training in TB diagnosis received in the last 3 years .....
.....
.....

Comments:

A. Laboratory activities		
Next, mark the cells that correspond to the activities developed in your laboratory. Where applicable, complete the requested information		
a. Smear microscopy Method:	Estimated number of tests per month ___	☐
b. Preparation of staining solutions Distribution of staining solutions How many laboratories?		☐ ☐
c. Xpert MTB / Rif or Xpert MTB Ultra / Rif	Estimated number of tests per month ___	☐
d. Do you receive samples derived from other health centers?	Estimated number of tests per month ___	☐
e. Others Describe:	Estimated number of tests per month ___	☐
f. Do you derive samples to reference laboratories for more complex tests? If yes, mention to which service the referral is made).....		☐
g. The laboratory has some process in	YES ☐ NO ☐ Comments ☐	
accreditation stage	YES ☐ NO ☐ Comments ☐	
accredited		
Comments:		

B. Documents for the standardization of procedures
Required for smear microscopy, Xpert MTB / Rif

B.1 Organization chart and technical standards	
a. Organizational chart including all laboratory personnel involved in TB diagnostic activities	Available: YES ☐ NO ☐ Comments ☐
b. Description of responsibilities of each worker and replacement systems.	Available: YES ☐ NO ☐ Comments ☐
c. Manual of standards for all techniques performed at the lab	Available: YES ☐ NO ☐ Comments ☐
d. Work Algorithm / s d.1 Of the NTP d.2 Internal of the laboratory	Available: YES ☐ NO ☐ Comments ☐
B.2 Standard operating procedures (SOP) Availability of SOPs (that consider the technique, disposal of pathological and chemical wastes, maintenance of the necessary equipment, internal quality controls and external quality evaluation) for:	
a. Smear microscopy	Available in the work area: YES ☐ NO ☐ Incomplete ☐ Comments ☐ Updated version: (date of this updated version must be register) YES ☐ NO ☐ Incomplete ☐ Comments ☐
b. Xpert MTB/Rif	Available in the work area: YES ☐ NO ☐ Not applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐
c. Are the SOPs reviewed periodically and corrected appropriately when necessary?	YES ☐ NO ☐ Comments ☐
d. Are the versions of SOPs that were replaced withdrawn and archived?	YES ☐ NO ☐ Comments ☐
e. Are all personnel informed about the contents of the Laboratory SOPs?	YES ☐ NO ☐ Comments ☐
f. Is there a record to document that staff members of the laboratory have read and understood the SOPs?	YES ☐ NO ☐ Comments ☐
g. Is a SOP / Laboratory Biosafety Manual available?	YES ☐ NO ☐ Comments ☐
Comments	

C. Quality assessment Required for Smear microscopy, Xpert MTB / Rif	
C.1 Validation / verification studies	
a. Was the validation performed (non-standardized methods, methods designed or developed by the laboratory, standardized methods used outside its intended field of application or validated methods subsequently modified) before starting its use for routine diagnosis? (specify validated / verified techniques and parameters (sensitivity (S), specificity (E), efficiency (Efi), reproducibility (R))	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
b. Was verification carried out (validated commercial methods used without modification) before starting its use for routine diagnosis? (specify validated / verified techniques and parameters (sensitivity (S), specificity (E), efficiency (Efi), reproducibility (R))	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
c. Are there records that document these validations or verifications?	YES ☐ NO ☐ Comments ☐
C.2 External quality assessment (EQA)	
For smear microscopy	
a. Does the laboratory participate in external quality assessment tests?	YES ☐ NO ☐ Comments ☐
b. Are the tests corresponding to the EQA carried out by the technicians that execute them in the routine?	YES ☐ NO ☐ Comments ☐
c. Do results of the last two years show acceptable quality?	YES ☐ NO ☐ Comments ☐

d. Is any type of error repeated In the result reports of the last two years	YES ☐ NO ☐ Comments ☐ Indicate the type of error .....
e. Are the results of the EQA disseminated among the laboratory staff?	YES ☐ NO ☐ Comments ☐
f. is there documented evidence of this dissemination?	YES ☐ NO ☐ Comments ☐
For Xpert MTB/Rif	
a. Does the laboratory participate in external quality assessment tests?	YES ☐ NO ☐ Comments ☐
b. Are the tests corresponding to the EQA carried out by the technicians that execute them in the routine?	YES ☐ NO ☐ Comments ☐
c. Do results of the last two years show acceptable quality?	YES ☐ NO ☐ Comments ☐
d. Is any type of error repeated in the result reports of the last two years?	YES ☐ NO ☐ Comments ☐ Indicate the type of error .....
e. Are the results of the EQA disseminated among the laboratory staff?	YES ☐ NO ☐ Comments ☐
f. Is there documented evidence of dissemination?	YES ☐ NO ☐ Comments ☐
Comments	

D. Handling of laboratory data (Forms, instructions, records and reports)
Required for smear microscopy, Xpert MTB / Rif

D.1 Forms: Request for bacteriological and / or molecular studies Select 20 forms, review them and answer the following items:	
a. Do the laboratory receive the forms according to the NTP norms?	YES ☐ NO ☐ Comments ☐
b. Has It an adequate design?	YES ☐ NO ☐ Comments ☐
c. Usually, are they received complete? (at least 80% of data are included in the forms)	YES ☐ NO ☐ Comments ☐
d. Are they physically filed?	YES ☐ NO ☐ Comments ☐
e. Are they scanned and archived on computer?	YES ☐ NO ☐ Comments ☐
D.2. Instructions and time of sample collection. Transport and conservation of samples	
a. Are written instructions for sample collection available?	YES ☐ NO ☐ Not applicable ☐ Comments ☐
b. Are at least 2 sputum samples requested for each symptomatic respiratory person, the first one at the time of consultation, and the second one in the early morning at the patient's home?	YES ☐ NO ☐ Not applicable ☐ Comments ☐ If your answer was NO. Indicate the N° and moment of samples collection: .....
c. Are written instructions available for sample conditioning, preservation and transportation? Are they adequate?	YES ☐ NO ☐ Not applicable ☐ Comments ☐ YES ☐ NO ☐ Not applicable ☐ Comments ☐
d. It is a SOP for the rejection of samples available in the laboratory?	YES ☐ NO ☐ Comments ☐
D.3 Data record - Laboratory information system (LIS)	
a. Has the LIS basic information required by the NTP norms?	YES ☐ NO ☐ Comments ☐
b. If paper records are used	YES ☐ NO ☐ Comments ☐
b.1. Are they foliated?	YES ☐ NO ☐ Comments ☐
b.2. Is it complete with the samples received, at least from the previous day?	YES ☐ NO ☐ Comments ☐
c. If digital records are used	
c.1. Is there an exclusive system for TB?	YES ☐ NO ☐ Comments ☐
c.2. Is It complete with the samples received, at least from the previous day?	YES ☐ NO ☐ Comments ☐
c.3. Is there a backup system for the LIS in digital support? (describe in comments how it is done)	YES ☐ NO ☐ Comments ☐

d. Does each sample maintain a unique number for all procedures performed with it?	YES ☐ NO ☐ Comments ☐
e. Is the number of records rational and does it not generate unnecessary workload?	YES ☐ NO ☐ Comments ☐
f. Is access to LIS limited to authorized personnel?	YES ☐ NO ☐ Comments ☐
g. Is there a written SOP for the LIS use?	YES ☐ NO ☐ Comments ☐
Records for derivation of samples and/or isolates a. Has it adequate design?	YES ☐ NO ☐ Comments ☐
b. Is it complete with the necessary information and is legible?	YES ☐ NO ☐ Comments ☐
Records related to Biosafety	YES ☐ NO ☐ Comments ☐
a. Is there an adequate accident / incident record?	YES ☐ NO ☐ Comments ☐
b. Is there a record of the delivery of personal protection elements for each operator?	YES ☐ NO ☐ Comments ☐
c. Is there a record of the staff annual medical control?	YES ☐ NO ☐ Comments ☐
D.4 Reports	
a. Do the laboratory reports identify the laboratory that performs the tests?	YES ☐ NO ☐ Comments ☐
b. Do the laboratory reports identify the methods used?	YES ☐ NO ☐ Comments ☐
c. Have some people been designated to issue results reports?	YES ☐ NO ☐ Comments ☐
d. Are the reports verified by a second staff?	YES ☐ NO ☐ Comments ☐
e. Does the lab archive the results data (printed results, electronic records)? If yes, explain how they are archived and for how long.	YES ☐ NO ☐ Comments ☐ They are archived..... Time.....
f. Are the archived reports only accessible to authorized personnel?	YES ☐ NO ☐ Comments ☐
g. Estimation of the time from the reception of the sample to the report release Take 20 results produced during the last month	Smear microscopy Comments ☐ Within 24 hours ☐ Between 24 and 48 hours ☐ More than 48 hours ☐
	Xpert MTB/Rif Comments ☐ Less than 8 hours ☐ Between 8 and 12 hours ☐ More than 12 hours ☐
Comments	

E. Performance Indicators Monitoring	
E.1 Smear microscopy Record the following parameters for the total tests of performed during the last three months	
a. Total diagnostic smear microscopy	Number..... Comments ☐
b. Positive diagnostic smear microscopy	Number..... Comments ☐
c. Total microscopy of treatment monitoring	Number..... Comments ☐
d. Positive smear microscopy for treatment monitoring (The proportion of positive sputum smears should be close to 10-15% of all sputum smear microscopy)	Number..... Comments ☐
e. Estimation of the average number of smears read by each technician per day	Number..... Comments ☐
f. Does the lab analyze this information periodically?	YES ☐ NO ☐ Comments ☐
g. Does the lab send this information to the reference laboratory?	YES ☐ NO ☐ Comments ☐
h. If the answer is "Yes", how often?	Time..... Comments ☐
E.2 Applicable for Xpert MTB / Rif Record the following parameters for the total tests performed during the last three months	
a. Tests carried out	Number..... Comments ☐
b. Tests with Result MTB detected, resistance to R detected	Number..... Comments ☐
c. Are the cases identified as resistant to rifampicin reported immediately to the NTP?	YES ☐ NO ☐ Comments ☐
d. Tests with Result with errors (should not be greater than 3%)	Number..... Comments ☐
e. Tests with invalid results (should not be greater than 1%)	Number..... Comments ☐
f. Tests with no result (should not be greater than 1%)	Number..... Comments ☐
g. Does the lab analyze this information periodically?	YES ☐ NO ☐ Comments ☐
h. Does the lab send this information to the reference laboratory?	YES ☐ NO ☐ Comments ☐
i. If the answer is "Yes", how often?	Time..... Comments ☐
Comments	

F. Supplies and conservation of material for the different techniques				
F.1 Inputs **It is considered that the supply is adequate when there is current availability and there has been no fault during the last 6 months**				
F.1.1 Joint to smear microscopy and Xpert MTB / Rif				
	Available		Adequate supply	
	YES	NO	YES	NO
a. Sample collection containers Register N / C if the laboratory is not the place that delivers the sputum containers				
b. Markers				
c. Personal protection elements Gloves..... Masks N95..... Coats or overalls.....				
F.1.2 For smear microscopy				
a. Microscope slides				
b. Handles or sticks				
c. Funnel with filter paper				
d. Dyes and chemical products for preparing staining solutions (methylene blue, phenolic fuchsin, acid alcohol, auramine, potassium permanganate)				
e. Distilled water				
f. Immersion oil				
g. Paper tissues to clean microscope lenses				
F.1.3 Applicable for Xpert MTB / Rif				
a. Disposable Pasteur pipettes				
b. Xpert MTB / Rif or Xpert MTB Ultra / Rif kit				
c. Calibration kit				
F.2 Supplies Preservation				
Required for Smear Microscopy				
			Available	
			YES	NO
a. Staining solutions In clean containers protected from light, Correctly identified With date of preparation				
b. Distilled water in clean containers				

Applicable for Xpert MTB / Rif		
a. The cartridges are stored at a controlled temperature between 2 and 30 °C		
Comments		

G. Equipment **Verify the following as applicable to equipment used for specific laboratory activities, indicating Yes or No, as appropriate (List the manufacturer, model and date of installation in the preventive maintenance register)**		
Required for Smear microscopy and Xpert MTB / Rif		
	YES	NO
G.1 Refrigerator		
a. Preventive maintenance activities are carried out and documented (cleaning and disinfection).	☐ Frequency.....	☐
b. Are temperature readings taken and documented?	☐ Frequency.....	☐
c. Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. Is there documentation of corrective actions made in response to values out of range?	☐	☐
G.2 Autoclave		
a. Are annual check-up carried out to verify the complete sterilization of autoclaved materials, hydraulic testing and valve verification?	☐	☐
b. Are daily sterilization cycles recorded?	☐	☐
c. Are physico-chemical strips used as a sterile control in each carried out cycle?	☐	☐
d. Are biological controls used weekly or monthly as sterile control?	☐ Frequency of use:	☐

Required for Smear microscopy **G.3 Microscope**		
a. There is enough quantity for the workload (Consider that each bacilli colored by ZN requires at least 5 minutes to read, while fluorescence slides require an average of 2-3 minutes for observation)	☐	☐
b. It works properly (Look at a stained slide)	☐	☐
c. Is preventive maintenance (cleaning) performed and documented daily and annually?	☐	☐
d. If mercury lamp fluorescence microscopes are used, is the time of use of the mercury lamp recorded and is the lamp replaced, prior to the duration limit as described by the manufacturer?	☐	☐
G.4 Balance		
a. Are preventive maintenance activities/ services performed and documented daily and annually?	☐	☐
b. Are calibration procedures performed as described by the manufacturer?	☐	☐
c. Do calibration records exist and are periodically checked?	☐	☐
Applicable for Xpert MTB/Rif **G.5 GeneXpert** **N° of equipment modules.....**		
a. Is the latest software installed on the computer?	☐	☐
b. Have the equipment and the computer an auxiliary power system for the case of electricity interruption (UPS)?	☐	☐
c. Was the equipment calibrated? When was the last calibration?	☐ Date.../.../.....	☐
d. Is there a module that is not currently working?	☐	☐
e. Has there been a period, during the last year, in which the laboratory has interrupted the performance of the test due to equipment failure or lack of cartridges?	☐ Duration of interruption.....	☐
f. Is equipment maintenance performed and recorded in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
g. Is there an established sample bypass system in case the equipment breaks down?	☐	☐
Comments		

H. Characteristics of the laboratory (Basic infrastructure, biosafety, location of tasks according to the level of biological risk) Mark the cells that correspond to the characteristics of your laboratory)	
H.1 Place where the samples are processed	
a. In a laboratory dedicated to TB b. In a laboratory dedicated to other tasks, but there are tables or separate areas of a laboratory dedicated to TB (at least for the execution of smears or the preparation of the samples and inoculation to the Xpert MTB / Rif cartridges) c. Schedules of low personnel flow	☐ ☐
d. Separate spaces for the realization of microscopy, introduction of the cartridges in the GeneXpert equipment and reports	☐
H.2 Renovation, conditioning and addressing of laboratory air	
a. The laboratory has windows to renew the air or an extractor of air that allows making 6 to 12 changes of the volume of air per hour, not being the air flow directed to the countertop in which the smears are prepared or processed samples to inoculate the cartridges. b. If there is an air conditioner, the equipment does not generate air movement in the area of the smears preparation or of the sample processing to inoculate the Xpert cartridges	☐ ☐
H.3 Connection system	
a. Internet access continuously b. Access to telephone continuously	☐ ☐
H.4 Air temperature conditioning system for the equipment	
Applicable for Xpert MTB/Rif	
a. The laboratory where the equipment is located has an air conditioning system that allows to maintain the temperature between 15 and 30° C.	☐
b. The laboratory has enough space available, clean and with adequate temperature (up to 25 °C) for the storage of the cartridges.	☐
H.4 Lighting and general conditions of the work area	
a. The adequate lighting (it is considered 500 LUX without emission of reflections or brightness, which is equivalent to 50 Watt of a fluorescent lamp for 5 m2)	☐
b. Walls and ceilings are painted, clean and without humidity	☐

c. All work areas are clean, there is a daily cleaning service	☐
Comments	

I. Handling and transporting the sputum sample			
I.1 Collection of the sample **(Check the samples received during the day, see if the volume is adequate, if there are spills, if they are well conditioned, etc.)**			
a. Type of container used for sputum collection Does it comply with the standardized technical specifications?	YES ☐	NO ☐	Comments ☐
b. Is the containers label with the patient's identification on the side of the bottle and not on the lid?	YES ☐	NO ☐	Comments ☐
c. Is salivary sampling carried out by sputum smear / Xpert MTB / Rif and do reports indicate that the study was performed using a saliva sample?	YES ☐	NO ☐	Comments ☐
I.2 Conservation and transport of the sample			
a. Are the containers well conditioned with the samples that the laboratory receives, fulfilling the local standards for the shipment of samples? (Ex. transported in a strong, unbreakable and closed container, labeled with the international biohazard symbol)	YES ☐	NO ☐	Comments ☐
b. If they are referred from other centers, do they arrive at the laboratory within 24 hours of the sample collection?	YES ☐	NO ☐	Comments ☐
c. Are the samples stored in a cool place, preferably in the refrigerator, until they are processed?	YES ☐	NO ☐	Comments ☐
d. Are the samples stored in a refrigerator other than the one where the reagents are stored (or at least on an exclusive shelf)?	YES ☐	NO ☐	Comments ☐
e. Is there a regular transport system to transfer samples to the laboratory of culture/Xpert MTB/Rif?	YES ☐	NO ☐	Comments ☐
Comments			

J. Practice audit	
J.1 Procedure for Smear microscopy Ask, if possible, the technician to perform the technique. Otherwise, ask staff to tell you how to proceed. Check smears, observe stained smears on the microscope and the results records.	
a. Are new slide used to perform sputum smears?	YES ☐ NO ☐ Comments ☐
b. Are internal controls included according to standards?	Yes ☐ every time a staining is done ☐ each ___ days ☐ with each new batch of reagents ☐ never ☐
c. Is the identification of the microscope slide always carried out on the same edge of each slide with the identification number of each sample?	YES ☐ NO ☐ Comments ☐
d. Is the most dense or purulent particle selected from the sputum sample?	YES ☐ NO ☐ Comments ☐
e. Is the smear made with a size of approximately 2-3 cm long and 1-2 cm wide so that it is homogeneous?	YES ☐ NO ☐ Comments ☐
f. Is the smear air-dried, until there is no moisture left?	YES ☐ NO ☐ Comments ☐
g. Is the spread set 2 or 3 quick passes by the flame, without overheating or on an electric blanket at 60°C for one hour?	YES ☐ NO ☐ Comments ☐
h. For each batch of staining, are there up to 12 smears on the staining support?	YES ☐ NO ☐ Comments ☐
i. Is fuchsin/auramine solution filtered every day?	YES ☐ NO ☐ Comments ☐

j. Is the contrast solution placed for no more than one minute in the fluorescence technique?	YES ☐ NO ☐ Comments ☐
k. Are the smears with fluorescent staining examined for a period no longer than 24 hours after staining?	YES ☐ NO ☐ Comments ☐
l. Are the immersion lenses of the microscope cleaned with soft paper after each smear observation?	YES ☐ NO ☐ Comments ☐
m. In the ZN staining, when less than 5 bacilli/100 fields were observed, is the following attitude taken? - Extend the reading to 200 fields. - If with that reading no more bacilli are found another smear from the same sample is made, - If the reading of the second smear does not modify the result of the previous one, the sample is informed with the exact number of bacilli observed, and request a new sample. - If possible, the sample is derived for Xpert MTB / Rif, or culture.	YES ☐ NO ☐ Comments ☐
n. In fluorescence staining when less than 5 bacilli are read in a line at 200x amplification or less than 3 bacilli in a line at 400 x amplification, Is the following attitude taken?: - Expand the reading to another line of the smear - If with that reading no more bacilli are found, make another smear from the same sample - If the reading of the second smear does not modify the previous result, the sample must be reported as "Confirmation required" requesting a new sample - If possible, the sample is derived for Xpert MTB/ Rif, or culture.	YES ☐ NO ☐ Comments ☐
o. Are the slides kept according to standards for submission to the Reference Laboratory for external quality assessment by blinded rechecking?	YES ☐ NO ☐ Comments ☐
Comments	

J. Practice audit			
J.2 Procedure of the Xpert MTB / Rif Ask, if possible, the technician to perform the complete processing of the Xpert MTB/Rif test. Otherwise ask the staff to report how they process			
a. Is the sample reagent added in the amount set by the manufacturer?	YES ☐	NO ☐	Comments ☐
b. Is the sample mixture stirred at least twice with the sample reagent during the incubation time?	YES ☐	NO ☐	Comments ☐
c. Is the incubation time of the sample with the sample reagent 15 minutes (preferably using a stopwatch)?	YES ☐	NO ☐	Comments ☐
d. Is 2 ml of the mixture transferred to the cartridge, avoiding the transfer of solid particles and generating bubbles during the process?	YES ☐	NO ☐	Comments ☐
e. Can the laboratory analyze the data stored in the instrument? (For example, to verify results of individual patients, error codes, etc.)	YES ☐	NO ☐	Comments ☐
f. Are the curves analyzed and are peculiarities of these curves registered?	YES ☐	NO ☐	Comments ☐
g. Is the report done as established by the equipment information?	YES ☐	NO ☐	Comments ☐
h. Is the time between the loading of the samples in the cartridge and its location and processing in the equipment less than 8 hours?	YES ☐	NO ☐	Comments ☐
Comments			

K. Safety in the laboratory **Required for Smear microscopy, Xpert MTB/Rif**	
K.1 Safety practices	
a. Are the recommended disinfectants for tuberculosis available (5% phenol, 1% sodium hypochlorite, 70% ethanol)?	Available: YES ☐ NO ☐ Comments ☐
b. Are worktops cleaned and disinfected at least once before beginning and at the end of each work day?	YES ☐ NO ☐ Comments ☐
c. Are gloves used according to the laboratory's general biosafety work standards?	YES ☐ NO ☐ Comments ☐
d. Are respirators (type N95 or FFP2) used to work with samples? (Optional)	YES ☐ NO ☐ Comments ☐
e. Availability of respirators (type N95 or FFP2), for use in case of spills.	YES ☐ NO ☐ Comments ☐
f. Are the containers with sputum samples or Xxpert cartridges removed with the pathological waste by recommended methods (autoclaving or treatment with sodium hypochlorite before disposal with the rest of the pathological waste of the institution or, for special cases, open-air incineration)?	YES ☐ NO ☐ Comments ☐
g. Spill kit containing: an autoclavable bag, gloves, gowns, appropriate disinfectants, N95 or FFP2 respirators, cotton and adsorbent paper, soap, stick and container to collect waste, sharps container, DO NOT ENTER sign)	Available: YES ☐ NO ☐ Comments ☐
K.2 Staff security	
a. Regular annual program of medical control for health workers, following the labor regulations in the country (If there is no adopted policy, the supervisor must ensure that laboratory personnel have at least one annual medical evaluation that may include a chest x-ray).	Available: YES ☐ NO ☐ Comments ☐
b. Known and written instructions for accidents or incidents (may be included in the laboratory's Biosafety manual / SOP)	Available: YES ☐ NO ☐ Comments ☐
c. Initial safety / biosafety training program with records of the laboratory personnel participation	Available: YES ☐ NO ☐ Comments ☐

Comments	
L. Derivation of samples	
L.1 Required for Smear microscopy, Xpert MTB/Rif	
Are all samples indicated for subsequent studies referred to the reference laboratory? The following samples should be derived - samples for diagnosis: • of patients with persistent respiratory symptoms and two or more previous samples with negative smear microscopy or Xpert • of patients with suspected extrapulmonary TB • children's • of immunosuppressed particularly positive HIV • of patients with a history of antituberculosis treatment • of patients with exposure to infection with drug-resistant bacilli (contacts of cases with resistant tuberculosis, internees or workers from health institutions or prisons where there are cases of resistant tuberculosis), including those investigated by molecular methods • of gastric lavage and bronchoalveolar lavage • other risk groups defined by the country • patients with a rifampicin-resistant result to confirm the resistance and perform drug susceptibility testings to the rest of the first and second line drugs -samples for treatment control: • with smear positive at the end of the second month of chemotherapy or in a subsequent control	YES ☐ NO ☐ Comments ☐

b. Do you receive reports from the laboratories to which the samples are derived for more complex studies?	
c. What is the average time of results reception from the derivative studies?	Culture and identification ..... days Xpert MTB/Rif .....days LPAs: .....days Drug susceptibility testing (phenotypic methods) to Rifampicin .....days to Isoniazid .....days to second-line drugs .....days
Comments	

M. Other observations
Laboratory staff expressed the following concerns regarding their reference laboratory ....
Conclusions
The following strengths are highlighted ....
It highlights the following challenges or corrective measures to be implemented as a priority By the authorities ....
By the laboratory staff ....
The following training needs were identified ....
Agreements reached in relation to the challenges ....
Name and signature of the supervisor/s: .....
Name and signature of the laboratory manager: .....
Date: ..... /...../.....

Annex A.2: Guide for the technical visit to laboratories who perform culture and/or *Mycobacterium tuberculosis complex* (MTBC) identification

This guide has been developed to support the work of the supervisors during the technical visit to laboratories that perform culture and/or *Mycobacterium tuberculosis* complex identification. If the laboratory performs MTBC identification from samples by molecular methods in closed systems (Xpert MTB / Rif or Xpert MTB Ultra / Rif), refer to the questions presented at Annex A.1. Remember that in the case of having completed Annex A.1, the common questions to several methodologies should be ignored.

The contents of the questions cover some points of quality management in such a way that the laboratories incorporate these practices in their work routine.

Table of Contents

Summary of the visited laboratory

Visited laboratory: .....

Locality: .....

Province / State / Department: .....

Responsible of the lab: .....

Date of the visit: ..... / ..... / .....

Date of previous visit: ..... / ..... / .....

Staff dedicated to the diagnosis of tuberculosis

	Number	Daily hours / person dedicated to the diagnosis of tuberculosis
Professionals		
Technicians		
Technical assistants		
Administrative		

Name of professionals / technicians interviewed: .....
.....
.....

Training in TB diagnosis received in the last 3 years .....
.....
.....

Comments:

A. Laboratory activities

Next, mark the cells that correspond to the activities developed in your laboratory. Where applicable, complete the requested information		
a. Culture in solid medium	Estimated number of tests per month ___	☐
b. Culture in liquid medium	Estimated number of tests per month ___	☐ ☐
c. Culture medium preparation	Estimated number of tests per month ___	☐
d. Identification of the *M. tuberculosis* Complex by immunochromatographic methods	Estimated number of tests per month ___	☐
e. Identification of the *M. tuberculosis* by phenotypic methods	Estimated number of tests per month ___	☐
f. Identification of the *M. tuberculosis* Complex by commercial closed molecular methods *	Estimated number of tests per month ___	☐
g. Identification of the *M. tuberculosis* Complex by commercial and/or homemade open molecular methods. Name of the equipment.....	Estimated number of tests per month ___	☐
h. Others	Estimated number of tests per month ___	☐
i. Do you receive samples derived from other health centers?	Estimated number of samples per month ___	☐
j. Do you derive samples to reference laboratories for more complex tests? If yes, mention to which service the referral is made)..... to whom it is derived:	Estimated number of samples per month ___	☐

g. The laboratory possess a technical process in accreditation stage accredited	YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐
• Use Annex A.1 to monitor this methodology	
Comments:	

B. Documents for the standardization of procedures
Applicable for culture and *M. tuberculosis* complex identification

B.1 Organization chart and technical standards	
a. Organizational chart including all laboratory personnel involved in TB diagnostic activities	Available: YES ☐ NO ☐ Comments ☐
b. Description of responsibilities of each worker and replacement systems.	Available: YES ☐ NO ☐ Comments ☐
c. Manual of standards for all techniques performed at the lab	Available: YES ☐ NO ☐ Comments ☐
d. Work Algorithm / s d.1 Of the NTP d.2 Internal of the laboratory	Available: YES ☐ NO ☐ Comments ☐
B.2 Standard operating procedures (SOP) Availability of SOPs (that consider the technique, disposal of pathological and chemical wastes, maintenance of the necessary equipment, internal quality controls and external quality evaluation) for:	
a. Preparation of medium and/or reagents	Available in the work area: YES ☐ NO ☐ Incomplete ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐

b. Culture method used (decontamination and mediums used) Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐
c. Identification of *M. tuberculosis complex* by immunochromatographic and/or phenotypic methods Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐
d. Identification of *M. tuberculosis* complex by molecular methods with open systems Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐
e. Are the Sops reviewed periodically and corrected appropriately when necessary?	YES ☐ NO ☐ Comments ☐
f. Are the versions of SOPs that were replaced removed and filed?	YES ☐ NO ☐ Comments ☐
g. Is all staff informed about the contents of the Laboratory's SOPs?	YES ☐ NO ☐ Comments ☐
h. Is there a SOP/Laboratory Biosafety Manual?	YES ☐ NO ☐ Comments ☐
Comments	

C. Quality assessment **Applicable for culture and identification of *M. tuberculosis complex***	
C.1 Validation / verification studies	
a. a. Was the validation performed (non-standardized methods, methods designed or developed by the laboratory, standardized methods used outside its intended field of application or validated methods subsequently modified) before starting its use for routine diagnosis? (specify validated/verified techniques and parameters (sensitivity (S), specificity (Spe), efficiency (Efi), reproducibility (R)	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
b. Was verification carried out (validated commercial methods used without modification) before starting its use for routine diagnosis? (specify validated/verified techniques and parameters (sensitivity (S), specificity (Spe), efficiency (Efi), reproducibility (R)	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
c. Are there records that document these validations or verifications?	YES ☐ NO ☐ Comments ☐
C.2 External quality assessment (EQA)	
For culture	
a. Does the laboratory participate in external quality assessment tests?	YES ☐ NO ☐ Comments ☐
b. If your previous answer was affirmative, indicate the type of EQA test of which you participate: - external monitoring of performance indicators - evaluation of the quality of the medium produced - Other.	 YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐ Specify .....

c. Do the results of the last two evaluations show acceptable quality? - external monitoring of performance indicators - evaluation of the quality of the medium produced - Other	 YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐
d. Is any type of error or deviation repeated in the results of the last two years? - external monitoring of performance indicators - evaluation of the quality of the medium produced - Other	 YES ☐ NO ☐ Comments ☐ Indicate the type of deviation..... YES ☐ NO ☐ Comments ☐ Indicate the type of deviation..... YES ☐ NO ☐ Comments ☐ Indicate the type of deviation.....
e. Are the EQA results disseminated among the laboratory staff?	YES ☐ NO ☐ Comments ☐
f. Is there documented evidence of this dissemination?	YES ☐ NO ☐ Comments ☐
For identification *M. tuberculosis complex (In the case of an open molecular method (LPA), the detection of resistance to rifampicin and isoniazid is also considered.)*	
a. Does the laboratory participate in tests of external competence external quality assessment tests?	YES ☐ NO ☐ Comments ☐
b. Tests carried out: Phenotypic method Immunochromatographic method Open molecular method	 ☐ ☐ ☐ Comments ☐
c. Are the tests corresponding to the EQA performed by the technicians that execute them in the routine?	YES ☐ NO ☐ Comments ☐
d. Do the results of the last two years show acceptable quality?	YES ☐ NO ☐ Comments ☐
e. In the results of the last two years, is some kind of error reiterated?	YES ☐ NO ☐ Comments ☐ Indicate the type of error.....
f. Are the results of the EQA disseminated among the laboratory personnel?	YES ☐ NO ☐ Comments ☐
g. Is there documented evidence of the dissemination of the results?	YES ☐ NO ☐ Comments ☐

Comments

D. Handling of laboratory data (Forms, instructions, records and reports) **Required for culture and identification of *M. tuberculosis complex***			
D.1 Forms: Request for bacteriological and/or molecular studies Select 20 forms, review them and answer the following items:			
a. Do the laboratory receive the forms according to the NTP norms?	YES ☐	NO ☐	Comments ☐
b. Has It an adequate design?	YES ☐	NO ☐	Comments ☐
c. Usually, are they received complete? (at least 80% of data are included in the forms)	YES ☐	NO ☐	Comments ☐
d. Are they physically filed?	YES ☐	NO ☐	Comments ☐
e. Are they scanned and archived on digital format?	YES ☐	NO ☐	Comments ☐
D.2 Data record - Laboratory information system (LIS)			
a. Has the LIS basic information required by the NTP norms?	YES ☐	NO ☐	Comments ☐
b. If paper records are used			
b.1 Are they foliated?	YES ☐	NO ☐	Comments ☐
b.2 Is it complete with the samples received, at least from the previous day?	YES ☐	NO ☐	Comments ☐
c. If digital records are used			
c.1 Is there an exclusive system for TB?	YES ☐	NO ☐	Comments ☐
c.2 Is it complete with the samples and / or cultures or isolates received, at least from the previous day?	YES ☐	NO ☐	Comments ☐
c.3 Is there a backup system for the LIS in digital support? (describe in comments how the backup is done)	YES ☐	NO ☐	Comments ☐

d. Does each sample and/or culture or isolate maintain a unique number for all the procedures performed with it?	YES ☐ NO ☐ Comments ☐
e. Is the number of records rational and does it not generate unnecessary workload?	YES ☐ NO ☐ Comments ☐
f. Is access to the LIS limited to authorized personnel?	YES ☐ NO ☐ Comments ☐
g. Is there a written SOP for the use of LIS?	YES ☐ NO ☐ Comments ☐
Record for derivation of samples and/or isolates a. Has it an adequate design?	YES ☐ NO ☐ Comments ☐
b. Is it complete with the necessary information and is it legible?	YES ☐ NO ☐ Comments ☐
D.3 Records related to Biosafety	
a. Is there an adequate accident/incident record?	YES ☐ NO ☐ Comments ☐
b. Is there a record of the delivery of personal protection items for each operator?	YES ☐ NO ☐ Comments ☐
c. Is there a record of the staff annual medical control?	YES ☐ NO ☐ Comments ☐
D.4 Reports Select 20 reports, review them and respond	
a. Do the laboratory reports identify the laboratory that performs the tests?	YES ☐ NO ☐ Comments ☐
b. Do the laboratory reports identify the methods used?	YES ☐ NO ☐ Comments ☐
c. Have you designated certain people to issue results reports?	YES ☐ NO ☐ Comments ☐
d. Are the reports verified by a second staff?	YES ☐ NO ☐ Comments ☐
e. Does the laboratory archive the results data (printed results, electronic records)? If yes, explain how they are archived and for how long.	YES ☐ NO ☐ Comments ☐ They are archived..... ..... Time.....
f. Are the archived reports only accessible to authorized personnel?	YES ☐ NO ☐ Comments ☐

g. Estimation of the time from the reception of the sample to the report release Take 20 results produced during the last month	Culture Contaminated sample report (*) Number..... Between 0 and 72 hours ☐ N°..... More than 72 hours ☐ N°..... (*) For the calculation of response time, the date of the contaminated culture detection in the laboratory record is taken as time 0 Positive culture report Number of positives..... Within 48 hours of positivity°..... Negative culture report(*) Number of negatives..... Between 62 and 64 days (solid medium) N°..... More than 64 days N°..... Between 42 and 44 days (liquid medium) N°..... More than 44 days N°..... (*) For the calculation of the delay, the time of culture inoculation is taken as time 0 Comments ☐

	Report of MTBC by phenotypic methods (*) Between 20 and 40 days ❐ N°..... More than 40 days ❐ N°..... MTBC report by immunochromatography (*), (***) Between 24 to 48 hours ❐ N°..... More than 48 hours ❐ N°..... MTBC report by system molecular open (*), (**) Between 48 to 72 hours ❐ N°..... More than 72 hours ❐ N°..... Comments ❐ Report of resistance to isoniazid and rifampicin (LPA) (*), (**) Less than 48 hours ❐ Between 48 and 96 hours ❐ More than 96 hours ❐ Comments ❐ (*) For the calculation of the delay, the time at which the positive culture was detected is taken as time 0. (**) For the calculation of the delay, the time of reception of the isolation or of the sample (for molecular methods) is taken as time 0. (***) It may be higher if the samples are processed in batches
Comments	

E. Performance Indicators monitoring	
E.1 Culture Record the following parameters for the total of tests performed during the last three months or, if the workload is high, during the last month prior to the visit	
a. Total cultures	Number..... Comments ☐
b. Percentage of contamination (per tube)	%..... Comments ☐
c. Proportion of smear positive samples with positive culture	%..... Comments ☐
d. Contribution of the culture to the diagnosis of cases of pulmonary tuberculosis (in relation to smear microscopy)	%..... Comments ☐
e. Contribution of the culture to the diagnosis of cases of pulmonary tuberculosis (in relation to the Xpert MTB / Rif or Xpert Ultra MTB / Rif test)	%..... Comments ☐
f. Contribution of the culture to the diagnosis of cases of pulmonary tuberculosis (in relation to the Xpert MTB / Rif or Xpert Ultra MTB / Rif test)	Number..... Comments ☐
g. Does the lab analyze this information periodically?	YES ☐ NO ☐ Comments ☐
h. Does the lab send this information to the reference laboratory?	YES ☐ NO ☐ Comments ☐
i. If the answer is "Yes", with what periodicity?	Time..... Comments ☐
E.2 Required for identification *M. tuberculosis complex (In the case of LPA, the detection of resistance to rifampicin and isoniazid is also considered.)* Record the following parameters for the total of tests performed during the last three months or, if the workload is high, during the last month prior to the visit	
a. Tests carried out Phenotypic method Immunochromatographic method Open molecular method	 Number..... Comments ☐ Number..... Comments ☐ Number..... Comments ☐
b. Number and proportion of samples with non-interpretable results (invalid and indeterminate) by open molecular system (LPA). It is considered adequate when this value does not exceed 5%	Number..... %..... Comments ☐
c. Number and proportion of samples and/or isolates without results by the open molecular system (LPA). It is considered adequate when this value does not exceed 1%	Number..... Comments ☐
d. Tests with MTBC detected result and only Rifampicin resistance detected	Number..... Comments ☐
e. Tests with MTBC detected result and only Isoniazid resistance detected	Number..... Comments ☐
f. Tests with MTBC detected result and multidrug resistant TB detected	Number..... Comments ☐

g. Are the cases identified as resistant to rifampicin and multidrug resistant TB being reported immediately to the NTP?	YES ☐ NO ☐ Comments ☐
h. Do you analyze this information periodically?	YES ☐ NO ☐ Comments ☐
i. Do you send this information to the reference laboratory?	YES ☐ NO ☐ Comments ☐
j. If the answer is **"Yes"**, with what periodicity?	Time..... Comments ☐
Comments	

F. Supplies and conservation of material for the realization of the different techniques

F.1 Supplies
It is considered that the supply is adequate when there is current availability and there has been no fault during the last 6 months

F.1.1 Common to preparation of medium, culture and identification of *M. tuberculosis Complex*

	Available		Proper supply	
	YES	NO	YES	NO
a. Tubes or flasks for storing culture medium or for decontamination of samples				
b. Markers				
c. Distilled water				
d. Racks, baskets and trays				
e. Containers for autoclaving material				
f. Personal protection items Gloves..... Masks N 95/100..... Tyvec or waterproof gown.....				
F.1.2 For preparation of medium and/or reagents for different methodologies				
a. Glass material for measurement and storage (Erlenmeyer, test tubes, beaker, tubes)				

b. Manual or automatic systems for dispensing medium				
c. Strips to measure PH in different ranges				
F.1.3 Applicable for culture				
a. Hyssop				
b. Glass pipettes				
c. Disposable Pasteur pipettes				
d. Disposable tubes with screw cap of 15 ml or 50 ml				
e. Devices for pipetting (pipet aid)				
f. Mortars and pylons				
g. Reagents for the realization of the culture (decontaminating and regulatory solutions)				
h. Eggs from ecological farms				
i. Tubes, reagents and tube holders for culture in MGIT				
F.1.4 Required for identification by phenotypic and immunochromatographic methods				
a. Immunochromatographic strip				
b. 1.5 ml eppendorf tubes				
c. Disposable tips with aerosol protection of 100 µl and 200 µl				
d. Glass tubes with Bakelite lid 13 x 100 mm				
e. Niacin reagent strips				
f. Reagents for the nitratase, catalase and niacin test				
F.1.5 Required for identification by molecular methods (LPA or other home methods)				
a. Extraction equipment				
b. LPA equipment				
c. Reagents for the amplification mixture (buffers, taq polymerase, nucleotides, primers)				
d. Distilled water Milli-Q or double distilled				
e. Tubes of 0.2 ml ultra-thin wall				
f. 1.5 ml eppendorf tubes				
g. Disposable tips with aerosol protection of 20 µl, 100 µl and 1000 µl				
F.2 Supplies Conservation				
Applicable for culture and identification by phenotypic and immunochromatographic methods				
			Available	
			YES	NO
a. Reagents In clean containers protected from light, Correctly identified				

b. Distilled water in clean containers		
Required for identification by open molecular methods (LPA)		
a. The reagents for the identification of the amplicons by reverse hybridization are conserved at a controlled temperature between 2 and 8°C in an area where tubes with amplicons are opened.		
b. Reagents for the amplification reaction of nucleic acids or those of the mixture of the home-brew methods are stored at a controlled temperature between -20° C in the mixing preparation area		
c. Water Milli-Q in aliquots		
d. Reagents for electrophoretic run and for the loading and identification of amplicons are stored at room temperature in an area where tubes with amplicons are opened		
Comments		

G. Equipment

Verify the following as applicable to equipment used for specific laboratory activities, indicating Yes or No, as appropriate (List the manufacturer, model and date of installation in the preventive maintenance register)

Applicable for culture and identification of *M tuberculosis* Complex		
	YES	NO
G.1 Refrigerator (Ignore if it has already been completed in Annex A.1)		
a. Preventive maintenance activities are carried out and documented (cleaning and disinfection).	☐ Frequency.....	☐
b. Are temperature readings taken and documented?	☐ Frequency.....	☐
c. Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐

d. Is there documentation of corrective actions made in response to values out of range?	☐	☐
G.2 Autoclave (Ignore if it has already been completed in Annex A.1)		
a. Are annual check-ups performed to verify complete sterilization of autoclaved materials, hydraulic testing and valve verification?	☐	☐
b. Are daily sterilization cycles recorded?	☐	☐
c. Are physical-chemical strips used as a sterile control in each carried out cycle?	☐	☐
d. Are biological controls used weekly or monthly as sterile control?	☐ Frequency of use:	☐
Required for medium preparation **G.3 Egg-based medium coagulation oven or coagulator**		
a. It works constantly at 80-85ºC, with a water bath system around each shelf or forced ventilation that ensures uniform temperature	☐	☐
b. Perform and document preventive maintenance activities/services.	☐	☐
c. Are there records of temperature control of the coagulation process?	☐ Frequency of use use of the registry:	☐
G.4 Balance (ignore if it has already been completed in Annex A.1)		
a. Are preventive maintenance activities/ services performed and documented daily and annually?	☐	☐
b. Are calibration procedures performed as described by the manufacturer?	☐	☐
c. Do calibration records exist and are periodically checked?	☐ Frequency.....	☐
G.5 Thermostatic bath		
a. Has it an electronic temperature control?	☐	☐
b. Are preventive maintenance activities/ services performed and documented?	☐	☐
c. Are there records of temperature control of each process?	☐ Frequency.....	☐

Applicable for culture and identification of M tuberculosis Complex **G.6 Automatic pipettes**		
a. Are preventive maintenance activities/ services performed and documented annually/biannually?(Depending on the requirements for each quality body).	☐	☐
b. Are calibration procedures performed as described by the manufacturer?	☐	☐
c. Do calibration records exist and are checked?	☐ Frequency.....	☐
G.7 Vortex		
a. Are preventive maintenance activities/ services performed and documented annually?, including daily cleaning.	☐	☐
G.8 Biological safety cabinet		
a. Is it one of these models? Class I (EN12469 / NSF49) Class IIA2 (NSF49) o Class II (EN12469)	Model..... ☐ ☐	 ☐ ☐
b. Does it have an outside duct?	☐	☐
c. Is it certified at least annually? (verify certificates)	☐	☐
d. Does the equipment have an uninterruptible power supply (UPS) system?	☐	☐
e. Are preventive maintenance activities/ services performed and documented annually?	☐	☐
f. Do the laboratory technicians perform and document cleaning and operation daily?	☐	☐
G.9 Centrifuge		
a. It is refrigerated with a temperature range between 4 and 12 ° C and reaches a speed of at least 3000 g?	☐	☐
b. Has it tube holders covered with a lid and are they autoclavable?	☐	☐
c. Is there a record of use and temperature of each run?	☐	☐
d. Has it an uninterruptible power supply (UPS)?	☐	☐
e. Do the laboratory technicians perform and document the daily use of the equipment and annually the preventive maintenance services?	☐	☐

G.10 Culture camera or Incubators		
a. Has it enough space for the work load that the laboratory handles?	☐	☐
b. Has it minimum and maximum temperature control with a device that detects variations of ± 1ºC?	☐ Frequency.....	☐
c. Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. Are there temperature records and are corrective measures applied when the temperature parameters are out of range?	☐	☐
G.11 Bactec 320/960 equipment		
a. Has It a computer associated with installation of the latest software?	☐	☐
b. Do the computer and the equipment count with an uninterruptible power supply (UPS)?	☐	☐
c. Are calibration procedures carried out as described by the manufacturer?	☐	☐
d. Is the equipment maintenance carried out and registered in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
e. Are there calibration and maintenance records? Do they review periodically?	☐ Frequency.....	☐
G.12 Chronometer		
a. Are calibration procedures carried out as described by the manufacturer?	☐	☐
b. Are there recalibration records? Are they periodically reviewed?	☐	☐
Required for identification of *M tuberculosis* complex by open molecular methods (LPA) or home-brew tests **G.13 Thermocycler**		
a. Is the maintenance carried out and registered in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
b. Are the procedures for temperature verification of the thermal block carried out by external probes as described by the manufacturer?	☐	☐

G.14 Twincubator		
a. Is the equipment maintenance carried out and registered in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
G.15 Freezer		
a. Are the preventive maintenance activities/services performed and documented?	☐ Frequency.....	☐
b. Are temperature readings taken and documented?	☐ Frequency.....	☐
c. Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. Is there documentation of corrective actions made in response to values out of range?	☐	☐
G.16 Microcentrifuge (for the case of performing the molecular test from isolates)		
a. Are the preventive maintenance services performed annually? Is the daily use of the equipment documented?	☐	☐
b. It is refrigerated?	☐	☐
Comments		
H. Characteristics of the laboratory (Basic infrastructure, biosafety, location of tasks according to the level of biological risk) Mark the cells that correspond to the characteristics of your laboratory)		
H.1 Place where samples are processed for culture **H.1.1 Applicable for culture techniques that do not require concentration methods (Ogawa-Kudoh method)**		

a. In a laboratory dedicated to TB b. In a laboratory dedicated to other tasks, but there are tables or separate areas of a laboratory dedicated to TB c. Schedules of low personnel flow	☐ ☐ ☐
d. Separate spaces for reporting results	☐
H 1.2 Applicable for culture in solid medium involving procedures of liquefaction of samples, concentration of bacilli and / or extraction of DNA from biological samples (Moderate risk laboratories)	
It is adequate when these tasks are performed in laboratories with: a. Restricted access b. Floors, walls, ceilings, furniture and chairs have waterproof surfaces c. Specific space for the biological safety cabinet	 ☐ ☐ ☐
d. With the autoclave located in the place or in a nearby laboratory or accessible by a circulation route used only by laboratory staff. (The material transferred to be sterilized have to be conditioned in closed containers and protected against falls and blows)	☐
H.1.3 Applicable for culture, DNA extraction and identification of species from isolates (High risk laboratories)	
It is appropriate when these tasks are performed in laboratories with identical conditions as the laboratories of moderate risk, to which the following requirements are added: a. Isolation You enter the laboratory through two doors of an antechamber or a small previous laboratory, which separates the culture/species identification laboratory from the public and other areas of the institution	☐
b. Autoclave located inside the laboratory or in an adjacent laboratory	☐
H.1.4 Applicable for laboratories that performs DNA amplification of and opens amplification products for molecular methods (open systems (LPA) or home-brew methods)	
It is adequate when these tasks are performed in: a. Three physically separated areas (amplification mix preparation, DNA loading and amplification products opening))	☐
b. The DNA loading area is far from the other two areas.	☐
c. The preparation of the amplification mixture area must be completely separate from the amplification opening area	☐
d. Separate spaces for the realization of reports	☐

H.1.5 Required for the preparation of solid and / or liquid culture medium and / or reagents	
It is adequate when these tasks are performed in areas: a. Own a TB lab or shared areas with general medium preparation b. Where they are considered pathogen-free, separated from sample handling areas c. With floors, walls, ceilings, furniture and chairs easy to clean. d. With anti-vibration counter for the installation of a precision scale	☐ ☐ ☐ ☐
e. With the exclusive autoclave for clean material located in the place or in another shared sterilization area	☐
H.2 Renovation, conditioning and addressing of laboratory air **H.2.1 Applicable to culture methods that do not require concentration (Ogawa-Kudoh method)**	
It is adequate when: a. The laboratory has windows to renew the air or an extractor of air that allows making 6 to 12 changes of the volume of air per hour, not being the air current directed to the countertop in which the samples are manipulated. b. If there is an air conditioner, the equipment does not generate air movement in the area of manipulation and culture of samples	☐ ☐
H.2.2 Applicable for culture in solid or liquid medium that involve procedures of liquefaction of the samples and concentration of the bacilli, extraction of DNA and / or species identification from positive cultures	
It is suitable when the area for processing samples for culture and / or extraction and loaded with DNA has a. Directed air (from clean areas taken by the Biological Safety Cabinet in operation and expelled by a duct that filters the air through HEPA before being expelled to the exterior), or there is another more complex system that accomplishes this requirement by ensuring at least 6-12 changes in laboratory air volume / hour. b. Safe air conditioning (air conditioner (s) are split type and do not generate air movements in front of the BSC)	☐ ☐
H.2.3 Applicable for molecular methods with open systems and / or home methods	
It is suitable when: a.The areas of preparing the amplification mixture and amplification / detection of products are separated with independent ventilation between them	☐
H.3 Connection system	
a. Internet access continuously b. Access to telephone continuously	☐ ☐

H.4 Air temperature conditioning system for the equipment **H.4.1 Applicable for culture in solid and / or liquid medium where methods of liquefaction of the sample and concentration of the bacilli by centrifugation are used**	
It is suitable when the maximum and minimum laboratory temperature is recorded daily and where: a. the culture equipment (MGIT 320/960) has an air conditioning system that allows to maintain the temperature between 19 and 30°C	☐
b. the refrigerated centrifuge for concentration of bacilli, has an air conditioning system that allows to maintain the temperature between 19 and 30°C	☐
c. the lab in which the biological safety cabinet is placed has a comfortable ambient temperature to work	☐
H.4.2 Required for the preparation of solid and / or liquid culture medium	
It is suitable when the area keeps the right temperature to work comfortably a. With the presence of incubators with forced ventilation that ensures uniform temperature inside and / or coagulators for the solidification of egg-based medium and thermostatic baths.	☐
H.5 Lighting and general conditions of the work area	
a. The adequate lighting (it is considered 500 LUX without emission of re fl exes or brightness, which is equivalent to 50 Watt of a fluorescent lamp for 5 m^2)	☐
b. Walls and ceilings are painted, clean and without humidity	☐
c. All work areas are clean, there is a daily cleaning service	☐
Comments	

I. Handling and transporting the sputum sample and/or isolates	
I.1 Collection of the sample **(Check the samples received during the day, see if the volume is adequate, if there are spills, if they are well conditioned, etc.) (Ignore if it has already been completed in Annex A.1)**	
a. Type of container used for sputum collection. Does it comply with the standardized technical specifications?	YES ☐ NO ☐ Comments ☐
b. Is the containers label with the patient's identification on the side of the bottle and not on the lid?	YES ☐ NO ☐ Comments ☐
I.2 Collection of isolates (Verify which isolates are derived, if they are closed, if they are well labeled and conditioned)	
a. The tube is tightly closed; it has a screw cap or a cotton cap and a rubber stopper. In addition, it is sealed with parafilm.	YES ☐ NO ☐ Comments ☐
b. The label of the tubes is legible with the patient identification, the number of the laboratory of origin.	YES ☐ NO ☐ Comments ☐
I.3 Conservation and transport of the sample **(Ignore if it has already been completed in Annex A.1)**	
a. Are the containers well conditioned with the samples that the laboratory receives, fulfilling the local standards for the shipment of samples? (Ex. transported in a strong, unbreakable and closed container, labeled with the international biohazard symbol)	YES ☐ NO ☐ Comments ☐
b. If they are referred from other centers, do they arrive at the laboratory within 24 hours of the sample collection?	YES ☐ NO ☐ Comments ☐
c. Are the samples stored in a cool place, preferably in the refrigerator, until they are processed?	YES ☐ NO ☐ Comments ☐
d. Are the samples stored in a refrigerator other than the one where the reagents are stored (or at least on an exclusive shelf)?	YES ☐ NO ☐ Comments ☐
e. Is there a regular transport system to transfer samples to the laboratory that culture them?	YES ☐ NO ☐ Comments ☐
I.4 Isolates conservation and transport	
a. Are the isolates received by the laboratory properly conditioned, complying with local regulations for the shipment of isolates? (Ex. transported in a triple container labeled with the international biohazard symbol, with the culture tube fitted with absorbent and / or anti-shock material)	YES ☐ NO ☐ Comments ☐

b. Are the boxes with the isolates opened in a biological safety cabinet in case there is a broken tube?	YES ☐ NO ☐ Comments ☐
c. Is the outside part of the triple container boxes decontaminated with alcohol 70%? Is the primary container autoclaved before being recycled?	YES ☐ NO ☐ Comments ☐
d. Is there a regular transport system to transfer isolates to the reference laboratory that processes them for more complex tests?	YES ☐ NO ☐ Comments ☐
Comments	
J. Practice audit	
J.1 Preparation of reagents, egg-based solid/liquid media and conservation Ask, if possible, the technician to perform the operation. Observe the process, from the weighing of the reagents to the coagulation of the medium in the case of egg-based media. In case you do not have enough time, ask at least these questions about critical points	
a. Are the batches of the chemical compounds used for each one of the different reagents elaborated in the lab registered?	YES ☐ NO ☐ Comments ☐
b. Are the time and temperature of coagulation of the medium recorded? (Consider adequate if the coagulation is performed for 45 minutes at 80-85°C)	YES ☐ NO ☐ Comments ☐
c. Verify the absence of abundant bubbles in the solid media. (Consider that the presence of them is an indicator of overheating)	YES ☐ NO ☐ Comments ☐
d. Check color homogeneity or absence of lumps of malachite green (Consider that the presence of green dots indicates a poor homogenization of the medium)	YES ☐ NO ☐ Comments ☐

e. Are sterile controls of the adequately performed? (Consider adequate if after coagulation, a sample of tubes is incubated at 35-37°C for 24 hours and then at room temperature for 48 hours)	Yes ☐ every time a new batch is made ☐ Never ☐ Comments ☐
f. Is the time of employment of the medium from the date of preparation recorded?(Consider adequate if used up to 2 months after its preparation)	YES ☐ NO ☐ Comments ☐
g. Are the tubes with media well preserved? (Consider placing them in a clean and frequently disinfected refrigerator, inside plastic boxes with the lid, each of the tubes hermetically closed. Do not introduce cardboard boxes because of the possibility of fungi formation).	YES ☐ NO ☐ Comments ☐
Comments	

J. Practice audit	
J.2 Culture procedure Ask, if possible, the technician to perform the full processing of the culture method you are using. Observe the process	
a. Is the digestion / decontamination procedure established normatively for each type of sample used? (Sputum, LBA, LB, LCR or biopsies)	YES ☐ NO ☐ Comments ☐
b. Is the recommended order maintained for sample processing, handling smear-positive samples at the end of each round of cultures?	YES ☐ NO ☐ Comments ☐

c. Does the contact time between the decontaminant and the sample correspond to the standard and is it measured with a stopwatch. (Consider adequate if this time of contact does not exceed 30 minutes in the Petroff method and 2 minutes in the Ogawa-Kudoh method)	YES ☐ NO ☐ Comments ☐
d. Is the sample mixture stirred at least twice with the decontaminant reagent in the Petroff method during the incubation time?	YES ☐ NO ☐ Comments ☐
e. How many samples do you process per culture round? (Consider adequate if up to 12 samples are processed per serie)	YES ☐ NO ☐ Comments ☐
f. Do you control that the speed of the centrifuge is that suggested by the norms? (Consider adequate that at least reach 3000g)	YES ☐ NO ☐ Comments ☐
g. Is the temperature reached by the centrifuge during its operation control? (Consider adequate if it does not exceed 35ºC)	YES ☐ NO ☐ Comments ☐
h. Do you control the amount of decontaminated sample added to the culture media? (Consider adequate if 0.2-0.5 ml of digested sample is added per tube with solid medium and no more than 0.5 ml in the case of MGIT tubes)	YES ☐ NO ☐ Comments ☐
i. Are the inoculated tubes inspected 48 hs after their inoculation, in order to promptly verify the contamination of the cultures?	YES ☐ NO ☐ Comments ☐
j. How often do you review the culture tubes to detect growth?	Weekly ☐ Biweekly ☐ Monthly ☐ Comments ☐
k. When a suspected positive culture is identified Do you prepare ZN smears before reporting a positive culture result?	YES ☐ NO ☐ Comments ☐
l. In order to report a positive culture Do you performed the species identification at least at the MTBC level?	YES ☐ NO ☐ Comments ☐
Comments	

J. Practice audit	
J.3 Identification procedure of MTBC by lateral immunochromatography Ask, if possible, the technician to carry out the complete processing of the test. Observe the process	
a. Is protein extraction performed in water or buffer?	Water ☐ Buffer ☐ Comments ☐
b. Are filter tips used to add the supernatant to the cassette?	YES ☐ NO ☐ Comments ☐
c. Is the procedure performed following the steps established in the insert?	YES ☐ NO ☐ Comments ☐
d. Do the technicians await the time recommended in the insert to consider an isolate as negative for MTBC?	YES ☐ NO ☐ Comments ☐
e. Is the reading of the bands done carefully by the technicians?	YES ☐ NO ☐ Comments ☐
f. How often are the internal quality controls carried out? Consider adequate when such controls are carried out: - With each new batch of kits and with each new batch of extraction buffer. - Weekly, or with each batch of patient tests, if tests are performed less frequently.	Each run ☐ Every time the batch changes ☐ Once a month ☐ Comments ☐
g. What do you use as a negative and positive control?	Negative control Water ☐ Non-tuberculosis mycobacterium ☐ Positive control Strain H37Rv ☐ MTBC isolate ☐
Comments	

J. Practice audit			
J.4 Test procedure with line probe assays for MTBC identification (Genotype MTBDR) Ask, if possible, the technician to carry out the complete processing of the test. Observe the process			
a. Is DNA extraction done from isolates by heating at 100°C?	YES ☐	NO ☐	Comments ☐
b. Is the temperature of the bath or twincubator the one established in the insert?	YES ☐	NO ☐	Comments ☐
c. Is it expected that the hybridization and washing buffers are at the temperature recommended by the manufacturer and are homogenized before use? (It is considered adequate if hybridization buffers and wash buffers (STR buffer) were preheated to 37°C-45°C, while the rest of the solutions reached room temperature before use)	YES ☐	NO ☐	Comments ☐
d. Are you careful in dispensing the preheated hybridization buffer to avoid splashing into neighboring channels?	YES ☐	NO ☐	Comments ☐
e. Is the reading of the strips carefully done by the technicians?	YES ☐	NO ☐	Comments ☐
f. Are the results analyzed by evaluating the patient's clinical and epidemiological data before reporting?	YES ☐	NO ☐	Comments ☐
g. Are invalid and indeterminate results analyzed to try to decipher the causes of these results?	YES ☐	NO ☐	Comments ☐
h. Are invalid or indeterminate results repeated?	YES ☐	NO ☐	Comments ☐
i. Are reports made according to the rules?	YES ☐	NO ☐	Comments ☐
Comments			

K. Safety in the laboratory **Applicable for culture and identification of *M. tuberculosis* Complex**	
K.1 Safety practices	
a. Laboratory biosafety manual	Available: YES ☐ NO ☐ Comments ☐
b. Use of recommended disinfectants for tuberculosis (5% phenol, 1% sodium hypochlorite, 70% alcohol).	Available: YES ☐ NO ☐ Comments ☐
c. Cleaning at least once before beginning and at the end of each working day.	YES ☐ NO ☐ Comments ☐
d. Use of gloves according to the general biosafety work standards of the laboratories.	YES ☐ NO ☐ Comments ☐
e. Use of respirators (type N95 or FFP2).	YES ☐ NO ☐ Comments ☐
f. Availability of respirators (type N95 or FFP2), (when they are not used in the work routine), for their use in case of spills.	YES ☐ NO ☐ Comments ☐
g. Form of availability and use of personal protection elements.	Available: YES ☐ NO ☐ Comments ☐
h. Disposal of pathological waste and all the elements used for the different methodologies by recommended methods (autoclaving or treatment with sodium hypochlorite before disposal with the rest of the pathological waste of the institution or, discarding of solutions in special containers for liquid waste). The material transferred for autoclaving must be transported in secure containers.	YES ☐ NO ☐ Comments ☐
i. Disposal and transport of material (potentially infectious) in secure containers that resist autoclaving and autoclaving of contaminated material daily for 1 hour at 121ºC.	YES ☐ NO ☐ Comments ☐
j. Chemical indicators for heat sterilization (autoclaving)	Available: YES ☐ NO ☐ Comments ☐ Frequency of use.....
k. Biological indicators for heat styling (autoclaving)	Available: YES ☐ NO ☐ Comments ☐ Frequency of use.....

l. Kit in case of spills containing: an autoclavable bag, gloves, gowns, appropriate disinfectants, N95 or FFP2 respirators, cotton and adsorbent paper, soap, stick to collect waste, sharps container, DO NOT ENTER sign)	Available: YES ☐ NO ☐ Comments ☐
m. Written rule for the management of biohazard waste and regulated chemical waste	Available: YES ☐ NO ☐ Comments ☐
K.2 Staff security (Ignore if it has already been completed in Annex A.1)	
a. Regular annual program of medical control for health workers, following the labor regulations in the country (If there is no adopted policy, the supervisor must ensure that laboratory personnel have at least one annual medical evaluation that may include a chest x-ray).	Available: YES ☐ NO ☐ Comments ☐
b. Known and written instructions for accidents or incidents (may be included in the laboratory's Biosafety manual / SOP)	Available: YES ☐ NO ☐ Comments ☐
c. Initial safety / biosafety training program with records of the laboratory personnel participation	Available: YES ☐ NO ☐ Comments ☐
Comments	

L. Derivation of samples and / or isolates	
L.1 Applicable for culture and identification of M. tuberculosis Complex Depending on the diagnostic algorithm established in each country, the same patient could be studied by one or more of these techniques	
a. Are all the samples indicated by the standards derived for MTBC identification or drug susceptibility testing? The following isolates are considered suitable for derivation - positive cultures with cultural characteristics compatible with MTB or already identified as MTBC of patients with: • history of previous treatment (relapses, failures, loss in follow-up) • history of contacts with patients with multidrug-resistant or extensively resistant TB • smear positive at the end of the second month of treatment or in a subsequent control • cases diagnosed with negative sputum smear microscopy and that have a smear microscopy positive during treatment • immunosuppression, particularly positive HIV and diabetic patients • with exposure to infection by drug-resistant bacilli (internees or workers from health institutions or prisons where there are cases of resistant tuberculosis) • previous residence in countries with a high level of drug resistance (Ecuador, Peru, some Asian and Eastern European countries). • addiction to alcohol and/or other drugs • age under 15 years (children) • drug intolerance	YES ☐ NO ☐ Comments ☐
b. When a patient is identified by molecular methods as having a TB resistant to isoniazid and/or rifampicin, are samples derived to the NRL in order to confirm the specie identification and/or isoniazid/rifampicin resistance and to perform a drug susceptibility testing to the rest of the first and second line drugs?	YES ☐ NO ☐ Comments ☐

c. Is there a regular transport system to transfer the isolates to the laboratory that performs species identification and drug susceptibility testing?	YES ☐ NO ☐ Comments ☐
d. Laboratory to which samples/isolates are derived for species identification and / or drug susceptibility testing.	.....
e. What is the average time of results reception from the derivative studies?	Culture and identification ..... days Xpert MTB/Rif ..... days LPAs ..... days Drug susceptibility testing (phenotypic methods) to Rifampicin .....days to Isoniazid .....days to second-line drugs .....days
Comments	
M. Other observations	
Laboratory staff expressed the following concerns regarding their reference laboratory	

Conclusions
The following strengths are highlighted ....
It highlights the following challenges or corrective measures to be implemented as a priority By the authorities ....
By the laboratory staff ....
The following training needs were identified ....
Agreements reached in relation to the challenges ....
Name and signature of the supervisor/s: ..... Name and signature of the laboratory manager: ..... Date: ..... /...../.....

Annex A.3: Technical visit guide to laboratories performing drug susceptibility testing to first and second line antituberculosis drug

This guide has been developed to support the work of the supervisors during the technical visit to the laboratories that perform these methods. If the laboratory performs the detection of resistance to Rifampicin and/or isoniazid by molecular methods with closed system (Xpert MTB/Rif or Xpert MTB Ultra/Rif) or open methods (LPA), refer to the questions presented in Annex A.1 and A.2. Remember that in the case of having completed Annex A.1 and/or A.2, common questions to several methodologies must be ignored; they have been identified with an asterisk, so that they can be easily recognized in the document.

The contents of the questions cover some points of quality management in such a way that laboratories incorporate these practices in their work routine.

Table of Contents

Summary of the visited laboratory

Visited laboratory: .....

Locality: .....

Province / State / Department: .....

Responsible of the lab: .....

Date of the visit: ..... / ..... / .....

Date of previous visit: ..... / ..... / .....

Staff dedicated to the diagnosis of tuberculosis

	Number	Daily hours / person dedicated to the diagnosis of tuberculosis
Professionals		
Technicians		
Technical assistants		
Administrative		

Name of professionals / technicians interviewed: .....
.....
.....

Training in TB diagnosis received in the last 3 years .....
.....
.....

Comments:

A. Laboratory activities

Next, mark the cells that correspond to the activities developed in your laboratory. Where applicable, complete the requested information		
a. Drug susceptibility testing on solid medium to detect resistance to isoniazid and rifampicin	Estimated number of tests per month ___	☐
b. Drug susceptibility testing in liquid medium to detect resistance to isoniazid and rifampicin	Estimated number of tests per month ___	☐ ☐
c. Drug susceptibility testing in solid medium to detect resistance to	Estimated number of tests per month ___	☐
Ethambutol	per month ___	☐
Amikacin	per month ___	☐
Kanamycin	per month ___	☐
Capreomycin	per month ___	☐
Levofloxacin	per month ___	☐
Moxifloxacin	per month ___	☐
Others.....	per month ___	☐
d. Drug susceptibility testing in liquid medium to detect resistance to	Estimated number of tests per month ___	☐
Ethambutol	per month ___	☐
Amikacin	per month ___	☐
Kanamycin	per month ___	☐
Capreomycin	per month ___	☐
Levofloxacin	per month ___	☐
Moxifloxacin	per month ___	☐
Others.....	per month ___	☐
e. Test for susceptibility to pyrazinamide by Wayne's method	Estimated number of tests per month ___	☐
f. Test for susceptibility to pyrazinamide by the liquid method	Estimated number of tests per month ___	☐
g. Preparation of culture medium with drugs	Estimated number of tubes per month ___	☐

h. Others	Estimated number of tests per month ___	☐
i. Do you receive samples or isolates derived from other health centers?	Estimated number of samples per month ___ Isolations per month _____	☐ ☐
j. Do you derive isolates to reference laboratories for more complex tests? If yes, mention to which service the referral is made).....	Estimated number of isolates per month ___	☐
k. The laboratory possesses a technical process in accreditation stage accredited	YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐	
Comments:		

B. Documents for the standardization of procedures
Enforceable for drug susceptibility testing to first and second line anti-tuberculosis drugs

B.1 Organization chart and technical standards	
a. * Organizational chart including all laboratory personnel involved in the diagnostic activities of resistant TB	Available: YES ☐ NO ☐ Comments ☐
b. * Description of responsibilities of each worker and replacement systems.	Available: YES ☐ NO ☐ Comments ☐
c. Manual of procedures for all techniques performed at the lab	Available: YES ☐ NO ☐ Comments ☐
d. * Work Algorithm / s d.1 From the NTP d.2 Internal of the laboratory	Available: YES ☐ NO ☐ Comments ☐

B.2 Standard operating procedures (SOPs) Availability of SOPs (that consider the technique, elimination of pathological and chemical residues, maintenance of the necessary equipment, internal quality controls and external quality evaluation):	
a. Preparation of medium and/or reagents	Available in the work area: YES ☐ NO ☐ Incomplete ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐
b. Drug susceptibility testing for first-line anituberculosis (Rifampicin and/or isoniazid) Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐
c. Drug susceptibility testing for second-line anituberculosis drugs (ethambutol, injectables and/or quinolones) Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐
d. Test for susceptibility to pyrazinamide Method.....	Available in the work area: YES ☐ NO ☐ No applicable ☐ Comments ☐ Updated version: YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐ YES ☐ NO ☐ Incomplete ☐ Comments ☐
e. * Are the Sops reviewed periodically and corrected appropriately when necessary?	YES ☐ NO ☐ Comments ☐
f. * Are the versions of SOPs that were replaced removed and filed?	YES ☐ NO ☐ Comments ☐
g. * Is all staff informed about the contents of the Laboratory's SOPs?	YES ☐ NO ☐ Comments ☐

h. * Is there a SOP/Laboratory Biosafety Manual?	YES ☐ NO ☐ Comments ☐
Comments	
C. Quality assessment **Required for drug susceptibility testing to first and second line antituberculosis drugs**	
C.1 Validation / verification studies	
a. Was the validation performed (non-standardized methods, methods designed or developed by the laboratory, standardized methods used outside its intended field of application or validated methods subsequently modified) before starting its use for routine diagnosis? (specify validated/verified techniques and parameters (sensitivity (S), specificity (Esp), efficiency (Efi), reproducibility (R)	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
b. Was verification carried out (validated commercial methods used without modification) before starting its use for routine diagnosis? (specify validated/verified techniques and parameters (sensitivity (S), specificity (Spe), efficiency (Efi), reproducibility (R)	YES ☐ NO ☐ Comments ☐ Techniques and parameters ....
c. Are there records that document these validations or verifications?	YES ☐ NO ☐ Comments ☐
C.2 External quality assessment (EQA)	
For drug susceptibility testings to first and second line antituberculosis drugs	
a. Does the laboratory participate in tests of external competence?	YES ☐ NO ☐ Comments ☐
b. Are the tests corresponding to the EQA performed by the laboratory technicians that carry out them in the routine?	YES ☐ NO ☐ Comments ☐

c. Do the results of the last two years show acceptable quality?	YES ☐ NO ☐ Comments ☐
d. In the results of the last two years is there any kind of deviation or poor quality?	YES ☐ NO ☐ Comments ☐ Indicate the type of deviation .....
e. Are the results of the EQA disseminated among the laboratory staff?	YES ☐ NO ☐ Comments ☐
f. Is there documented evidence of dissemination?	YES ☐ NO ☐ Comments ☐
Comments	
D. Handling of laboratory data (Forms, instructions, records and reports) **Required for drug susceptibility testing to first and second line antituberculosis drugs**	
D.1 Forms: Request for bacteriological and/or molecular studies Select 20 forms, review them and answer the following items:	
a. * Does the laboratory receive the forms according to the NTP norms?	YES ☐ NO ☐ Comments ☐
b. * Has It an adequate design?	YES ☐ NO ☐ Comments ☐
c. * Usually, are they received complete? (at least 80% of data are included in the forms)	YES ☐ NO ☐ Comments ☐
d. * Are they physically filed?	YES ☐ NO ☐ Comments ☐
e. * Are they scanned and archived on digital format?	YES ☐ NO ☐ Comments ☐
D.2 Data record - Laboratory information system (LIS) Skip if answered in Annex A.1 or A.2	
a. * Has the LIS basic information required by the NTP norms?	YES ☐ NO ☐ Comments ☐

b. * If paper records are used	
b.1 Are they foliated?	YES ☐ NO ☐ Comments ☐
b.2 Is it complete with the samples/isolations received, at least from the previous day?	YES ☐ NO ☐ Comments ☐
c. * If digital records are used	
c.1 Is there an exclusive system for TB?	YES ☐ NO ☐ Comments ☐
c.2 Is it complete with the samples and / or cultures or isolates received, at least from the previous day?	YES ☐ NO ☐ Comments ☐
c.3 Is there a backup system for the LIS in digital support? (describe in comments how the backup is done)	YES ☐ NO ☐ Comments ☐
d. * Does each sample and/or culture or isolate maintain a unique number for all the procedures performed with it?	YES ☐ NO ☐ Comments ☐
e. * Is the number of records rational and does it not generate unnecessary workload?	YES ☐ NO ☐ Comments ☐
f. * Is access to the LIS limited to authorized personnel?	YES ☐ NO ☐ Comments ☐
g. * Is there a written SOP for the use of LIS?	YES ☐ NO ☐ Comments ☐
Record for derivation of samples and/or isolates a. * Has it an adequate design?	YES ☐ NO ☐ Comments ☐
b. * Is it complete with the necessary information and is it legible?	YES ☐ NO ☐ Comments ☐
D.3 Records related to Biosafety	
a. * Is there an adequate accident/incident record?	YES ☐ NO ☐ Comments ☐
b. * Is there a record of the delivery of personal protection items for each operator?	YES ☐ NO ☐ Comments ☐
c. * Is there a record of the staff annual medical control?	YES ☐ NO ☐ Comments ☐
D.4 Reports Select 20 reports, review them and respond	
a. * Do the laboratory reports identify the laboratory that performs the tests?	YES ☐ NO ☐ Comments ☐
b. * Do the laboratory reports identify the methods used?	YES ☐ NO ☐ Comments ☐
c. * Have you designated certain people to issue results reports?	YES ☐ NO ☐ Comments ☐
d. * Are the reports verified by a second staff?	YES ☐ NO ☐ Comments ☐

e. * Does the laboratory archive the results data (printed results, electronic records)? If yes, explain how they are archived and for how long.	YES ☐ NO ☐ Comments ☐ They are archived..... ..... Time.....
f. * Are the archived reports only accessible to authorized personnel?	YES ☐ NO ☐ Comments ☐
g. Estimation of the time delay from the DST realization to the report release Take 20 results produced during the last month	Drug susceptibility testing (Analysis of 20 DST results) 1. Reports of results For DST in Löwenstein Jensen N° of reports analyzed ..... Within 42 days N°..... For DST in Middlebrook agar, N° analyzed reports ..... Within 23 days N°..... For DST in MGIT N° analyzed reports ..... Within 16 days N°..... It is considered appropriate when at least 95% of the reports were issued within the deadlines established above. Comments ☐

h. Estimation of the time from receipt of the sample or isolate/ positivization of the culture to the report release Take 20 results produced during the last month	1- Molecular methods report 1.1- rifampicin resistance detection by closed system report (see Annex A.1-D4-G) 1.2- isoniazid and Rifampicin resistance detection by open system report (see Annex A.2-D4-G) 1.3-Report on injectables and quinolones resistance detection by open system (LPA) (*) Less than 48 hours ☐ Between 48 and 96 hours ☐ More than 96 hours ☐ (*)To calculate the response time, the time at which the resistance to rifampicin and/ or isoniazid was detected by any genotypic method is taken as time 0. Comments ☐
i. Do the reports contain an item of observations specifying (in cases of rifampicin and / or isoniazid resistance detection) at least that the samples and / or isolates were derived to other laboratories for the study of their susceptibility to second-line drugs?	YES ☐ NO ☐ Comments ☐
Comments	

E. Performance Indicators Monitoring Record the following parameters for the total of tests performed during the last three months or, if the workload is high, during the last month prior to the visit **For the case of detection of resistance to rifampicin by closed systems complete Annex A.1 and for the detection of isoniazid and Rifampicin resistance by open systems complete Annex A-2**	
E.1 Required for drug susceptibility testings to first and second line antituberculosis drugs	
a. Tests carried out	Number..... Comments ☐
b. Number and proportion of samples and/or isolates that resulted with Resistance to rifampicin Multidrug resistance (MDR) MDR plus resistance to quinolones MDR more resistance to one second line injectable drug Extensively resistant TB (MDR, plus resistance to one second-line injectable drug and quinolones)	 Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐
c. Number and proportion of patients with samples and/or isolates that resulted in Mono-resistance to rifampicin Multidrug resistance (MDR) MDR plus resistance to quinolones MDR plus resistance to one second line injectable drug Extensively resistant TB (MDR, more resistance to one second line injectable drug and quinolones)	 Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐ Number..... %..... Comments ☐
d. Does the lab analyze this information periodically?	YES ☐ NO ☐ Comments ☐
e. Does the lab send this information to the reference laboratory?	YES ☐ NO ☐ Comments ☐
f. If the answer is **Yes**, How often?	Time..... Comments ☐
g. Are the cases identified as multidrug resistant, resistant to Rifampicin, but not to H and extensively resistant TB, reported immediately to the NTP?	YES ☐ NO ☐ Comments ☐
E.2 Required for drug susceptibility testing to first and second line antituberculosis drugs by phenotypic methods	
a. Number and proportion of isolates that were contaminated by phenotypic methods. It is considered adequate when this value does not exceed 3%	Number..... %..... Comments ☐

b. Number and proportion of isolates that resulted with non-interpretable results (insufficient growth in the controls) or invalid (lack of correlation in the number of colonies between the dilutions used) by phenotypic methods. It is considered adequate when this value does not exceed 3%	Number..... %..... Comments ☐
E.3 Enforceable for drug susceptibility testings to second-line anti-tuberculosis drugs by molecular methods	
a. Tests carried out	Number..... Comments ☐
b. Number and proportion of samples and/or isolates with non-interpretable results (invalid and indeterminate) by open molecular system (LPA). It is considered adequate when this value does not exceed 5%	Number..... %..... Comments ☐
c. Tests with MTB detected result and only resistance to injectables detected	Number..... Comments ☐
d. Tests with MTB detected result and only resistance to quinolones detected	Number..... Comments ☐
e. Tests with MTB detected result and extensively drug resistant TB	Number..... Comments ☐
Comments	

F. Supplies and conservation of material for the realization of the different techniques **For the case of closed detection methods, respond in Annex A.1**				
F.1 Supplies **It is considered that the supply is adequate when there is current availability and there has been no fault during the last 6 months**				
F.1.1 Common to medium preparation and realization for drug susceptibility testings				
	Available		Proper supply	
	YES	NO	YES	NO
a. Antibiotics				
b. *eppendorf* tubes of 2 ml for the conservation of stock of drugs				
c. * Tubes or bottles for the preparation of culture medium with drugs and for the distribution of water for dilutions				
d. * Markers				
e. * Distilled water				
f. * Racks, baskets and trays				
g. * Containers for autoclaving material				
h. * Personal protection items Gloves..... Masks N 95/100..... Tyvec or waterproof gown.....				
F.1.2 For preparation of medium and / or reagents for different methodologies				
a. * Glass material for measurement and storage (Erlenmeyer, test tubes, beaker, tubes)				
b. Disposable material for the distribution of medium (Petri dishes)				
c. * Manual or automatic systems for dispensing medium				
d. * Disposable 10 ml pipettes				
e. * Strips to measure PH in different ranges				
F.1.3 Applicable for phenotypic methods				
a. Solution Turbidity standard Mc Farland 1 or BCG 1mg / ml				
b. Bottles of 5 to 10 ml with 5 glass beads and screw cap				
c. * Disposable Pasteur pipettes				

d. * Sterile disposable bacteriological loops				
e. * Disposable pipettes of 2 ml				
f. * Pipetting devices (rubber swabs, rubber or electric proppets)				
g. * Containers for autoclaving and discarding of material (pipettes and tips with protection)				
h. * Containers for autoclaving and recycling of material (tubes and jars)				
i. * Plastic bags for incubation of inoculated plates (proportions method in Middelbrook 7H10 medium)				
j. Reagents for the detection of viability or compounds of bacillus activity (nitrate reductase method (Griess) or Wayne test)				
k. *Tubes, reagents and tube holders for the use of MGIT				
F.1.4 Enforceable for drug susceptibility testing to second-line drugs by open molecular methods. Skip if you have answered Annex A.2 F 1.5.				
a. * Extraction equipment				
b. LPA equipment				
c. Reagents for the amplification mixture (buffers, taq polymerase, nucleotides, primers)				
d. * Distilled water Milli-Q or double distilled (quality molecular biology)				
e. * Ultra thin wall tubes of 0.2 ml				
f.* eppendorf tubes de 1,5 ml				
g. Disposable tips with aerosol protection of 20 µl, 100 µl and 1000 µl				
F.2 Supplies Conservation				
Applicable for phenotypic drug susceptibility testing				

	Available	
	YES	NO
a. Pure antibiotics stored at the temperature established by the manufacturer and within the expiration date - Room temperature - 4°C - -20°C		

b. Diluted antibiotics stock solutions to -20°C		
c. Reagents In clean containers protected from light, Correctly identified		
b. Distilled water in clean containers		
e. Reagents for tests in MGIT		
Required for drug susceptibility testing to second line drugs by open molecular methods (LPA) *To avoid having completed item F 2 of Annex A.2.*		
a. * The reagents for the identification of the amplicons by reverse hybridization are conserved at a controlled temperature between 2 and 8°C in an area where tubes with amplicons are opened.		
b. * Reagents for the amplification reaction of nucleic acids or those of the mixture of the home-brew methods are stored at a controlled temperature between -20°C in the mixing preparation area		
c. * Ultra pure water Milli-Q in aliquots		
d. * Reagents for electrophoretic run and for the loading and identification of amplicons are stored at room temperature in an area where tubes with amplicons are opened		
Comments		

G. Equipment Verify the following as applicable to the equipment used for specific laboratory activities, indicating Yes or No, as appropriate (List the manufacturer, model and date of installation in the preventive maintenance register) ***Ignore those items that have been completed in Annex A.1 and A.2***		
Required for drug susceptibility testing to first and second line antituberculosis drugs		
	YES	NO
G.1 Refrigerator		
a. * Preventive maintenance activities are carried out and documented (cleaning and disinfection).	☐ Frequency.....	☐
b. * Are temperature readings taken and documented?	☐ Frequency.....	☐
c. * Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. * Is there documentation of corrective actions made in response to values out of range?	☐	☐
G.2 Freezer		
a. * Preventive maintenance activities are carried out and documented.	☐ Frequency.....	☐
b. * Are temperature readings taken and documented?	☐ Frequency.....	☐
c. * Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. * Is there documentation of corrective actions made in response to values out of range?	☐	☐
G.3 Autoclave		
a. * Are annual check-up carried out to verify the complete sterilization of autoclaved materials, hydraulic testing and valve verification?	☐	☐
b. * Are daily sterilization cycles recorded?	☐	☐
c. * Are physical-chemical strips used as a sterile control in each carried out cycle?	☐	☐
d. * Are biological controls used weekly or monthly as sterile control?	☐ Frequency of use:	☐

Required for preparation of medium with drugs for phenotypic methods **G.4 Egg-based medium coagulation oven or coagulator**		
a. * It works constantly at 80-85ºC, with a water bath system around each shelf or forced ventilation that ensures uniform temperature	☐	☐
b. * Perform and document preventive maintenance activities/services.	☐ Frequency.....	☐
c. * Are there records of temperature control of the coagulation process?	☐ Frequency.....	☐
G.5 Balance		
a. * Are preventive maintenance activities/ services performed and documented daily and annually?	☐	☐
b. * Are calibration procedures performed as described by the manufacturer?	☐	☐
c. * Do calibration records exist and are periodically checked?	☐ Frequency.....	☐
G.6 Thermostatic bath		
a. * Has it an electronic temperature control?	☐	☐
b. * Are preventive maintenance activities/ services performed and documented?	☐	☐
c. * Are there records of temperature control of each process?	☐ Frequency.....	☐
Applicable for phenotypic tests **G.7 Automatic pipettes**		
a. * Are preventive maintenance activities/ services performed and documented annually/biannually?	☐	☐
b. * Are calibration procedures performed as described by the manufacturer?	☐	☐
c. * Do calibration records exist and are checked?	☐ Frequency.....	☐
G.8 Vortex		
a. * Are preventive maintenance activities/ services performed and documented annually?	☐	☐

G.9 Biological safety cabinet		
a. * Is it one of these models? Class I (EN12469 / NSF49) Class IIA2 (NSF49) o Class II (EN12469)	Model..... ☐ ☐	 ☐ ☐
b. * Does it have an outside duct?	☐	☐
c. * Is it certified at least annually? (verify certificates)	☐	☐
d. * Does the equipment have an uninterruptible power supply (UPS) system?	☐	☐
e. * Are preventive maintenance activities/ services performed and documented annually?	☐	☐
f. * Do the laboratory technicians perform and document cleaning and operation daily?	☐	☐
G.10 Centrifuge		
a. * It is refrigerated with a temperature range between 4 and 12 ° C and reaches a speed of at least 3000 g?	☐	☐
b. * Has it tube holders covered with a lid and are they autoclavable?	☐	☐
c. * Is there a record of use and temperature of each run?	☐	☐
d. * Has it an uninterruptible power supply (UPS)?	☐	☐
e.* Do the laboratory technicians perform and document the daily use of the equipment and annually the preventive maintenance activities/ services?	☐	☐
G.11 Culture camera or Incubators		
a. * Has it enough space for the workload handled by the laboratory?	☐	☐
b. * Has it minimum and maximum temperature control with a device that detects variations of ± 1ºC?	☐ Frequency.....	☐
c. * Have tolerance limits been established and documented for temperature readings?	☐ Rank.....	☐
d. * Are there temperature records and are corrective measures applied when the temperature parameters are out of range?	☐	☐
e. *Do you have enough space for the workload handled by the laboratory?	☐	☐

G.12 Bactec 320/960 equipment		
a. * Has It a computer associated with installation of the latest software?	☐	☐
b. * Do the computer and the equipment count with an uninterruptible power supply (UPS))?	☐	☐
c. * Are calibration procedures carried out as described by the manufacturer?	☐	☐
d. * Is the equipment maintenance carried out and registered in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
e. * Are there calibration and maintenance records? Do they review periodically?	☐ Frequency.....	☐
G.13 Chronometer		
a. * Are calibration procedures carried out as described by the manufacturer?	☐	☐
b. * Are there recalibration records? Are they periodically reviewed?	☐	☐
Required for drug susceptibility testing to second line drugs by open molecular methods *(LPA). Ignore if item G of Annex A.2 was completed* **G.14 Thermal cycler**		
a. * Is the equipment maintenance performed and recorded in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
b. * Are the procedures for temperature verification of the thermal block carried out by external probes as described by the manufacturer?	☐	☐
c. * Are there calibration records and are they reviewed periodically?	☐ Frequency.....	☐
G.15 Twincubator		
a. * Is the equipment maintenance performed and recorded in the periods established by the manufacturer (daily, weekly, monthly)?	☐	☐
G.16 Microcentrifuge (for the case of performing the molecular test from isolates)		
a. * Are the preventive maintenance activities/ services performed annually? Is the daily use of the equipment documented?	☐	☐
b. * It is refrigerated?	☐	☐

Comments	
H. Characteristics of the laboratory (Basic infrastructure, biosafety, location of tasks according to the level of biological risk) Mark the cells that correspond to the characteristics of your laboratory) Ignore if you have completed the points in Annex A.2 for high-risk laboratories (which manipulate isolates for the realization of species identification or molecular tests) and open molecular systems	
H.1 Place where the isolates are processed for drug susceptibility testing to first and second line antituberculosis drugs	
H.1.1 *Required for phenotypic drug susceptibility testing and DNA extraction from isolates (High risk laboratories)	
It is appropriate when these tasks are performed in laboratories with identical conditions as the laboratories of moderate risk (see requirements described in item H.1.2 of Annex A.2), to which the following requirements are added: a. Isolation You enter the laboratory through two doors of an antechamber or a small previous laboratory, which separates the culture laboratory from the public area and other areas of the institution	☐
b. autoclave located inside the laboratory or in an adjacent laboratory	☐
H 1.2 *Required for laboratories that perform DNA amplification and open of amplification products for molecular methods (open systems (LPA)	
It is adequate when these tasks are performed in: a. Three physically separated areas (amplification mix preparation, DNA loading and amplification products opening)	☐
b. The DNA loading area is far from the other two areas.	☐
c. The preparation of the amplification mixture area must be completely separated from the amplification opening area	☐
d. Separate spaces for the realization of reports	☐

H.1.3 *Required for the preparation of solid and/or liquid culture medium and/or reagents	
It is adequate when these tasks are performed in areas: a. of a TB laboratory or shared areas with the preparation of general medium b. considered clean of pathogens, separated from areas of samples handling c. with floors, walls, ceilings, furniture and chairs with easy cleaning surfaces. d. with anti-vibration counter for the installation of precision scales	☐ ☐ ☐ ☐
e. with the exclusive autoclave for clean material located in the place or in another shared sterilization area	☐
H.2 Renovation, conditioning and addressing of laboratory air	
H.2.1 *Required for drug susceptibility testing to first and second line antituberculosis drugs by phenotypic methods and extraction of DNA from isolates	
It is suitable when the area for the processing of isolates or and / or extraction and loaded of the DNA has a. Directed air (from clean areas taken by the Biological Safety Cabinet in operation and expelled by a duct that filters the air through HEPA before being expelled to the exterior), or there is another more complex system that achieves this requirement by ensuring at least 6-12 changes in laboratory air volume / hour. b. Safe air conditioning (air conditioner (s) are split type and do not generate air movements versus the BSC)	☐ ☐
H.2.3 *Applicable for molecular methods with open systems and/or home	
It is suitable when: a.the areas of amplification mixture preparation and amplification-detection of products) are separated with independent ventilation between them.	☐
H.3 * Connection system	
a. Internet access continuously b. Access to telephone continuously	☐ ☐
H.4 * Air temperature conditioning system for the equipment **H.4.1 Required for phenotypic tests**	
It is suitable when the maximum and minimum laboratory temperature is recorded daily and where: a. the culture equipment (MGIT 320/960) has an air conditioning system that allows to maintain the temperature between 19 and 30°C	☐
b. the refrigerated centrifuge for concentration of bacilli, has an air conditioning system that allows to maintain the temperature between 19 and 30°C	☐
c. the lab in which the biological safety cabinet is placed has a comfortable ambient temperature to work	☐

H.4.2 *Required for the preparation of culture medium with solid and/or liquid drugs	
It is suitable when the area keeps the right temperature to work comfortably a. With the presence of incubators with forced ventilation that ensures uniform temperature inside and / or coagulators for the solidification of egg-based medium and thermostatic baths.	☐
H.5 * Lighting and general conditions of the work area	
a. The adequate lighting (it is considered 500 LUX without emission of reflexes or brightness, which is equivalent to 50 Watt of a fluorescent lamp for 5 m^2)	☐
b. Walls and ceilings are painted, clean and without humidity	☐
c. All work areas are clean, there is a daily cleaning service	☐
Comments	

I. Handling and transporting the sputum sample and/or isolates Ignore if this item was answered in Annex A.1 or Annex A.2			
I.1 Collection of the sample **(Check the samples received during the day, see if the volume is adequate, if there are spills, if they are well conditioned, etc.)**			
a. * The type of container used for sputum collection Does it comply with the standardized technical specifications?	YES ☐	NO ☐	Comments ☐
b. * Is the containers label with the patient's identification on the side of the bottle and not on the lid?	YES ☐	NO ☐	Comments ☐
I.2 Management of the isolates (Verify which isolates are derived, if they are closed, if they are well labeled and conditioned)			
a. * The tube is tightly closed; it has a screw cap or a cotton cap and a rubber stopper. In addition, it is sealed with parafilm.	YES ☐	NO ☐	Comments ☐

b. *The label of the tubes is legible with the identification of the patient, the number of the laboratory of origin.	YES ☐ NO ☐ Comments ☐
I.3 Conservation and transport of the sample	
a. * Are the containers well conditioned with the samples that the laboratory receives, fulfilling the local standards for the shipment of samples? (Ex. transported in a strong, unbreakable and closed container, labeled with the international biohazard symbol)	YES ☐ NO ☐ Comments ☐
b. * If they are referred from other centers, do they arrive at the laboratory within 24 hours of the sample collection?	YES ☐ NO ☐ Comments ☐
c. * Are the samples stored in a cool place, preferably in the refrigerator, until they are processed?	YES ☐ NO ☐ Comments ☐
d. * Are the samples stored in a refrigerator other than the one where the reagents are stored (or at least on an exclusive shelf)?	YES ☐ NO ☐ Comments ☐
I.4 Isolates conservation and transport	
a. * Are the isolates received by the laboratory properly conditioned, complying with local regulations for the shipment of isolates? (Ex. transported in a triple container labeled with the international biohazard symbol, with the culture tube fitted with absorbent and / or anti-shock material)	YES ☐ NO ☐ Comments ☐
b. * Are the boxes with the isolates opened in a biological safety cabinet in case there is a broken tube?	YES ☐ NO ☐ Comments ☐
c. * Is the outside part of the triple container boxes decontaminated with alcohol 70%? Is the primary container autoclaved before recycling?	YES ☐ NO ☐ Comments ☐
d. * Is there a regular transport system to transfer isolates to the reference laboratory that processes them for more complex tests?	YES ☐ NO ☐ Comments ☐
Comments	

J. Practice audit	
J.1 Preparation of reagents, egg-based solid/liquid media and conservation Ask, if possible, the technician to perform the operation. Observe the process, from the weighing of the reagents to the coagulation of the medium in the case of egg-based media. In case you do not have enough time, ask at least these questions about critical points	
a. Are the batches of the chemical compounds used for each one of the different reagents elaborated in the lab registered?	YES ☐ NO ☐ Comments ☐
b. Are batches of drugs or chemical compounds used for each preparation of solid and / or liquid medium registered?	YES ☐ NO ☐ Comments ☐
c. Are the time and temperature of coagulation of the medium recorded? (Consider adequate if the coagulation is performed for 45 minutes at 80-85°C)	YES ☐ NO ☐ Comments ☐
d. Verify the absence of abundant bubbles in the solid media. (Consider that the presence of them is an indicator of overheating)	YES ☐ NO ☐ Comments ☐
e. Verify color homogeneity or absence of malachite green clumps (consider that the presence of green dots indicates a bad homogenization of the medium)	YES ☐ NO ☐ Comments ☐
f. Are the critical concentrations of drugs recommended by WHO used?	YES ☐ NO ☐ Comments ☐
g. To dissolve the drugs, Are the solvents recommended by the regional guidelines used?	YES ☐ NO ☐ Comments ☐
h. If the solvent is not water, Is only a minimum amount of solvent sufficient to solubilize the drug used? Is the final stock concentration then diluted with distilled water?	YES ☐ NO ☐ Comments ☐

i. Are portions of stock solutions of thawed and unused drugs discarded (not refrozen)?	YES ☐ NO ☐ Comments ☐
j. Do you check the Middelbrook 7H10 medium temperature at the time of incorporating the enrichment and each of the drugs?	YES ☐ NO ☐ Comments ☐
k. Are sterile controls of the medium performed? (consider appropriate if after coagulation, a sample of tubes is incubated at 35-37°C for 24 hours and then at room temperature for 48 hours)	YES ☐ every time a new batch is made ☐ Never ☐ Comments ☐
l. Is the time of use of the medium from the date of preparation recorded? (consider adequate if the media is used up to 1 month after its preparation)	YES ☐ NO ☐ Comments ☐
m. Is the elaborated medium kept in the proper place? (Consider adequate if the tubes with media are placed in a clean and frequently disinfected refrigerator, inside plastic boxes with the lid and each one of the tubes hermetically closed. The box can be protected by a nylon bag if the medium has a cotton plug to prevent drying. Do not introduce cardboard boxes for the possibility of fungal growth).	YES ☐ NO ☐ Comments ☐
Comments	
J. Practice audit	
J.2 Procedure of the drug susceptibility testing using the proportion method in Löwenstein Jensen medium or 7H10 Ask, if possible, the technician to complete the processing of the method you are using. Observe the process	
a. Is drug susceptibility testing performed using the drugs indicated in the laboratory's working algorithm?	YES ☐ NO ☐ Comments ☐

b. Are drug susceptibility testings carried out from smear positive samples 2 (+) or 3 (+)?	YES ☐ NO ☐ Comments ☐
c. Is the culture procedure used for each type of sample established if the test is made from positive smear samples? (Sputum, LBA, LB, LCR or biopsies)	YES ☐ NO ☐ Comments ☐
d. Is a homogeneous inoculum generated without the presence of lumps? (by leaving the suspension at rest before its inoculation to ensure that the clumps decant at the bottom of the tube)	YES ☐ NO ☐ Comments ☐
e. Is the turbidity pattern recommended by each methodology used to have representative colonies to infer the resistance?	YES ☐ NO ☐ Comments ☐
f. Are the stock suspension dilutions indicated by norms used taking into account if samples or isolates are being used?	YES ☐ NO ☐ Comments ☐
g. Are the dilutions of the bacillary suspensions made by mixing them with the closed tube?	YES ☐ NO ☐ Comments ☐
h. Is the test for each sample and/or isolate performed using at least two dilutions for the drugs tubes and three for the controls without drugs according to the method used?	YES ☐ NO ☐ Comments ☐
i. Is the media inoculation of each corresponding dilution performed using different pipettes?	YES ☐ NO ☐ Comments ☐
j. Are the absorption and contamination controls carried out 48 hours after sowing the tests?	YES ☐ NO ☐ Comments ☐
k. When is the review of the tests performed to detect resistance?	For medium in LJ to the 20 days ☐ 40 days ☐ Comments ☐ 7H10 to the 7 to 10 days☐ 21 days☐ Comments☐
l. Is the number of colonies developed in each tube quantified and recorded?	YES ☐ NO ☐ Comments ☐
m. Do the lab technicians verify that there were development of more than 100 colonies in the medium-drug-free tube with the most concentrated dilution?	YES ☐ NO ☐ Comments ☐

n. Is the critical ratio used to define resistance?	YES ☐ NO ☐ Comments ☐
o. Are internal quality controls carried out and registered for the drugs tested?	YES ☐ NO ☐ Comments ☐
p. Is the drug susceptibility testing reported with the species identification at the MTBC level?	YES ☐ NO ☐ Comments ☐
q. What results are reported in the first reading?	Resistant ☐ Sensible ☐ Comments ☐
Comments	

J. Practice audit	
J.3 Procedure of the drug susceptibility testing by the proportion method in MGIT Ask, if possible, the technician to complete the processing of the method. Observe the process	
a. Is the susceptibility testing performed on the drugs indicated in the laboratory's working algorithm?	YES ☐ NO ☐ Comments ☐
b. Are reconstituted drugs stored at -20°C or less for up to 6 months, or until the expiration date of the drugs (if it was before 6 months)?	YES ☐ NO ☐ Comments ☐
c. For drugs that are not included in the commercial equipment, are the critical concentrations recommended by WHO used?	YES ☐ NO ☐ Comments ☐
d. To dissolve drugs that are not included in the commercial equipment, Are the solvents recommended by the regional guidelines used? If the solvent is not water, Is only a minimum amount of solvent sufficient to solubilize the drug used? Then, to reach the final stock concentration, Is distilled water employed?	YES ☐ NO ☐ Comments ☐ YES ☐ NO ☐ Comments ☐

e. Are portions of stock solutions of thawed and unused drugs discarded (not refrozen)?	YES ☐	NO ☐	Comments ☐
f. For the preparation of the inoculum, are the recommendations of the regional guidelines followed, depending on whether isolates in liquid or solid medium are being used?	YES ☐	NO ☐	Comments ☐
g. Is a homogeneous inoculum generated without the presence of lumps (by leaving the suspension at rest before its inoculation to ensure that the lumps decant at the bottom of the tube)?	YES ☐	NO ☐	Comments ☐
h. Is the turbidity pattern recommended for this methodology used to have representative colonies to infer the resistance?	YES ☐	NO ☐	Comments ☐
i. Are the dilutions made by mixing them with the closed tube?	YES ☐	NO ☐	Comments ☐
j. Is the test performed for each isolate using the corresponding inoculum dilution for drug medium and a hundred times more diluted for growth control (for all drugs except for pyrazinamide in which the inoculum is ten times more diluted than the employee for the mediums with drugs)?	YES ☐	NO ☐	Comments ☐
k. When resistance to pyrazinamide is detected in a patient for the first time, is the test repeated when a lower density inoculum to ensure that it is not a false resistant result?	YES ☐	NO ☐	Comments ☐
l. Is the inoculation of each corresponding dilution performed using different pipettes?	YES ☐	NO ☐	Comments ☐
m. Are the results that the equipment emits checked daily?	YES ☐	NO ☐	Comments ☐
n. Are the growth controls checked for the presence or not of characteristic lumps of *M. tuberculosis* in order to inform the results?	YES ☐	NO ☐	Comments ☐
o. Are microscopic controls carried out (by making a smear with the broth of the tubes, stained by ZN) in case of doubt that the growth in the tubes corresponds to a contamination?	YES ☐	NO ☐	Comments ☐
p. Are the curves emitted by the equipment with the visual data corroborated?	YES ☐	NO ☐	Comments ☐

q. Are the curves emitted by the equipment saved and / or the results recorded in an Excel spreadsheet?	YES ☐ NO ☐ Comments ☐
r. Do you follow the recommendations of the technical guide to interpret the results?	YES ☐ NO ☐ Comments ☐
s. Are the internal quality controls for the tested drugs carried out and registered following the indications of the regional guides? These guides recommend: - At least every batch of MGIT medium put into use should be controlled, - Then, if the lot is used for several months, at least, a monthly check should be made	YES ☐ NO ☐ Comments ☐
t. Is the drug susceptibility testing reported with the result of the species identification at the MTBC level?	YES ☐ NO ☐ Comments ☐
Comments	
J. Practice audit	
J.4 Drug susceptibility testing procedure for the detection of resistance to rifampicin and isoniazid by the fast Griess method Ask, if possible, the technician to carry out the complete processing of the test. Observe the process	
a. Is the drug susceptibility testing performed only for isoniazid and rifampicin?	YES ☐ NO ☐ Comments ☐
b. Are drug susceptibility testings carried out from smear positive samples 2 (+) or 3 (+)?	YES ☐ NO ☐ Comments ☐
c. Is the culture procedure used for each type of sample established if the test is made from positive smear samples? (Sputum, LBA, LB, LCR or biopsies)	YES ☐ NO ☐ Comments ☐

d. Is a homogeneous inoculum generated without the presence of lumps?	YES ☐ NO ☐ Comments ☐
e. Is the turbidity pattern recommended by the methodology used to have representative colonies to infer the resistance?	YES ☐ NO ☐ Comments ☐
f. Are the dilutions of the stock suspension indicated by norms used?	YES ☐ NO ☐ Comments ☐
g. Are the dilutions made by mixing them with the tube closed?	YES ☐ NO ☐ Comments ☐
h. Is the test performed for each sample and / or isolate using at least three tubes for the controls and one tube with the drug medium?	YES ☐ NO ☐ Comments ☐
i. Is the inoculation of each dilution done using different pipettes?	YES ☐ NO ☐ Comments ☐
j. When is the reading of the test performed?	First revealed control to Direct method 14 days ☐ other ☐ Indirect method 7 days ☐ other ☐ Second development of control to the Direct method 21 days ☐ other ☐ Indirect method 10 days☐ other ☐ Third development of control to the Direct method 28 days ☐ other ☐ Indirect method 14 days ☐ other ☐ Comments ☐
k. Is the color between the control and the drug tube compared?	YES ☐ NO ☐ Comments ☐
l. Are internal quality controls carried out and registered for the drugs tested?	YES ☐ NO ☐ Comments ☐
m. Is the susceptibility test reported with the result of the species identification test at the MTBC level?	YES ☐ NO ☐ Comments ☐
Comments	

J.5 Procedure of the susceptibility testing for the detection of resistance to pyrazinamide by Wayne's rapid method Ask, if possible, the technician to complete the processing of the method. Observe the process			
a. Is the susceptibility testing performed on the isolates indicated in the laboratory's working algorithm?	YES ☐	NO ☐	Comments ☐
b. Is the susceptibility testing carried out from isolates on solid medium of up to 1 month of development?	YES ☐	NO ☐	Comments ☐
c. Is the incubation performed 7 days before the test is revealed?	YES ☐	NO ☐	Comments ☐
d. Is it left 4 hours before reading the reaction?	YES ☐	NO ☐	Comments ☐
e. Are internal quality controls carried out and registered?	YES ☐	NO ☐	Comments ☐
f. Is the susceptibility test reported with the result of the species identification test at the MTBC level?	YES ☐	NO ☐	Comments ☐
g. Are pyrazinamide-resistant results reported when the isolate was sensitive to isoniazid and rifampicin?	YES ☐	NO ☐	Comments ☐
h. If YES, Is the identification test at species level within the *M tuberculosis* complex performed before reporting?(In case *M. bovis* and/or *M. bovis BCG* is suspected).	YES ☐	NO ☐	Comments ☐
Comments			

J.6 Test procedure with line probe assay for injectable and quinolone drug susceptibility testing (LPA) Ask, if possible, the technician to carry out the complete processing of the test. Observe the process			
a. Is DNA extraction done from isolates by heating at 100°C?	YES ☐	NO ☐	Comments ☐
b. Is the temperature of the bath or twincubator the one established in the insert?	YES ☐	NO ☐	Comments ☐
c. Are the hybridization and washing buffers are at the temperature recommended by the manufacturer and are homogenized before use? (it is considered adequate that hybridization and buffers (buffer STR) were preheated to 37°C - 45°C, while the rest of the solutions have to reach room temperature before use)	YES ☐	NO ☐	Comments ☐
d. Are you careful in dispensing the preheated hybridization buffer to avoid splashing into neighboring channels?	YES ☐	NO ☐	Comments ☐
e. Is the reading of the strips carefully done by the technicians and / or professionals?	YES ☐	NO ☐	Comments ☐
f. Are the results analyzed by evaluating the patient's clinical and epidemiological data before reporting?	YES ☐	NO ☐	Comments ☐
g. Are the invalid and indeterminate results analyzed to try to decipher the problem?	YES ☐	NO ☐	Comments ☐
h. Are the invalid or indeterminate results repeated?	YES ☐	NO ☐	Comments ☐
i. The report is made according to the norms.	YES ☐	NO ☐	Comments ☐
Comments			

K. Safety in the laboratory **Required for drug susceptibility testing to first and second line antituberculosis drugs** To ignore if it was completed in Annex A.1 or A.2	
K.1 Safety practices	
a. * Laboratory biosafety manual	Available: YES ☐ NO ☐ Comments ☐
b. * Use of recommended disinfectants for tuberculosis (5% phenol, 1% sodium hypochlorite, 70% alcohol).	Available: YES ☐ NO ☐ Comments ☐
c. * Cleaning at least once before beginning and at the end of each working day.	YES ☐ NO ☐ Comments ☐
d. * Use of gloves according to the general biosafety work standards of the laboratories.	YES ☐ NO ☐ Comments ☐
e. * Use of respirators (type N95 or FFP2).	YES ☐ NO ☐ Comments ☐
f. * Availability of respirators (type N95 or FFP2), (when they are not used in the work routine), for their use in case of spills.	YES ☐ NO ☐ Comments ☐
g. *Form of availability and use of the elements of personal protection.	Available: YES ☐ NO ☐ Comments ☐
h. * Disposal of pathological waste and all the elements used for the different methodologies by recommended methods (autoclaving or treatment with sodium hypochlorite before disposal with the rest of the pathological waste of the institution or, discarding of solutions in special containers for liquid waste). The material transferred for autoclaving must be transported in secure containers.	YES ☐ NO ☐ Comments ☐
i. * Disposal and transport of material (potentially infectious) in secure containers that resist autoclaving and autoclaving of contaminated material daily for 1 hour at 121ºC.	YES ☐ NO ☐ Comments ☐
j. * Chemical indicators for heat sterilization (autoclaving)	YES ☐ NO ☐ Comments ☐ Frequency of use.....
k. * Biological indicators for heat styling (autoclaving)	YES ☐ NO ☐ Comments ☐ Frequency of use.....
l. * Kit in case of spills containing: an autoclavable bag, gloves, gowns, appropriate disinfectants, N95 or FFP2 respirators, cotton and adsorbent paper, soap, stick to collect waste, sharps container, DO NOT ENTER sign).	Available: YES ☐ NO ☐ Comments ☐
m. * Written rule for the management of biohazard waste and regulated chemical waste	Available: YES ☐ NO ☐ Comments ☐

K.2 Staff security	
a. * Regular annual program of medical control for health workers, following the labor regulations in the country (If there is no adopted policy, the supervisor must ensure that laboratory personnel have at least one annual medical evaluation that may include a chest x-ray).	Available: YES ☐ NO ☐ Comments ☐
b. * Known and written instructions for accidents or incidents (it can be included in the laboratory's Biosafety manual / SOP)	Available: YES ☐ NO ☐ Comments ☐
c. * Initial safety / biosafety training program with records of the laboratory personnel participation	Available: YES ☐ NO ☐ Comments ☐
Comments	

L. Derivation of samples	
L.1 Required for susceptibility tests to first and second line anti-tuberculosis drugs Depending on the diagnostic algorithm established in each country, the same patient could be studied by one or more of these techniques Ignore if Annex A.1 or A.2 was completed	
a. *Are all samples and / or isolates derived from the group of patients indicated by the standards derived for drug susceptibility testing to first-line drugs? The following samples and / or isolates are considered adequate for derivation - positive smear samples 2 or 3 crosses of patients with: • history of previous treatment (relapses, failures, loss in follow-up) • contact history with patients with multidrug resistant or extensively resistant TB • smear positive at the end of the second month of chemotherapy or in a subsequent control - positive cultures with cultural characteristics compatible with MTB or already identified as MTBC of patients with: •history of previous treatment (relapses, failures, loss in follow-up) •history of contacts with patients with multidrug-resistant or extensively resistant TB • smear positive at the end of the second month of chemotherapy or in a subsequent control • cases diagnosed with negative sputum smear microscopy and that have a smear microscopy positive during treatment •immunosuppression, particularly positive HIV and diabetic patients •with exposure to infection by drug-resistant bacilli (internees or workers from health institutions or prisons where there are cases of resistant tuberculosis) • previous residence in countries with a high level of drug resistance (Ecuador, Peru, some Asian and Eastern European countries). •addiction to alcohol and / or other drugs •age under 15 years (children) •drug intolerance	YES ☐ NO ☐ Comments ☐

b. When a sample/isolate is identified with a result of monoresistance to rifampicin or isoniazid or multidrug resistance TB. Are samples derived from the NRL, to confirm the identification and resistance/s and to perform drug susceptibility testing to the rest of the first and second line drugs?	YES ☐ NO ☐ Comments ☐
c. *Is there a regular transport system to transfer the isolates to the laboratory that performs identification and drug susceptibility testing?	YES ☐ NO ☐ Comments ☐
d. *Laboratory to which samples/isolates are derived for identification and / or drug susceptibility tests	.....
e. What is the average time of results reception from the derivative studies?	LPAs (quinolones and ISL):..... days Drug susceptibility testing (phenotypic methods) to Rifampicin ..... days to Isoniazid..... days to second-line drugs ..... days
Comments	
M. Other observations	
Laboratory staff expressed the following concerns regarding their reference laboratory	

Conclusions
The following strengths are highlighted ....
It highlights the following challenges or corrective measures to be implemented as a priority by the authorities ....
by the laboratory staff ....
The following training needs were identified ....
Agreements reached in relation to the challenges ....
Name and signature of the supervisor/s: .....
Name and signature of the laboratory manager: .....
Date: ..... /...../.....

ANNEX B - SMEAR MICROSCOPY- Periphery-center supervision

Annex B.1. Example of a register used to document the schedule of laboratories to be monitored during a semester.

This record can be used when the sampling of the slides to be rechecked is done only over a given period of the year (e.g. one month). In this example, laboratories to be monitored from January to June were included in the schedule; in this way, the supervising laboratory at the end of each month will be able to identify the laboratories to which slides must be requested. The laboratories that are going to be evaluated should not know in advance in which months they will be supervised. In this example, the register has been used, moreover, to record the date of the slides request to the service to be monitored, the reception of the acknowledgment of receipt (in case the request has been given by email) by the laboratory to be monitored and the date of receipt of the slide sat the supervisory laboratory.

Schedule of Laboratories to be Supervised - Semester = 1 Year = 2017				
Month	Service	Date Request	Acknowledgment of Receipt of the Request (Yes / No)	Date of slides reception
January	Laboratory A	31/01/2017	Yes	25/02/2017
	Laboratory B	31/01/2017	Yes	21/02/2017
	Laboratory C	31/01/2017	Yes	03/03/2017
February	Laboratory D	26/02/2017	No	
	Laboratory E	28/02/2017	Yes	22/02/2017
	Laboratory F	28/02/2017	Yes	04/03/2017
March	Laboratory G	30/03/2017	Yes	28/04/2017
	Laboratory H	30/03/2017	Yes	16/04/2017
	Laboratory I	30/03/2017	Yes	05/04/2017
April	Laboratory J	30/04/2017	Yes	03/06/2017
	Laboratory K	30/04/2017	Yes	03/06/2017
	Laboratory L	30/04/2017	Yes	05/06/2017
May	Laboratory M	29/05/2017	Yes	25/06/2017
	Laboratory N	29/05/2017	Yes	21/06/2017
	Laboratory Ñ	29/05/2017	Yes	04/05/2017
June	Laboratory O	02/07/2018	Yes	25/07/2017
	Laboratory P	02/07/2018	Yes	21/07/2017
	Laboratory Q	02/07/2018	Yes	03/08/2017

Suggested forms for EQA for smear microscopy using the rechecking method

The following five instruments, corresponding to Annexes B.2 to B.6, are designed to guide the supervisory laboratory in the following activities:

Annex	Activities
B.2	List the slides sample that will be reread and register the rechecking results obtained by the controllers
B.3	List the slides with discordant results between the supervised laboratory and the first controller, so that they are read by the second controller
B.4	Consolidate results of the re-checking of all the participant laboratories in a certain area and those of the controller corresponding to those laboratories with the purpose of evaluating the performance of the controllers
B.5	Report annually the workload and performance of each of the laboratories that were supervised
B.6	Consolidate the annual global results of the performance of all laboratories in an area

These instruments may be available in paper format, but it is advisable, as stated above, that they can be included electronically.

Due to the self-explanatory characteristics of these forms, they do not have additional instructions to complete them; only a description of its use has been placed in the context of the “Global procedure of EQA by the method of rechecking SM", as well as some footnotes to define the abbreviations that appear in them.

Annex B.2.Controllers rechecking results

This form is used by the local coordinator to list the numbers of the slides that will be reread (without the results of the laboratory to be monitored) and will be delivered to the first controller along with the slides. Upon receiving the copy with the results of the first controller, the coordinator will complete the results column of the supervised laboratory and identify the smears with discordant results, which will be listed in the Form shown in Annex B.3 "List of discrepancies", to be reread by the second controller. After the reading of the second controller, the local coordinator will complete the tables located at the bottom of the form with the numbers and types of errors committed by the supervised laboratory and the first controller and the recommendations related to the identified findings (errors, quality of the sample, smear and staining).

EQA by rechecking

Annex B.2: Controllers rechecking results

Supervised laboratory: _____ **First Controller:** _____

Local technician / s: _____ Laboratory: _____

Date _____ **Second Controller:** _____

Period evaluated: _____ Laboratory _____

Supervised Lab		Result		Sample Quality	Smear Quality	Staining Quality	Commentary
Slide Number	Result	First Controller	Second Controller				

Supervised Laboratory

Total results reported in the sample				
Positive:	Countable:	Negative:		
Summary of identified errors				
HFP	LFP	HFN	LFN	QE
High false positive, Low false positive, High false negative, Low false negative ,Quatification error				

First Supervisor

Total results reported in the sample				
Positive:	Countable:	Negative:		
Summary of identified errors				
HFP	LFP	HFN	LFN	QE

Summary of specimen, smear and staining quality

Sputum samples with adequate quality: .....%

Good Smear .....%

Good staining .....%

Observations and Recommendations:

Annex B.3. List of discordant slides

This form is used by the coordinator to list the slides with discordant results between the supervised laboratory and the first controller, together with both results. It can be used to place the discordant slides of several laboratories, since the laboratory whose smears present discordant results can be identified in the same form. To ensure that the second controller does not know to whom each result corresponds (laboratory or controller), it is

recommended to alternatively place the results of the controller and the laboratory in the columns whose headings are "Results 1 and 2". For example, place the laboratory results of one group of microscope slides in the "Results 1" column, and in the "Results 2" column, those of the controllers, while, for another group of microscope slides, place in the column of "Results 1", the results of the controller and in the "Results 2" column introduce those of the supervised laboratory.

EQA by rechecking

Annex B.3: List of discordants

Province / Area .....

First **controller:** .....

Second controller: .....

Date: .....

Collection period of the sample .....

Supervised Lab	**Nº Slide**	Result 1	Result 2	**Second Controller**	
				Result	

Annex B.4. Evaluation of the first controller

It is used by the coordinator to condense the re-reading results of all the supervised laboratories in a specific area and of the first controller who performed the re-readings. It is used as a support to evaluate the competences of the controller. Only consolidated results from the laboratories are placed, not smear lists. The information that must be included in this form is:

List with the names of all supervised laboratories

For each supervised laboratory: total of positive, countable and negative smears reported by each laboratory in the slide sample and total of identified errors (from the Form of Annex B.2).

Name of the first controller, second controller and coordinator

Summary of the total of positive, countable and negative smears reported by the first controller for each laboratory evaluated and errors committed by the first controller (of the Form of Annex B.2).

Totals of each column calculated at the bottom of the form

EQA by rechecking

Annex B.4: Evaluation of the first controller

Area/Provinc _____ **First controller** _____

Second controller: _____

Period: _____ **Coordinator**

	Evaluation of laboratories								Evaluation of the first controller							
Name of participant laboratories	Reported results in the rechecked smears(No.) (*)			Errors detected in the EQA (N °) (**)					Reported results in the rechecked smears(No.) (***)			Errors detected in the EQA(No.) (****)				
	Pos.	Countable	Neg.	HFP (N°)	HFN (N°)	LFP (N°)	LFN (N°)	QE	Pos.	Countable	Neg.	HFP (N°)	HFN (N°)	LFP (N°)	LFN (N°)	QE
Totals																

Date _____

Sign _____

(*) Complete with the numbers of positive, countable and negative results registered by the Laboratory evaluated in the rechecked smears(Summary table "Supervised laboratory" Annex B.2)

(**) Complete the number of errors found in the supervised laboratory after the dicordant slides were read by the second controller (HFP: high false positive, HFN: High false negative,LFP: Low false positive , LFN: Low false negative , QE , quantification error) (Summary Table "Supervised Laboratories" Annex B.2)

(***) Complete with the positive, countable and negative results numbers registered by the first controller in the relegated extensions (Summary table "First controller" Annex B.2)

(****) Fill in the number of errors found for the first controller after discordant slides were read by the second controller (Summary table "First controller" Annex B.2)

Annex B.5. Performance of sputum smear labs

This instrument is used to annually report the workload and performance of each of the laboratories that were supervised. It includes a list of each laboratory, the workload and positivity, the size and positivity of the sample of re-checked smears and the errors identified, by type of error.

EQA by rechecking

Annex B.5: Performance of sputum smear labs

ANNUAL ANALYSIS

Year ..... Supervisory laboratory .....

N	Name of participant laboratories	Slides processed during the year (Nº)			Rechecked slides (Nº)			Errors identified in the rechecked sample of slides				
		Pos	Countable	Neg-	Pos	Countable	Neg-	HFP (Nº)	HFN (Nº)	LFP (Nº)	LFN (Nº)	QE
1	0											
2	0											
3	0											
4	0											
5	0											
6	0											
7	0											
8	0											
9	0											
10	0											
11	0											
12	0											
13	0											
14	0											
15	0											
	0											
	0											
	Total											

HFP: High false positive; HFN: High false negative; LFP: Low false positive; FLN: Low false negative; QE: Quantification error

Annex B.6. Summary of rechecking results

This instrument is used to consolidate the annual global results of the laboratory performance in an area. The report includes indicators of EQA program coverage, workload and positivity of participating laboratories, rechecking sample size and positivity, percentage of errors (by type of error) and number of laboratories with more than one major error (by type of error). Using the information gathered in the reports made in previous years, it is possible to make a trend of the indicators of coverage and overall performance of the laboratories, in order to be able to identify if the corrective activities applied have been effective to maintain and / or improve the quality of the services.

EQA by rechecking

Annex B.6: Summary of rechecking results

Year:

Number of laboratories in the network	
Number of laboratories evaluated by rechecking	
Average percentage of positivity in the participating laboratories	
Number of positive smears rechecked	
Number of negative smears rechecked	
Average percentage of HFP	
Average percentage of HFN	
Number (%) of laboratories with more than one HFP	
Number (%) of laboratories with more than one HFN	

HFP: High false positive; HFN: High false negative.

Annex B.7. Guide of actions to be carried out during the technical visit to investigate the causes of the errors detected by the rechecking method (Table translated and adapted from the Document "External quality assessment for AFB Smear microscopy". Washington, DC: APHL; 2002).

Errors	**Possible causes**	**Investigations / actions to be carried out during the technical visit**
One only HFP	1. Administrative error 2. The same causes as when you find regular amount of HFP(see below)	1. Compare the laboratory record with the list submitted for the slides rechecking. Right or wrong result? 2. Exclude other causes described for the finding of several HFP
Scarce FPL	Limitation of the EQA technique by rereading	Ignore if they occur in numbers comparable to those obtained by controllers.
Some HFP with or without LFP	1. Problems with staining Are there artifacts? Was AFB discolored before rereading? Are the smears restaining before performing the EQA by rereading? 2. Problems with the microscope 3. The technician cannot recognize the AFB 4. Problems with the laboratory record	1. Check the staining solutions and the staining procedure. Restain and examine the HFP results. Are they positive? 2. Examine an smears with few bacilli using the laboratory microscope 2. Reread several smears with few bacilli to analyze the technician's ability to recognize bacilli 4. Compare the laboratory record with the list submitted for the slides rereading. Right or wrong results?
Only HFN (2–3+)	1. Administrative error as in the case of a single HFP	1. Compare the laboratory record with the list submitted for the smear rechecking. Right or wrong result?
More than one HFN and / or several LFN Quantification lower than the RL	1. Poor dye quality/inadequate staining technique 2. Inadequate preparation of the smears. 3. Problems with the microscope 4. Rough microscopy	1. Evaluate whether positive smears recently stained in the laboratory are seen in deep red. Observe the staining procedure: enough time, warming up? Time with the right contrast stain? 1. Stain positive smears with staining solutions prepared in the RL. 2. Check the thickness of the smears. Are they observed intense blue? 3. Use the laboratory microscope to observe smears that are known to be positive. Intensity of adequate light? Clear image? 4. Exclude other causes.
QE serious	1. Staining solutions/poor staining 2. Problems with the microscope	1. Those mentioned in the above section 2. Those mentioned in the above section

Annex B.8. Examples of rechecking reports

Example 1

Smear microscopy EQA report by rechecking

Laboratory: Hospital A
Date: 20-8-16
Evaluated period: June (January -June 2016)
Province: Fictional

First controller: N. A
Laboratory: regional
Second controller: F. A
Laboratory: regional

Laboratory		Result		Specimen	Smear	Staining
N°	Result	First controller	Second controller			
8022	Neg (-)	Pos (8 AFB)	Pos (5 AFB)	Mucopurulent	Good	Good
8024	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8026	Pos (++)	Pos (+++)		Mucopurulent	Not homog.	Good (lack of discoloration)
8028	Neg (-)	Neg (-)		Mucous	Good	Good
8030	Neg (-)	Neg (-)		Mucous	Not homog.	Good (lack of discoloration)
8032	Neg (-)	Neg (-)		Mucous	Good	Good
8034	Neg (-)	Pos (6 AFB)	Pos (6 AFB)	Mucous	Good	Good
8036	Neg (-)	Neg (-)		Saliva	Fine	Good
8038	Neg (-)	Neg (-)		Mucous	Thick	Good (lack of discoloration)
8040	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8042	Neg (-)	Neg (-)		Mucous	Thick	Good (lack of discoloration)
8044	Neg (-)	Neg (-)		Mucopurulent	Thick	Good (lack of discoloration)
8046	Neg (-)	Neg (-)		Mucous	Good	Good
8048	Neg (-)	Neg (-)		Mucous	Good	Good
8050	Neg (-)	Neg (-)		Mucopurulent	Thick	Good (lack of discoloration)
8052	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8054	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8056	Neg (-)	Neg (-)		Mucous	Not homog.	Good (lack of discoloration)
8058	Neg (-)	Neg (-)		Mucopurulent	Not homog.	Good (lack of discoloration)

8060	Neg (-)	Neg (-)		Mucous	Not homog.	Good (lack of discoloration)
8062	Neg (-)	Neg (-)		Mucopurulent	Thick	Good
8064	Neg (-)	Neg (-)		Mucous	Thick	Good (lack of discoloration)
8066	Neg (-)	Neg (-)		Mucous	Good	Good (fuchsin crystals)
8068	Neg (-)	Neg (-)		Mucopurulent	Thick	Good (lack of discoloration)
8070	Neg (-)	Neg (-)		Mucous	Good	Good
8072	Neg (-)	Neg (-)		Mucous	Not homog.	Good (lack of discoloration)
8074	Neg (-)	Neg (-)		Mucous	Good	Good
8076	Neg (-)	Neg (-)		Mucopurulent	Not homog	Good (lack of discoloration)
8078	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8080	Neg (-)	Neg (-)		Saliva	Good	Good
8082	Neg (-)	Neg (-)		Mucous	Not homog	Good
8084	Neg (-)	Neg (-)		Mucous	Thick	Good
8086	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8088	Neg (-)	Neg (-)		Mucous	Not homog	Good (lack of discoloration)
8090	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8092	Neg (-)	Neg (-)		Mucous	Good	Good
8094	Neg (-)	Neg (-)		Mucopurulent	Thick	Good (lack of discoloration)
8096	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8098	Pos (++)	Pos (+++)		Mucopurulent	Not homog	Good (lack of discoloration)
8100	Neg (-)	Neg (-)		Mucopurulent	No homog	Good (lack of discoloration)
8102	Neg (-)	Neg (-)		Mucous	No homog	Good (lack of discoloration)
8104	Neg (-)	Neg (-)		Mucous	Good	Good
8108	Pos (++)	Pos (+++)		Saliva	Fine	Good
8110	Neg (-)	Neg (-)		Mucopurulent	Good	Good
8112	Neg (-)	Pos (5 AFB)	Pos (9 AFB)	Saliva	Fine	Good
8114	Neg (-)	Pos (7 AFB)	Pos (5 AFB)	Mucous	Good	Good

8118	Neg (-)	Neg (-)		Mucous -	Not homog	Good (lack of discoloration)
8120	Neg (-)	Pos (+)	Pos (+)	Mucopurulent	Good	Good

Total results reported in the sample					
Positive: _____3_		Countable: _____0_		Negative: ___45_____	
Summary of identified errors					
HFP 0	LFP 0	HFN 1	LFN 4	QE 0	
HFP= High False Positive; HFN= High False Negative; LFP= Low False Positive; LFN= Low False Negative; QE: Quantification error					
Summary of specimen, smear and staining quality Sputum samples with adequate quality: 92% Good smears: 50% Good and good staining with objections: 100%					

Observations and recommendations

1. Quality of the samples: Good. The majority (92%) of the samples have been classified as mucous or mucopurulent, a fact that would indicate that the patients´ instructions for obtaining the sputum specimens are given in improper way and that the particle for the smear preparation is chosen appropriately in the laboratory.

2. Smear quality: There is a tendency to make thick and uneven smears. Fields with too much sample can generate an intense contrast coloration that can hide the AFB, causing false negative results. On the other hand, in thick smears, part of the material can be detached during the staining process, causing possible false negative results. It is recommended to review the smear preparation procedure. Refer to the technical standards manual http://www1.paho.org/Spanish/AD/DPC/CD/tb-labs-baciloscopia.pdf

3. Staining quality: A high proportion of smears with insufficient discoloration are observed, a fact that is associated with the preparation of thick or non-homogeneous smears. Additionally, the lack of discoloration can be caused by the drying of the fuchsin solution over the smears, or due to an acid concentration in the discoloration solution lower than the established in the norms or an inadequate exposure time of the discoloration solution on the smears. The presence of background staining can cause confusion in the observation of AFB and give rise to false positive and negative results.

4. Quality of the readings: Four LFN and one HFN errors were identified. Additionally, there is a tendency to lower semiquantitative readings than those of the Reference Laboratory. These findings could be associated with the reading of fewer fields than those established in the norms or without the use of a micrometer screw for smear reading. These results could also be due to the conditions of the microscope in use, in particular to the lack of adequate light intensity for the observation of AFB. Although in smears with few bacilli, the reading reproducibility is close to 50%, the fact of identifying four LFN errors together with an HFN is an alarm signal that must be evaluated. It is recommended to read more carefully, using the micrometer screw and observing at least 100 fields to declare a smear as negative. A technical visit to the laboratory will be coordinated.

Example 2

Smear microscopy EQA report by rechecking

Laboratory: Hospital B
Date: 30-1-17
Evaluated period: November (July-December 2016)
Province: Fictional

First controller: N. B
Laboratory: regional
Second controller: F. B
Laboratory: regional

Laboratory		Result		Specimen	Smear	Staining
N°	Result	First controller	Second controller			
809	Neg (-)	Neg (-)		Mucopurulent	Good	Good
811	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
813	Pos (+)	Neg (-)	Neg (.)	Saliva	Fine	Good (fuchsin crystals)
815	Neg (-)	Neg (-)		Mucopurulent	Good	Good
817	Pos (5 AFB)	Neg (-)	Neg (-)	Mucopurulent	Good	Good (fuchsin crystals)
819	Neg (-)	Neg (-)		Mucopurulent	Good	Good (lack of discoloration
821	Neg (-)	Neg (-)		Mucous	Good	Good
823	Pos (6 AFB)	Neg (-)	Neg (-)	Mucous	Good	Good (fuchsin crystals)
825	Neg (-)	Neg (-)		Mucous	Good	Good
827	Neg (-)	Neg (-)		Mucopurulent	Good	Good
829	Pos (++)	Pos (++)		Mucous	Good	Good
831	Neg (-)	Neg (-)		Mucous	Good	Good (fuchsin crystals)
833	Neg (-)	Neg (-)		Mucopurulent	Good	Good
835	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
837	Neg (-)	Neg (-)		Saliva	Fine	Good
839	Neg (-)	Neg (-)		Mucopurulent	Good	Good
841	Pos (5 AFB)	Neg (-)	Neg (-)	Mucopurulent	Good	Good (fuchsin crystals)
843	Neg (-)	Neg (-)		Mucopurulent	Good	Good
845	Pos (++)	Pos (++)		Mucopurulent	Good	Good (lack of discoloration

847	Neg (-)	Neg (-)		Mucous	Good	Good
849	Neg (-)	Pos (6 AFB)	Pos (+)	Mucous	Good	Good (fuchsin crystals)
851	Neg (-)	Neg (-)		Saliva	Fine	Good
853	Pos (7 AFB)	Neg (-)	Neg (-)	Saliva	Fine	Good (fuchsin crystals)
855	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
857	Neg (-)	Neg (-)		Mucopurulent	Good	Good
859	Neg (-)	Neg (-)		Mucopurulent	Good	Good
861	Neg (-)	Neg (-)		Mucous	Good	Good
863	Pos (+++)	Pos (+++)		Mucopurulent	Fine	Good (fuchsin crystals)
865	Neg (-)	Neg (-)		Mucous	Good	Good
867	Neg (-)	Neg (-)		Mucous	Good	Good
869	Neg (-)	Neg (-)		Mucous	Good	Good
871	Neg (-)	Neg (-)		Mucopurulent	Good	Good (lack of discoloration
873	Neg (-)	Neg (-)		Mucous	Good	Good
875	Neg (-)	Neg (-)		Saliva	Fine	Good
877	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
879	Neg (-)	Neg (-)		Mucous	Good	Good
881	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
883	Neg (-)	Neg (-)		Mucous	Good	Good
885	Neg (-)	Neg (-)		Mucous	Good	Good
887	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)
891	Neg (-)	Neg (-)		Mucopurulent	Good	Good
893	Neg (-)	Neg (-)		Mucopurulent	Good	Good
895	Neg (-)	Neg (-)		Mucopurulent	Good	Good (fuchsin crystals)

897	Neg (-)	Neg (-)		Mucopurulent	Good	Good (lack of discoloration
899	Neg (-)	Neg (-)		Mucous	Good	Good
901	Neg (-)	Neg (-)		Mucous	Good	Good
903	Neg (-)	Neg (-)		Saliva	Fine	Good (fuchsin crystals)

Total results reported in the sample									
Positive: _____4_			Countable: _____4_			Negative: ___40_____			
Summary of identified errors									
HFP	1	LFP	4	HFN	0	LFN	1	QE	0
HFP= High False Positive; HFN= High False Negative; LFP= Low False Positive; LFN= Low False Negative; QE: Quantification error									
Summary of specimen, smear and staining quality Sputum samples with adequate quality: 87% Good smears: 98% Good and good staining with objections: 100%									

Observations and recommendations

1. Quality of the specimens: good. Most of the samples have been classified as mucous or mucopurulent (85%), a fact that would indicate that the patients´ instructions for obtaining the sputum specimens are given in improper way and that, the particle for the smear preparation is chosen appropriately in the laboratory.

2. Smear quality: good. Most smears prepared from good samples have the proper thickness and size.

3. Staining quality: a high proportion of smears with fuchsin deposits are observed. The presence of these artifacts makes the observation of AFB difficult and may cause false negative results. On the other hand, these deposits of dyes can be confused with bacilli, causing false positive results. It is recommended to check the fuchsin concentration in the preparation of the staining solution and filter a portion of the fuchsin solution before each working day or to dispense the fuchsin on a funnel with filter paper on top of each smear. Review the results recorded in the internal quality control Register of staining solutions and staining technique.

4. Quality of the readings: Six disagreements were found: a low false negative and five false

positive errors, one of them, a high false positive one. All the false positive errors were identified in smears with fuchsin deposits, emphasizing the importance of correcting this problem in the staining. Additionally, the confusion of AFB with these artifacts could be due to the conditions of the microscope in use, in particular the sharpness and lack of light intensity suitable for the observation of AFB. A technical visit to the laboratory will be coordinated.

Annex B.9. Smear microscopy quality monitoring form

This form is used to perform an annual monitoring of the performance of each of the microscopy laboratories that are regularly monitored by each supervising laboratory. From the data included in the Form at Annex B.2, information is collected on the annual performance of each laboratory in order to temporarily evaluate the occurrence of systematic errors and the progress made by each service.

EQA by rechecking

Annex B.9: Smear microscopy quality monitoring form

Service name: .....

Location: .....

Province, area, region:.....

Year	N° of smears evaluated	Pos. (N°)	Countable (N°)	% Good specimens	% Good smears	% Good Staining	HFP (N°)	LFP (N°)	HFN (N°)	LFN (N°)	Comments

HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative

Annex B.10. Example of a smear microscopy quality monitoring form

Smear microscopy quality Monitoring Form

Service Name: Hospital A
Location: Fictitious
Province, area, region: Ficticia

Year	N° of smears evaluated	Pos. (N°)	Countable (N°)	% Good specimens	% Good smears	% Good Staining	HFP (N°)	LFP (N°)	HFN (N°)	LFN (N°)	Comments
2015	96	9	7	80	95	95	1	5	0	1	Presence of fuchsin crystals in a significant proportion of the smears. Possible cause of FP. Technical Visit Planned
2016	96	8	2	75	96	96	0	1	0	0	
2017	96	9	3	78	93	98	0	0	0	1	

ANNEX C –Smear microscopy. Center-periphery supervision

Records, forms and procedures for preparation of slides batches and panels testing sets for proficiency testing.

The following annexes include a procedure for preparing slides batches and panel testing sets and several records and forms to collect information on the quality of the slide batches and the test results

The instruments presented in Annexes C.2 to C.7 have been designed to guide the Reference Laboratory in the following sequence:

Annexed	Activity
C.2	Produce the stock positive suspension that will be used for the production of slide batches with different degrees of positivity
C.3	Produce and validate a collection of AFB positive and negative slides that will be used to prepare panel testing sets.
C.4	Produce panels, record the expected results for each slide and the results obtained by the participating microscopists
C.5	Send a record to register the results obtained by local service laboratories along with the panels. Includes the instructions for conducting the test.
C.6	Make the results report of the proficiency testing.
C.7	Record in a condensed form, the results obtained by the participating laboratories of the test in an area / region / department during a given period

Annex C.1. Procedure for the preparation of slides batches and panels sets for SM proficiency testings

This procedure is a self-explanatory method of producing multiple slides from AFB positive and negative specimens. The team of technicians must read and understand the process and protocols before making the slides. If there is any difficulty in producing slides that meet the consistency requirements, they should: 1) review the processes, especially the warming and homogenization of suspensions; 2) select less mucous samples (mucopurulent). Before proceeding to the development of panel sets, the laboratory must demonstrate efficiency to produce consistent batches of a minimum of 50-100 slides, especially those corresponding to countable AFB.

Required Materials:

- Water bath or dry bath incubator (thermal block) at 55-60 ° C
- Plastic tubes with 50 ml screw cap
- Plastic tubes with screw cap of 15 ml
- 3mm glass beads
- Formaline (Formaldehyde solution 40%)
- 4% NaOH solution
- Distilled water
- Bovine albumin 2% in distilled water
- Biological safety centrifuge with a capacity of 3000 G
- Serological pipettes of 10ml, 5ml and 1ml.
- Propipette for serological pipettes.
- Micropipette of 20-200 µl and 200-1000 µl.
- Tips for micropipettes of 20-200 µl and 200-1000 µl.
- One-channel multidispenser micropipette with a capacity of 50 µl or greater that allows dispensing 30 µl.
- Tips for the multi-dispensing pipette
- Permanent marker
- Glass marker
- Microscope slide
- Vortex type agitator
- Timer
- Heating plate with capacity to maintain the temperature at 60ºC.

Note: the processing must be carried out in a biological safety cabinet in laboratories with adequate biosafety conditions. The procedure applied to the specimens for the production of the bacillary suspensions that will give origin to the batches of slides, causes moderate risk of generation of infectious aerosols, so all the procedures must be carried out in a laboratory with infrastructure, equipment and practices corresponding to a "moderate risk" service.

Positive sample

Although fresh samples with no more than 2 or 3 days after collection are preferred, samples stored for up to a week at 4 °C and then stored in a freezer at -20ºCcan also be used. Sputum must have the following characteristics:

- Volume: 3 ml or more.
- AFB load: ≥2 + by ZN.
- Color: White to light green. Do not use blood samples.
- Consistency: samples whose consistency is not very mucous are preferred because they hinder the production of stock of consistent positives.

Negative Sample:

It is very important that they are fresh, of no more than 2 days after collection, and with the following characteristics:

- Volume: 3-4 ml (if the volume is lower, several negative sputum of good quality can be collected to reach this volume).
- Color: white to light green.
- Negative samples having 20 leukocytes or more per field are preferred since this number of cells favors the appearance of the slides to be similar to that of a smear prepared from a sputum sample; the leukocytes must have preserved morphology, otherwise, they are lysed during the process.

Operational Sequence

Preparing the stock of positives

Sample volume: 3 ml

1. If the sample volume is greater than 3 ml, separate aliquots of that amount in different tubes.

2. Place the 3 ml of the sputum in a 15 ml graduated tube. Add 5-10 glass beads (of about 3mm diameter) and 50 µl formalin per ml of positive sputum. Mix using a vortex type stirrer.

3. Incubate for one hour at room temperature, mixing with the vortex type agitator every 10 minutes in order to thoroughly homogenize the material.

4. Add 1 ml of 4% NaOH (for consistent sputches add up to 2 ml of NaOH).

5. Stir 4-5 minutes with a vortex-type shaker.

6. Transfer the contents of the 15 ml tube to a plastic tube with a 50 ml screw cap using a pipette (do not pierce the glass beads).

7. Add distilled water to complete a volume of 20 ml.

8. Incubate in a water bath or thermal block at 55-60ºC for 30 minutes, mixing occasionally by inversion (3 times in 30 minutes).

9. Add distilled water to a volume of 40 ml and mix by inversion.

10. Centrifuge at 3000 G for 15 minutes at room temperature (if you do not have a tube holder for 50 ml tubes, can distribute the 40 ml contained in the 50 ml tube in 3 tubes of 15 ml before centrifuging).

11. Let stand 5 minutes and carefully decant the supernatant.

12. Add 0.5 to 1 ml of distilled water and resuspend using a vortex-type stirrer for 2 minutes (if the centrifugation procedure was performed with 15 ml tubes, 0.2 to 0.4 ml of distilled water should be added to each tube and collect its contents in a single tube).

13. Before evaluating the amount of bacilli in the stock suspension, carry out a verification of the disposition of the bacilli taking 30 µl of material to make a smear of this preparation. To do this, shake the bacillary suspension for 2 minutes using a vortex-type shaker, dispense 30 µl of the material in the center of a slide and extend the suspension using a clean tip to perform a smear of approximately 2x1cm. Allow to dry in a level horizontal position, then set the slide for 1 hour at 60ºC placing it on the surface of a heating plate and stain by ZN. Verify that most of the bacilli are dispersed, since the appearance of bacilli in a grouped form makes difficult to count the number of bacilli of the stock positive suspension. If a significant proportion of bacilli arranged in groups are observed, it is preferable to eliminate the processed suspension and start the procedure again.

14. If more than one tube of positive samples were being processed, those suspensions whose disposition of the bacilli in the smears may have been suitable can be brought

together, after agitation with a vortex-type stirrer of 2 minutes each tube.

15. To quantify the bacilli in the stock suspension, take 30 µl of material and dispense it in the center of a slide, as explained above. Extend the suspension using a clean tip to perform an approximately 2x1 cm extension. Using this procedure perform three smears, which will be used to evaluate the number of bacilli of the stock suspension. It is necessary to use a well leveled surface for the drying of the smears.

16. Fix the smears for 1 hour at 60ºC using the heating plate.

17. Stain the smears by the ZN technique.

18. Keep the positive suspension at 2-8ºC if it is not going to be used immediately.

19. Each of the three smears will be read by two or three readers, in such a way that the average of the 6 or 9 readings made by the 2-3 readers will be taken to calculate the bacillary load of the stock positive suspension.

20. Ideally, the stock of positives should have a concentration of between 60-80 AFB per field. If a suspension containing a lower amount of bacilli / field is obtained, centrifuge and suspend in a smaller volume of water than the original volume of the suspension. If more bacilli are obtained, dilute the suspension with distilled water. If the concentration is adjusted, carry out steps 15 to 19 of this procedure to calculate the bacillary concentration of the stock of positives.

21. Identify the tube with the stock of positives with an Arabic number and record the volume and date of preparation data in the form shown in Annex C.2.

22. This preparation can be stored in the refrigerator for several months.

Preparing the stock of negatives

Sample volume: 3-4 ml

1. Distribute aliquots of 3-4 ml of negative sputum for AFB in plastic tubes with a screw cap of 50 ml capacity.

2. Add one drop (50 µl) of formalin for each ml of sputum. Mix using a vortex type stirrer.

3. Incubate for one hour at room temperature, stir by vortex type stirrer every 10 minutes.

4. Add 1 ml of 4% NaOH (if the sputum is very consistent, add up to 2 ml to reach a final concentration always close to 1-2%).

5. Stir using the vortex type stirrer 4-5 min.

6. Add albumin 0.2% to complete the 20 ml and mix by inversion *(NOTE: this suspension can also be done using distilled water, but the use of the bovine albumin solution is recommended to favor the adhesion of the suspension to the slide).*

7. Incubate in a water bath or thermal block at 55-60ºC for 10 minutes. *(NOTE: the negative sputum should be heated for a shorter period than the positive to preserve the integrity of the leukocytes)*. This preparation is used as a

diluent in the process of dilution of the stock of positives.

8. Prepare two smears using 30 µl of the stock of negatives (following the instructions mentioned above) in order to check that the preparation is fixed to the slide and that the leukocytes have preserved their shape, before using it to dilute the stock of positives. Slides should be set for at least one hour at 60ºC. If after staining them by ZN, the preparation maintains good adherence to the slide and the appearance of the slide is similar to that of a smear prepared from a sputum sample. (with leukocytes of conserved morphology), it is considered that this suspension can be used as a diluent for the preparation of slide batches.

Notes:

- For the reading and interpretation of the results, it is important that the appearance of the smears was more or less consistent, and therefore it is beneficial to keep the number of leukocytes as stable as possible in the different dilutions. For that, it is suggested to dilute the negative sputum with distilled water (prior to the addition of NaOH), when the amount of leukocytes is high.

- Record the characteristics of the negative sample used to prepare the stock of negatives (Collection date, sample registration number and sample quality) in the Form shown in Appendix C.3. Information about the quality of the original sample (e.g. mucous, mucopurulent, white, greenish) can be useful to determine later which of them can be associated with consistent slide batches.

Dilutions

1. Using the negative suspension, make dilutions of the stock of positives to obtain concentrations of bacilli that conform to the four categories of positive results of the semiquantitative scale recommended by WHO for SM (it is recommended to prepare at least 50-100 sheets of each suspension to have enough available for the duplicates (5 stained and 5 unstained) in each panel set, (in case you have decided to send both stained and unstained panel sets).

2. Prepare about 4 ml of each suspension to be used for the Positive (3+), Positive (2+), Positive (1+) and countable(1-9 AFB) slides. With these quantities it will be possible to obtain around 120 slides of each degree of positivity (if 30 µl of suspension are used for each slide).

3. For the preparation of the most concentrated suspension use the following calculation:

$$N = DC*A / AC$$

N: ml of the concentrated positive suspension to be added
DC: desired concentration of the AFB suspension to be prepared
A: total volume of the desired suspension to be prepared
AC: real concentration AFB in the stock suspension of positives

For example, for the preparation of the Positive 3+ suspension from a stock suspension of positives with a real concentration of 80 AFB / field:

DC: the desired concentration of AFB to prepare is 50 AFB / field
A: is the volume to be prepared from the desired dilution. For ex. 5 ml
AC: the concentration of AFB in the concentrated positive suspension, in this case of the stock of positives whose concentration was obtained from the average of the 6 or 9 readings made by the 2-3 readers. For example: 80 AFB / field
N (ml of the stock of positives to add) = 50 AFB/field x 5 ml/ 80 AFB/field = 3.1 ml of stock solution of positives

The stock volume of negatives to be added can be calculated with the following formula:

X (stock volume of negatives) = final volume – N
In this example X= 5 ml – 3.1 ml = 1.9 ml

That is, to obtain 5 ml of Positive 3+ suspension, add 3.1 ml of positive stock and 1.9 ml of negative diluent.

4. For the rest of the dilutions proceed in a similar way, considering that from the Positive suspension (3+) the Positive suspension is prepared (2+), from the Positive suspension (2+) the Positive (1+) is prepared and of the Positive suspension (1+), the Positive suspension (1-9 AFB) is prepared, following the following guide:

Dilutions to obtain the positive slides					Denomination of the obtained bacillary suspension
Scale	Expected concentration of AFB	Concentration to be prepared to obtain the expected concentration of AFB (DC)	Calculation of volumes to obtain 4 ml of each suspension		
			Calculation of the volume of each dilution to be added to obtain 5 ml of each suspension	Negative stock volume to add (X)	
Positive (3+)	>10 AFB/field	50 AFB/field	50 AFB/field x 5ml/ AC= N	5ml - N	I
Positive (2+)	1-10 AFB/field	10 AFB/field	10 AFB/field x 5 ml/50 AFB/field= 1ml de I	5 ml – 1ml = 4ml	II
Positive (1+)	10-99 AFB/100 field	2 AFB/field	2 AFB/field x 5 ml / 10 AFB/field= 1ml de II	5 ml – 1ml = 4ml	III
Positive (1 -9 AFB)	1-9 AFB/100 fields	40 AFB/100 fields	40 AFB/100 fields x 5 ml/2 AFB/field= 1ml de III	5 ml – 1ml = 4ml	IV

AC: the actual concentration of AFB in the stock of positives obtained by calculating the average of the 6 or 9 microscopic readings performed by the 2-3 readers
N: ml of the stock of positives to add

Note: Note that the desired concentrations (DC) of each bacillary suspension correspond to suspensions with N° of bacilli / field greater than what would be expected according to the categories of the smear quantification scale (expected concentration of bacilli). The overall experience of several laboratories shows that the use of concentrations lower than those established in the table gives rise to batches of slides with a lower amount of bacilli per field than expected.

Operating procedure for the realization of the dilutions

1. Mark 4 tubes with 15 ml screw cap as Positive (3+) (I), Positive (2+) (II), Positive (1+) (III) y 1-9 AFB (IV) and place in a rack.

2. Place the suspensions of the stock of positives and stock of negatives in the same rack.

3. Shake by vortex-type stirrer the stock of negatives for 20 seconds and place X ml in the tube identified as Positive 3+ (I) (in the example 1.9 ml of stock of negatives)

4. Shake by vortex-type stirrer the stock of positives for 2 minutes and add the N ml of the stock of positives in the same tube (I) (in the example 3.1 ml of stock of positives)

5. Shake the stock of negatives with a vortex-type stirrer for 20 seconds and distribute 4 ml in each of the tubes II, III and IV.

6. Place 1 ml of I (previously homogenized by shaking using a vortex-type stirrer for 2 minutes) in tube II (Positive 2+) and stir 2 minutes by vortex-type shaker.

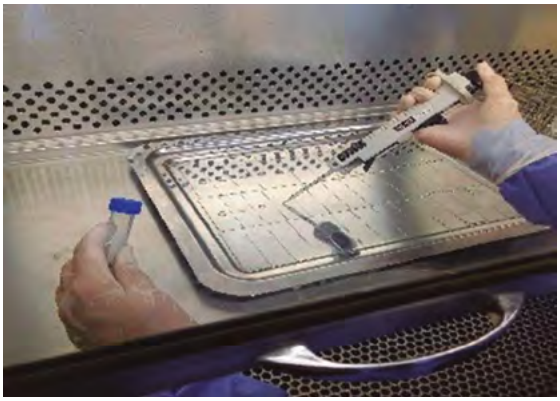
7. Place 1 ml of II in the tube marked as tube III (Positive 1+) and shake for 2 minutes using a vortex-type shaker.

8. Place 1 ml of III in the tube marked IV (Accounting (1-9 AFB)) and shake for 2 minutes using a vortex-type shaker.

9. Identify each dilution of the stock of positives (I, II, III and IV) with a unique number that corresponds to the identification of the batch of slides that will be prepared. Place each number in each tube of 15 ml containing the corresponding bacillary suspension and write it down in the "Development and validation of slide batches record" (Annex C.3). It is recommended to use sequential two-digit numbers (01, 02, 03 ... .22, 23, etc.).

10. Mix for 30 seconds each dilution before loading the tip of the multidispensing pipette to make the smear ones.

11. Dispense 30 µl of each suspension (measured with multidispensing pipette) in the center of the slides, which must have been previously placed on aluminum / plastic trays identified with the assigned lot number for the corresponding dilution (I, II, III y IV), contained in the "Development and validation of slide batches record" (Annex C.3).



Procedure for the distribution of 30 µl of bacillary suspensions on the slides using a multidispensing pipette

12. Using a clean tip of a micropipette for each of the dilutions draw an oval 2-3 cm wide by 1 cm long.

13. Allow to dry in a level horizontal position.

Validation of the slide batches

1. Take 6 slides of each batch of dilutions, identify the batch corresponding to each sheet using a marker and fix them for one hour at 60 ° C by placing them on a heating plate.

Fixing the slides on the heating plate at 60ºC

2. Staining by ZN

a. Slides must be read by at least 2 different technicians.
b. For positive 2+ and 3+ technicians can estimate the average number of bacilli per field by reading 50 fields.
c. For weak positives (Positive 1+ or 1-9 AFB) read at least 300 fields.

3. Record the readings that each reader made in the smear 6 using the Form shown in Annex C.3.

4. Calculate the average reading and the standard deviation (SD) of the readings of the total readers and register them in the Form of Annex C.3.

The calculation of the standard deviation can be done with a scientific calculator, using a spreadsheet of the Excel program or similar or using the following formula:

$$\sqrt{\frac{n \sum x^2 - (\sum x)^2}{n\,(n-1)}}$$

Where x = is the reading obtained by each reader expressed in average AFB / field counted in each slide and n is the number of readings made by readers (for example, from two readers who read 6 slides will generate 12 readings, i.e. n=12).

If the average minus two standard deviations (X - 2SD) is greater than 0, consider that the batch has consistency and make the decision to "accept" it, that is to say that in that batch the variation in the N ° of AFB per slide is considered It is small, so it can be used in a reliable way for the preparation of panel sets to evaluate the performance of microscopists.

In the following example, the readings of 2 microscopists for 6 slides of two slide batches (38 and 39) with expected results of Positive (1+) and Positive (1-9 AFB), respectively, have been recorded.

			Smear evaluation																
	Preparation of slide batches		Results of the readings (AFB average / 100 fields)																
Batch N°	N° of slides prepared	Expected result	1	2	3	4	5	6	7	8	9	10	11	12	X	SD	Consistency Yes/No	Decision	Reading result
38	120	Pos (1+)	0,1	0,07	0,15	0,50	0,30	0,54	0,80	0, 10	0,09	0,45	0,15	0,60	**0,32**	**0, 25**	Not	Rejected	
39	120	Pos (1-9)	0,0 5	0,04	0,03	0,06	0,03	0,04	0,05	0,02	0,05	0,05	0,06	0,04	0,04	0,01	Yes	Accepted	Pos (1-9)

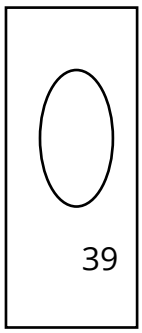
In the case of batch38, the value of the average minus 2 SD gives less than 0, so the batch lacks consistency and is rejected, while batch39 is accepted since the average of the 12 readings minus 2 SD gives greater than 0.

5. When the batch has been accepted, record this finding in the Form of Annex C.3.

6. Fix the slides with heat.

7. Identify all the slides prepared with the N° assigned to the corresponding dilution that has been noted in the Register of Annex C.3. It is recommended to engrave the assigned number with diamond pencil on the right end of the width of the slide.

In the case of the previous example, the slides prepared with the bacillary suspension corresponding to the batch 39 must be recorded as suggested in the following scheme:



6. If the batch is rejected, discard the sides.

Storage and conservation of slides batches.

1. Keep the slides fixed to the heat. They can be stored for months if stored in a cool, dry place. The exact time of conservation of the slides has not been determined. However, the experience of some regional laboratories shows that the convenient storage time is 4-6 months.

Numbering of slides for the preparation of the panel sets

Two methodologies can be used to prepare the panel sets from the slide batches:

• The NRL can produce many identical panels; In other words, all laboratories receive the same slide number, from the same batch and with the same expected results. When identical panels are used, the NRL can

complete a single Registry (Annex C.4) that can contain the results for all the slides of a certain panel set that were sent to different laboratories.

• The NRL may decide to send different panels to different laboratories, that is, prepared from the same slide batches, but numbered differently. The panel sets may differ in the order of the slides, in such a way that the number assigned to each slide will be different for each laboratory. When this methodology is used, each panel set will receive a unique number and the NRL must fill out a separate Form (Annex C.4) for each panel set sent. Although this procedure is useful to prevent the laboratories of different services from sharing the results, it is important to consider that their use will considerably an increase in the logistics and workload for the NRL.

For the numbering and registration of each of the slides of the panel set it is recommended to proceed as detailed below:

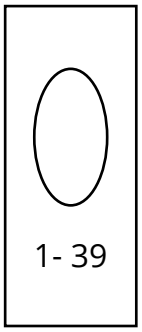
1. Identify each new panel set, assigning it a number in the Register that is shown in Annex C.4.

2. If the conformation of the panel is 10 slides, place numbers from 1 to 10 in the section "Slide N°" of the Register.

3. Using the information in the Form of Annex C.3, record in the Form of Annex C.4, the slides that will make up the panel set, identifying them for the slide batches and the expected result. Indicate also, if the panel is made up of stained or unstained smears.

4. Using the information recently registered in the Form of Annex C.4, identify each of the panel slide, by engraving the number corresponding to the slide(number from 1 to 10 for the example of 10 slides / panel set) in front of Batch N° (previously recorded with the number corresponding to the Form of Annex C.3). For example, when a slide is identified as 1-39, this would mean that "1" is the slide number of the Form in Annex C.4 and "39" is the number of the Form in Annex C.3, that is, the batch number . It is recommended to record these numbers using a diamond-tipped pencil.

Identification scheme
of the slide of a panel set (*)



(*) N° "1" corresponds to the slide number of the panel set, and "39" indicates that slide was prepared with the bacillary suspension corresponding to batch 39

Annex C.2. Positive stock registration

EQA for SM by proficiency testing

Annex C.2: Positive stock record

Stock Nº	Sample / s employed	AFB count / field	Volume	Date of process	Laboratory responsible

Instructive

Stock N°: identify with a code the stock of positives.
Sample/s used/s: identify the sample/s used for the preparation of the stock of positives.
AFB count/field: indicate the final count that was obtained. Ideally, get between 60-80 AFB per field.
Volume: indicate the final volume of the prepared stock.
Date of process: Place the processing date. Keep in mind that this preparation can be stored in refrigerator at 4-8 ° C for several months.
Laboratory responsible for the procedure: the technician responsible for the procedure.

Annex C.3: Development and validation of slide batches register

								Smear evaluation															
	Characteristics of the sample (-)				Slides preparation			Results of the readings															
Lot N°	Date of collection	Registration number of the sample	Sample quality	Stock k (+) N°	N° slides prepared	Expected result	Process date	1	2	3	4	5	6	7	8	9	10	11	12	X	DS	Consistency	Decision	Reading Result

Annex C.3. Development and validation of slide batches register

Instructive

Batch Number: Corresponds to the sequential numbers of two digits (01, 02, 03 ... .22, 23, etc.) that is assigned to each dilution of the stock of positives.

Characteristics of the negative sample

Date of collection: date on which the patient's sample was collected.

Registration number of the sample: number with which that sample was recorded in the laboratory book.

Sample quality: mucous, mucopurulent, saliva

Preparation of the slides

Stock number of positives: indicate the N° of stock of positives that has been used for the preparation of the batch (of the Form that appears in Annex C.2).

Number of slides prepared: the laboratory should note how many slides have been made from each dilution of the stock of positives to determine how many panel sets will be able to prepare

Expected result: indicate the semiquantitative result of the batch to be prepared, i.e. Pos 3+, Pos 2+, Pos 1+ o Pos (1 to 9 AFB).

Date of process: is the day they are made.

Smear evaluation

Results of the readings (AFB / 100 fields) (Columns 1-12): each column represents the average number of AFB/100 fields for 6 different slides selected from the sample and preferably read by at least 2 different readers. For 2+ and 3+ positives, the technician can estimate the AFB number / 100 fields by selecting 50 representative fields, for weak positives ("countable" or 1+) and for the negative ones, at least 300 fields should be read per spread and Record the average AFB number in 100 fields

Average and standard deviation: the laboratory can use the program in Excel or another similar one that automatically generates the data of the average columns, standard deviation and consistency. The standard deviation can also be calculated using the data in columns 1-12, with the formula:

$$\sqrt{\frac{n\sum^{2} - (\sum x)^2}{n\,(n-1)}}$$

Where x= is the reading obtained by each reader and n is the number of readings made by the readers (for example, from two readers who read 6 slides will be generated 12 readings, i.e. n = 12).

Consistency (average minus 2 standard deviations): If the average minus two standard deviations (X - 2DS) is greater than 0, the batch is considered to have good consistency. In this case, "Yes" will be placed in this column. If, on the other hand, the

consistency is not good, that is, there is great variation in the number of AFB per slide, the slides of that dilution cannot be used for the proficiency testing and they must be discarded. In this case, "No" will be placed in this column.

Decision: If the batch was consistent with this column, "Accepted" will be placed. If instead the consistency was negative, "Rejected" will be placed in this column.

Reading result: This space is reserved to indicate the result of the reading in semiquantitative terms i.e. Pos 3+, Pos 2+, Pos1+, Pos (1 to 9 AFB) and Negative.

EQA for SM by proficiency testing

Annex C.4: Registration of panel sets.

Panel Nº
Date of sending the set

Result of the panels

Slide Nº	Stained Yes/No	Batch Nº	Expected result	Laboratories										
				1	2	3	4	5	6	7	8	9	10	Observations
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														

Annex C.4: Registration of panel sets

This record is useful for recording the slides that make up the panel set and recording the expected results for the slide of each panel. It must also be used to record and evaluate the results of one or more laboratories participating in the test.

Instructive

Panel set number: This is a unique identification number for each panel set. If identical panel sets are sent to several laboratories they must all carry the same number.

Panel submission date: date the slides panel set is sent to the peripheral laboratories.

Slide N°: this is a unique number that must be marked on the sheet, in front of the Batch number. If the panel is 10 slides, this N° will be from 1 to 10.

Stained Yes / No: his column is used to record if the slides will be sent stained or unstained.

Batch N°: is the number assigned to the slides of each batch, which appears in the corresponding column in the Form of Annex C.3.

Expected results: it is the result validated by the reference laboratory that appears in the column "Reading results" in the Registry of Annex C.3. This is the expected result that must be compared with that obtained by the Laboratory that participates in the proficiency testing.

Results of readings: is the result of reading each slide reported by each Laboratory that performs the proficiency testing

Observations: optional space to evaluate the consistency of a certain slide after reading the panel sets or for other notes.

EQA for SM by proficiency testing

Annex C.5: Form for recording the reading results of panel sets

For use of the Reference Laboratory

Slide panel set N°: ....
Shipping date: .... /.... /....
Receipt date results: .... /.... /....
Service name: .....
Address: .....
City: ..... Postal Code: .....

To be completed in the service that receives the panel set

Date of receipt of the panel set: ...../...../.....
Date of sending results to the Reference Laboratory: ...../...../.....
Name of the professional/technician who performs the proficiency testing:
.....

(If there are several people in your laboratory who read sputum smears, each of them should read the microscope slides separately, and write down their results in a different form. Please do not reveal each reader or compare the results of each reader until you have sent the forms with the results to the Reference Laboratory).

Number	Readings result of the slides (*)	Comments
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

(*)Use the semiquantitative scale suggested by national standards:
Neg (-); 1-9 AFB; Pos 1+; Pos 2+; Pos 3+

Date: ...../...../..... Signature: .....

Annex C.5. Form for recording the reading results of the panel sets

It is a standard form that is sent together with the panel set to be completed by the participating laboratory technicians and returned to the Reference Laboratory. Part of this information must be completed by the Reference Laboratory and the rest by the participating Laboratory. Include the INSTRUCTION FOR THE READING OF PANELS SETS on the back of this form.

Instructive

To be completed by the Reference Laboratory

Slide panel set number: it is the same panel number as the Registry in Annex C.4. This allows the RL to compare the results of the Laboratory technicians with the expected results recorded in the Registry of Annex C.4.

Shipping date: date the sheet panel is sent.

Receipt date of results: date on which the results are received in the RL.

Name and address of the service to which the panel set is being sent.

To be completed in the service the receives the panel set

Date of reception of the panel: date on which the panel set is received by the participating Laboratory

Date of the results submission to the Reference Laboratory: date on which the results of the panel set are sent to the RL.

Name of the professional / technician: name of the professional / technician who performs the test. If there are several technicians that participate on the test, they must register their results on separate forms.

Table of results of the test.

Slide number: the numbers of the slides of each panel set and usually correspond to 1 to 10.

Results of the local laboratory: The microscopist must record the result of the slides reading

Date: the date on which the table with the reading results was completed by the microscopist.

Signature: is the signature of the technician who made the reading.

INSTRUCTION FOR THE READING OF SLIDES PANEL SETS (*)

1. Concept

This test is used to determine the skills of the laboratory personnel in performing the reading and reporting of smear results; it is done by sending a slide panel set prepared at the national reference laboratory (NRL).

2. Process

2.1. Readings

You received a panel of slides prepared in the NRL, composed of stained and unstained slides. We suggest that they have to be read by the person or persons who are in charge

of this task in your laboratory and, as far as possible, with the same dedication as they usually do.

Each technician must examine the slides and report separately; the results should not be shared with other technicians. In case there are more than one people who make readings, we recommend duplicating this form in as many copies as microscopists are going to be evaluated. In this case it is convenient that each form is identified by the name of the reader or by a code chosen at random by the reader himself.

The results of the readings must be placed in the column "Readings result of the slides".

2.2. Slides conservation

If the slides are not going to be stained immediately, we recommend keeping them in a dry place, since, in the presence of excessive humidity, the smears tend to detach during the staining. The unstained slides panel must be stained by one of the technicians and read by the person/s that are responsible for this task independently, as mentioned above.

3. Sending of forms with the readings reports

After completing the test, we recommend that you remove the excess of oil from the slides, leaving them upright on an absorbent paper until the next day and then gently placing on another clean absorbent paper over them. Once clean, the panel set, together with the forms with the reports, should be sent to the Reference Laboratory.

The term for the test realization has been established in a month from the reception of the panel at the laboratory.

4. Results comparison

You will receive as soon as possible a form in which the slides reading results made at the Reference Laboratory were added. This report will be confidential and individual.

We already appreciate your willingness to achieve together the best quality in the diagnosis of tuberculosis.

(*) Example for a proficiency testing that includes stained and unstained slides, and includes the return of the panel to the RL along with the results report. This instruction must be modified according to the characteristics of the test designed for each laboratory network.

EQA for SM by proficiency testing

Annex C.6: Form of the results report of the reading of panel sets

Service name: .....
Address: .....
City: .....Postal Code: .....
Slide panel set N°: ....
Date of receipt of the set: ...../...../.....
Date of sending results to the Reference Laboratory: ...../...../.....
Name of the professional or technician who performs the proficiency testing: .....
.....

Number	Participant laboratory result	Reference laboratory result	Type of error (*)	Score
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Discordances (**)
Nº LFP= Nº HFP= Nº LFN= Nº HFN= Nº QE=
Score obtained =
Observations:
Recommendations:

Date: ...../...../..... Signature: .....

(*) Complete with the type of error identified after the disagreements were corroborated by the LR (HFP: High False positive; HFN: High false negative; LFP: Low False positive; LFN: Low false negative; QE; quantification error).
(**) Complete with the number of errors of each type after the disagreements were corroborated by the RL.

Annex C.6. Form of the results report of the reading of panels sets

This form has been prepared to make the report of the results of the readings of each microscopist.

It has a header with the name and address of the laboratory where the participating microscopist/s works. It also includes the identification of the panel set read, the date of receipt of the set and the date of sending results to the RL (both dates must be obtained from the information provided in the Form in Annex C.5). In addition, the professional or technician who performs the proficiency testing is identified.

Instructive

Table of results of the proficiency testing

Slide number: the numbers of the slides of each panel set and usually correspond to 1 to 10.

Participant laboratory result: the result of the slides reading informed by the microscopist (of the Form of Annex C.5).

Reference laboratory results: the expected reading for each slides is placed here, after rereading the smear in the RL in case of discordance (when the protocol of the test foresees the return of the slides).

Type of error: in the case of disagreements, these will be indicated in this column, identifying the type of error as described in Table 5 of the Manual.

Score: is the score assigned to each slide according to the score system established by the RL.

Disagreements: complete the number of errors of each type.

Score obtained: is the total score obtained according to the scoring system established by the RL based on the classification of the errors and the sum of the scores obtained for each slide that appear at the "Score" column of the Table.

Observations: the probable causes of error and their consequences.

Recommendations: the recommendations to correct the possible causes of the identified errors.

EQA for SM by proficiency testing

Annex C.7: Annual evaluation report by panels

Province / state / department _____

Period in wich the test was performed _____

Approval criteria: _____

Laboratory	Annual volume of smear microscopy	Positive rate (%)	Technicians participating in the test	Score	HFP	HFN	LFP	LFN	QE	Total errors
Totals and district averages										

HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative; QE: Quantification error

Report prepared by _____ Date _____

Annex C.7. Annual evaluation report by panels

This form is used to record in condensed form the results obtained by the participating laboratories of the test in an area / region / department during a given period.

Instructive

Province / state / department: identify the area to which the information corresponds.

Period in which the test was performed: indicate the period to which the report corresponds.

Approval criteria: establish the score and requirements for the passing of the proficiency testing.

Summary information table

Laboratory: indicate the name of the laboratories participating in the proficiency testing.

Annual volume of smear microscopy: indicate the total number of sputum smears performed by the participating laboratory during the period covered by the results report.

Positive rate: indicate the percentage of positive sputum smears of the participating laboratory during the period covered by the results report.

Technicians participating in the test: identify the technicians participating in the test in each of the laboratories included in the evaluation. One line per participant is used. Observe that the data of annual volume of smear microscopy and percentage of positivity correspond to the laboratories in which the participating microscopist of the proficiency testing works.

Score: indicate the score obtained by each microscopist.

HFP, HFN, LFP, LFN, QE and Total errors: Complete the number of errors of each type found.

Totals and district averages: indicate the total number of sputum smears and the average positivity rate of the laboratories in the area to which the report corresponds. Also indicate the totals for each type of error and for all the errors committed by the microscopists.

Report prepared by: Indicate the person responsible for the report and the date of its completion.

Annex C.8. Examples of the proficiency testing report for the smear microscopy

Example 1

The following is a model of the results report of the Regional Laboratory prepared by the RL based on the results of the reading of two panels of slides, one stained and the other not stained. Each of them was composed of 10 slides.

Results report of the reading of panel sets

Service Name: Regional Hospital
Address: XXX
City: AAAA **Postal Code:** OOOO.
Slide panel set N° 8/17
Date of receipt of the set: 15/11/17
Date of sending results to the Reference Laboratory: 31/11/17
Name of the professional or technician who performs the proficiency testing: AN
Stained panel

Number	Participant laboratory result	Reference laboratory result	Type of error (*)	Score
1	Pos (+)	Pos (+)		10
2	Neg	Neg		10
3	Pos (+)	Pos (+)		10
4	Neg	Neg		10
5	Pos (6 AFB)	Pos (1-9 AFB)		10
6	Neg	Neg		10
7	Pos (++)	Pos (++)		10
8	Neg	Neg		10
9	Neg	Neg		10
10	Pos (++)	Pos (++)		10

(*)HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative; QE: Quantification error

Discordances
Nº LFP= 0 Nº HFP= 0 Nº LFN= 0 Nº HFN= 0 Nº QE= 0

Score obtained = 100

Observations: Excellent reading!

Results report of the reading of panel sets

Service Name: Regional Hospital
Address: XXX
City: AAAA **Postal Code:** OOOO.
Slide panel set N° 9/17
Date of receipt of the set: 15/11/17
Date of sending results to the Reference Laboratory: 31/11/17
Name of the professional or technician who performs the proficiency testing: AN

Unstained panel

Number	Participant laboratory result	Reference laboratory result	Type of error (*)	Score
11	Neg	Neg		10
12	Pos (+)	Pos (++)		10
13	Neg	Pos (1-9 AFB)	FNL	5
14	Neg	Neg		10
15	Neg	Pos (+)	FNH	0
16	Neg	Neg		10
17	Neg	Neg		10
18	Pos (++)	Pos (+++)		10
19	Neg	Pos (+)	FNH	0
20	Neg	Neg		10

(*)HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative; QE: Quantification error

Discordances
Nº LFP= 0 Nº HFP= 0 Nº LFN=1 Nº HFN=2 Nº QE=

Score obtained = 75

Observations: Two HFN and one LFN errors were identified. These errors could be determined only after restaining the slides of the panel, clearly indicating the existence of problems in the quality of the staining solutions or in the staining technique. Additionally, in the positive slides (N° 12 and 18) returned by the local laboratory to the RL, the bacilli were observed pale pink, coinciding with the identification of problems in the staining solutions/staining. The fact that, using the stained panel (N° 8/17), the technician has shown adequate performance, evidence that the errors identified in this panel are associated with staining problems.

Recommendations: Check the concentration of fuchsin in the staining solution, ensure that fuchsin heating is up to the release of white vapors, control the exposure time of hot fuchsin on the smear (which should not be less than 5 minutes), check the expiration date of the staining solutions, as well as to the conditions of their preservation. Additionally it is recommended not to overheat the smears during suffixing. The accomplishment of a visit to the laboratory will be coordinated.

Date: 14/12/17 SIGNATURE: .....

Example 2

The following is a model of report of results of the Regional Hospital Laboratory elaborated by the LR based on the results of the reading of a stained panel set composed of 10 slides.

Results report of the reading of panel sets

Service name: Regional hospital
Address: XXXX
City: AAA **Postal Code:** OOOO
Slide panel set N°: 3/17
Date of receipt of the slides: 14/03/17
Date of sending results to the RL: 25/03/17
Name of the professional or technician who performs the proficiency testing: O.P

Number	Participant laboratory result	Reference laboratory result	Type of error (*)	Score
1	Neg (-)	Pos (1-9)	FNL	5
2	Pos (+)	Pos (+)		10
3	Neg (-)	Neg (-)		10
4	Neg (-)	Neg (-)		10
5	Neg (-)	Neg (-)		10
6	Pos (+)	Pos (+)		10
7	Pos (++)	Pos (++)		10
8	Neg (-)	Neg (-)		10
9	Pos (++)	Pos (++)		10
10	Neg (-)	Neg (-)		10

(*) HFP = High False Positive; HFN = High False Negative; LFP = Low False Positive; LFN = Low False Negative; QE: Quantification error

Discordances
Nº LFP= 0 Nº HFP= 0 Nº LFN= 1 Nº HFN= 0 Nº QE=0

Score obtained = 95

Observations: An LFN error was identified. This type of finding is considered a minor error. Its occurrence is associated with a limitation of the sputum smear technique; because AFB are distributed in sputum in non-homogeneous form, when the number of bacilli is scarce, as in a sample whose result has been reported with the exact number of 1 to 9 AFB, it is possible for a technician to identify these AFB when reading 100 microscopic fields, while another technician, that examines 100 different microscopic fields is not able to find them.

Recommendations: It is recommended to read carefully using the micrometer screw to observe all the planes of the smear, examining at least 100 fields to declare a slide as negative.

Date: 20/03/17 SIGNATURE: .....

ANNEX D - CULTURE

Annex D.1. Data collection form for culture monitoring

Culture performance for the diagnosis of tuberculosis

Complete the following information and send before **XX/XX/20XX**, to the following address:
Name of the laboratory and Institution that organizes the control or collects the information
Address of the Institution that organizes or collects the information
Tel/Fax: ***Email:***

Participating laboratory: .....

A. METHOD APPLIED FOR CULTURE:

A.1 –Use own mediums yes ☐ no ☐

In the event that your answer is NO to include the name of the laboratory medium provider or trademark .....

A.2 - What samples do you grow?

a) ALL which you receives to investigate AFB/ SM ☐
b) CHOOSE The following samples (indicate): .....
.....
.....

A.3 - Do you receive derived samples? yes ☐ no ☐

Name the sampling centers, health services or laboratories that derive from it
.....
.....
.....
.....
.....

Number of samples of other services received during the period to be analyzed in B2a, B2b, B2c o B2d.....

A.4 - What method (s) do you usually use to process the samples you grow?

a) decontamination and concentration by the Petroff technique ☐
b) decontamination and concentration by the modified Petroff technique ☐
c) the one indicated by the manufacturer for the BACTEC MGIT system ☐
d) the one indicated by the manufacturer for the BacT/ALERT system ☐
e) decontamination and planting by the Kudoh technique ☐
f) others: ..... ☐

A.5 - What type of sample is sown in liquid medium (if available)?
.....
.....

A.6 -Receive in the culture application form, in most cases, information about:

	yes	no
a) If the samples you grow are for diagnosis or treatment control		
b) the month of anti-TB treatment in which the patient is		
c) if the patient has a history of previous treatment		
d) if the patient has immunosuppression		
e) if the patient belongs to some other risk group for resistance		

B- WORK ROUTINE:

B.1 - How many sputum samples are asked to diagnose tuberculosis? .....

B.2 - Select the results obtained from **CASES** with **RESPIRATORY SAMPLES OF ADULT PATIENTS PROCESSED FOR DIAGNOSIS** corresponding to the period **(XX/XX/20XX - XX/XX/20XX)**, and complete (according to the methodology used) the following tables with the cultivated samples:

B.2a - If you could distinguish **to patients whose samples were processed for diagnosis,** classify **THE LUNG CASES of adults (NOT the samples)** in the next table:

	Cases with:	Number (cases)
a	Positive smear microscopy and Positive culture of MTBC	
b	Positive smear microscopy and culture not performed	
c	Negative smear microscopy and Positive culture of MTBC	
d	Positive smear microscopy and Negative culture	
e	Positive smear microscopy and contaminated culture	
f	Smear microscopy not performed and Positive culture of MTBC	
	Patients with negative smear and negative culture	

Contribution of the culture to diagnosis = $\frac{c}{a + b + c + d + e}$ **x 100 = ... %**

B.2b - If you could distinguish **to patients whose samples were processed for diagnosis,** classify **the lung CASES of adults (NOT the samples)** in the next table:

	Cases with:	Number (cases)
a	Xpert positive and Positive culture of MTBC	
b	Xpert positive and Culture not performed	
c	Xpert negative and Positive culture of MTBC	
d	Xpert negative and Positive culture of MTBC	
e	Xpert positive and Contaminated culture	
f	Xpert not performed and positive Culture of MTBC	
	Patients with negative Xpert and negative culture	

Contribution of the culture to diagnosis = $\frac{c}{a + b + c + d + e}$ **x 100 = ... %**

B.3. Consider all respiratory samples (diagnostic and control treatment) seeded in liquid medium (if applicable) and solids and indicate

a) N° of tubes of solid medium seeded: .....
b) N° of tubes of solid medium seeded that were contaminated: .....
c) N° of tubes of liquid medium seeded: .....
d) N° of tubes of liquid medium seeded that were contaminated: .....

B.4 - Identify the patients included in categories a and b:

- Check if you received sputum samples from each of them to perform the treatment control - smear microscopy, Xpert and culture (during the 6 months after diagnosis).
- How many samples did you receive in total from all of them for treatment control? ....

B5. Analysis of the delay in the delivery of culture reports

B5.a. Samples planted on solid medium

N° of culture reports issued at term reported within the

- 21 days (smear positive and / or positive Xpert) of processed sample: .....
- 63 days (negative smears or Xpert negative or traces) of processed sample: .....

Total number of culture reports issued: .....

B5.b. Samples seeded in liquid medium

N° of culture reports issued at term reported within the

- 8 to 10 days (smear positive and / or positive Xpert) of processed sample: .....
- 43 days (negative smears and/or negative Xpert or traces) of processed sample: ....

Total number of culture reports issued: .....

B.6 - Please attach a photocopy to this form:

- The form model you receive, requesting culture of the sample.
- The last complete sheet you have of the record your laboratory uses for samples received for TB diagnosis by culture.
- The last positive culture report and the last negative culture report that your laboratory has issued.

SIGNATURE: .....

Clarification .....

Annex D.2. Culture performance form

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
														Baciloscopy (B)								Gene Xpert Rif TB (X)							
1	Laboratory		Period	Processed samples (n)	Worked days	Total seeded tubes	Samples processed per day (Average)	Tubes		Samples				Cases						Patients	% Contribution of the culture to the diagnosis	Cases						Patienets	% Contribution of the culture to the diagnosis
2								Contaminated tubes		Total bacteriology +		B+C+		B+ C+	B+ C nr	B- C+	B+ C-	B+ Cctdo	Bnr C+	B- C-		X+ C+	X+ Cnr	X- C+	X+ C-	X+ Cctdo	Xnr C+	B- C-	
3	Name	Code						n	%	n	%	n	%																
4	Hospital xxxxxxxx	c	01/09/2009 al 31/12/2009	406	85	1218	4,8	5	5,9	16	3,9	13	81,3	13	0	2	0	1	0	390	12,5	16	0	2	0	1	0	370	10,5
5							D5/E5		H5/F5 *100		J5/D5 *100		N5/J5 *100								Q5/ (N5+ P5 +Q5 +R5 +S5) *100								Y5/ (W5 +X5 +Y5+Z5+ AA5) *100
6							D6/E6		H6/F6 *100		J6/D6 *100		D6/J6 *100								Q6/ (N6+ P6 +Q6 +R6 +S6) *100								Y6/ (W6 +X6 +Y6+Z6+ AA6) *100
7																													
8																													

The values of this form are those extracted from Annex D.1. The formulas expressed in the columns are to minimize errors and represent the combination of different columns.

Thus, by way of example, to calculate the % of contaminated tubes in column I, arises from the division of the number of contaminated tubes recorded in column H and the number of tubes planted, recorded in column F, and multiplied by 100 to express the result as a percentage. These formulas can be easily placed with an Excel spreadsheet or similar.

Annex D.3. Template for the monitoring of culture performance in relation to the quality of the medium

The colors indicate the performance of laboratories in the different controls. In this way you can quickly visualize those laboratories that have problems.

In the case of laboratories that receive mediums of elaborating laboratories (code C in the example), it is important to clarify as comment the number and name of the laboratories to which they are given mediums (as specified in the blue table of the example). In this way, whenever there are problems in the contribution of the culture, the association or discard between this parameter and the sensitivity of the medium is facilitated.

Annex D.4.Culture quality indicator report

COOPERATIVE STUDY INTERLABORATORIES 8th control
NATIONAL NETWORK OF TUBERCULOSIS LABORATORIES
INSTITUTION XXXX
YEAR XXXX

Table 1: CULTIVATION QUALITY INDICATORS

Laboratories that identify samples for diagnosis and plant in solid medium

Laboratory Code	Period evaluated	Processed samples	Bacteriologically positive pulmonary samples			Contaminated tubes	Contribution of the Culture to the diagnosis of TB	Reports submitted to term (MS)	Reports submitted to term (ML)
			Total		B+ C+				
		n	n	%	% (*)	% (**)	% (***)	%	%
C	(a)	406	16	3,9	99,0	0,2	12,5	92,0	
E	(a)	357	15	3,2	89,0	3,9	50,0	89,0	
E2	(a)	8	0	0,0	97,0	12,5	0,0	98,0	
E3	(a)	111	14	12,6	96,0	8,1	35,7	96,0	
E4	(a)	130	6	4,6	99,0	3,8	9,1	95,0	

MS: Medium Solids - ML: Liquid Medium
(*) in relation to the total of pulmonary samples with smear positive and / or Xpert positive or cultured
(**) in relation to the total of tubes planted
(***) in relation to the total cases of pulmonary tuberculosis diagnosed by bacteriology (smear microscopy and / or Xpert)
(a) information corresponding to the period 01/01/2016 - 12/31/2016

Annex D.4.1 Example of culture performance results and observations made

QUALITY CONTROL OF CULTURE MEDIA
COOPERATIVE STUDY OF INTERLABORATORIES XXXX
NATIONAL NETWORK OF TUBERCULOSIS LABORATORIES
Institution:.....

Crop Quality Indicators
Laboratories that prepare culture media
June-September of XXXX
laboratories that identified samples for diagnosis and cultured all samples received from symptomatic

Laboratory Code	Processed samples	Bacteriologically positive pulmonary samples			Contaminated tubes	Contribution of the Culture to the diagnosis of TB	Reports submitted to term (MS)
		Total		B+ C+			
	n	n	%	%(*)	%(**)	%(***)	%
C	682	29	4.3	98.0	6.2	35.5	95.0
D	116	9	7.8	99.0	4.3	22.0	99.0
H	571	20	3.5	0.0	2.3	44.0	94.5
I	409	18	4.4	85.0	0.0	12.0	96.0
M	2505	159	6.3	96.0	2.4	18.3	96.0
AI	419	24	6.5	87.0	9.4	20.0	93.0
AJ	569	17	3.0	95.0	3.2	23.0	87.0
AM	434	31	7.1	97.0	2.9	38.2	96.0
AP	1677	106	3.9	99.0	4.5	13.0	94.0

MS: Medium Solids - ML: Liquid Medium
(*) in relation to the total of pulmonary samples with smear positive and / or Xpert positive or cultured
(**) in relation to the total of tubes planted
(***) in relation to the total cases of pulmonary tuberculosis diagnosed by bacteriology (smear microscopy and / or Xpert)

Annex D.4.2 Example of monitoring report on culture performance parameters

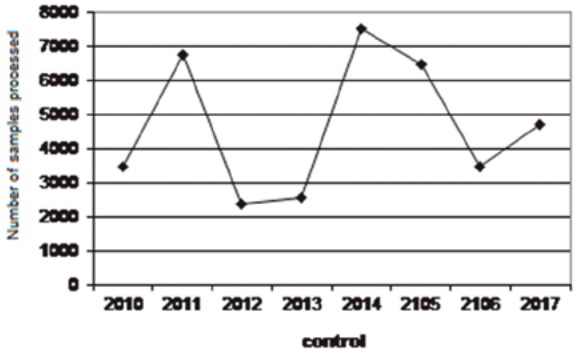
After the second or third year of data collection, follow up and report it. This allows to evaluate random or persistent deviations that can be produced by the personnel of the laboratory or alien to the same.

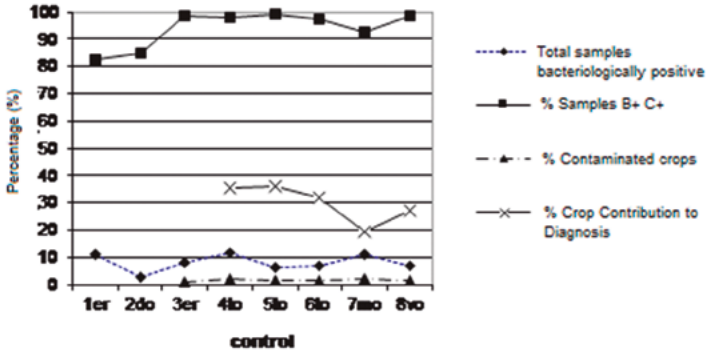
FOLLOW UP OF CULTIVATION QUALITY INDICATORS (2010 - 2016)

HOSPITAL XXXXXXXX

PULMONARY SAMPLES OF ADULT PATIENTS PROCESSED FOR DIAGNOSIS

Control	Evaluated Perior	Processed samples			Lung samples			Percentage of Contamination	Contribution of the Cultivation at TB diagnosis
		in the evaluated period	per day (estimated)	By year (estimated)	Total		B+ C+		
		n	n	n	n	%	% (*)	% (**)	% (***)
1er	01/01/2010 al 31/06/2010	1735	13	3470	195	11,2	82,5		
2do	01/05/2011 al 31/08/2011	2245	26	6735	58	2,6	85,0		
3er	01/07/2012 al 31/12/2012	1176	9	2352	96	8,2	98,4	0,9	
4to	01/01/2013 al 31/04/2013	853	10	2559	97	11,7	98,1	2,1	35,3
5to	01/06/2014 al 31/09/2014	2505	28	7515	159	6,3	99,0	1,6	35,9
6to	01/05/2015 al 31/08/2015	2155	24	6465	143	6,6	97,6	1,3	31,8
7mo	01/09/2016 al 31/12/2016	1145	13	3435	128	11,2	92,6	2,3	19,1
8vo	01/09/2017 al 31/12/2017	1559	18	4677	110	7,1	98,6	1,4	27,3
Expected values						4 - 8 %	>= 95%	3 - 4 %	at least 20%





(*) in relation to the total of pulmonary samples with smear positive and cultured

(**) in relation to the total of tubes planted

(***) in relation to the total cases of tuberculosis diagnosed by bacteriology

No information

Laboratory	Observations / Conclusion / Recommendations
C	The contribution of culture to the diagnosis continues to be very good and there are no signs of alarm detected in the indicators. However, we detected, between the results obtained in June-September of xxxx with samples of patients investigated for diagnosis, three highly positive samples per culture (++) that had negative sputum smear and three other samples with smear positive (+) that had culture negative. These signs could show some circumstantial problem in the culture, so we will keep the attention on them. But they could also show some defect in the smear reading. The institute has not registered its participation in smear quality control during the analyzed period. We suggest maintaining regularity in that external control.
D	The contribution of the culture in its work routine is good and the celerity of the diagnosis is adequate.
H	The high contribution of the culture to the diagnosis of tuberculosis and, on the contrary, the low contribution of the smear microscopy in its routine of work may be showing that it is investigating bacteriologically cases of pulmonary tuberculosis not advanced and not to the chronic respiratory syntomatic that are more frequent investigated by other laboratories of the tuberculosis network. Since the Xpert diagnosis has been implemented recently, it is recommended to review possible false negatives of the test associated with malfunction problems of some of the modules (see table A of the alarm signals on page 59 of the manual).
I	We draw attention to the three indicators corresponding to the period July-September xxxx: a level below that indicated in all was evidenced. Given that there is a decrease in the three indicators at the same time, it is recommended to review in particular point F of the alarm signal box on page 59 of the manual. Most likely, the decontamination that is being applied to the samples is too energetic. Also, check points G and H to rule out another cause. On the other hand, we called attention to a highly positive sample by culture (++) with negative sputum smear, in which case it could very probably have been rapidly diagnosed by smear microscopy. We suggest reviewing the sputum smear technique according to the observations of the institute.
M	The contribution of the culture to the diagnosis of tuberculosis is good. We call attention to a pulmonary sample of a patient investigated for highly positive diagnosis by culture (++) that had a negative sputum smear during the period June-September xxxx. We suggest investigating the possible cause of this result and reviewing the smear corresponding to that sample.

AI	The contribution of the culture in the work routine is good. Keep the percentage of contaminated culture under monitoring since it is a little high. It is recommended to check the points C, D and E of the alarm signals on page 59 of the manual given that the values are not excessively high it is recommended to analyze the percentage of contamination according to the places where the samples come in order to be able to show some problems related to the transport of the samples
AJ	The contribution of the culture in the work routine is good. The Stonebrink medium can be beneficial to increase culture sensitivity. The combination of media not only facilitates the development of some strains that have particular requirements but also minimizes the risk of false negative results in the culture caused by some batch of medium that may be defective. Try to improve the response time to the laboratories (If it is personnel or it is only an administrative inconvenience)
AM	His laboratory maintains good quality of culture evidenced as much by the good quality of the medium as by the good performance of the technique for the diagnosis of tuberculosis in pediatrics. The implementation of the molecular biology system (Xpert) in its institution is valued. We called attention to two pulmonary samples from two cases investigated for diagnosis during the June-September 2016 period, positive Xpert (high signal) and negative culture. We suggest checking if it is a patient in treatment.
AP	His laboratory evidenced low contribution of culture for the population and the prevalence that is handled in his area. In addition, the methodology you are using (smear microscopy) does not tend to lower culture efficiency. No deviations were observed with the values of the other two indicators, so it is recommended to check point B of the alarm signal box on page 59 of the manual.

Annex D.5. Culture Form - Medium elaborator

This form is used to collect information from those laboratories that are producers of culture medium for their own use or the network.

QUALITY CONTROL CULTURES INTERLABORATORIES
EMPLOYEE FOR THE DIAGNOSIS OF TUBERCULOSIS
National Network of Tuberculosis Laboratories

Take at random 12 tubes from each batch of medium prepared in your laboratory. Label them, arrange them in a box protecting them from breakage. This sample of culture medium must be sent before **XX / XX / 20XX,** accompanied by the information requested in form A, to the following address:

Enter the name of the laboratory and Institution that organizes the control, its address, Tel / Fax and Email

Information about the producer laboratory

Do you provide a laboratory? yes ☐ no ☐
If so:
To which? .....
.....

Did you stop providing culture medium to a network laboratory during the last two years?
yes ☐ no ☐
If so:
To which? .....

For what reason? .....
.....

Medium sent for control	**Batch nº**	**Preparation date**
Löwenstein Jensen		
Stonebrink		
Middelbrook 7H10		
Middelbrook 7H11		

Do you prepare another type of medium that you did not send for control?
yes ☐ no ☐

Which?.....
.....

A- METHODOLOGY APPLIED IN THE ELABORATION OF CULTURE MEDIUM:
A.1.Indicate the brand of the products used to produce the medium from which a sample is sent:

Drugs	**Brand**	**Lot nº**	**Expiration date**
Commercial medium packed in tubes (ready to use)			
Commercial base			
Prepare the medium with:			
Monopotassium phosphate			
Magnesium sulphate			
Magnesium Citrate			
L-asparagine			
Glycerin			
Malachite green			
Sodium pyruvate			
Disodium phosphate			

A.2. Where does the medium coagulate base on eggs? coagulator ☐ stove ☐ autoclave ☐

Other (describe): .....
Coagulation time and temperature: .....

A.3. Do you control the temperature of the equipment you use to coagulate during the process? yes ☐ no ☐

If yes, do you register it in a form? yes ☐ no ☐

A.4. How does the prepared medium preserve?
Room temperatura ☐ Refrigerator ☐ Stove ☐ Freezer ☐

How much time, on average, does the medium keep after being prepared? .....
.....

A.5. What quality controls do you perform on each batch of culture medium you prepare?
Development ☐ Sterile ☐ Sensitivity ☐ Consistency ☐

Specify how you perform them
.....
.....
.....
.....
.....

A.6. Detail all batches of medium prepared during **20XX** in the following table

Löwenstein Jensen neutro			Stonebrink neutro					
Lot	Preparation date	Volume ml	Lot	Preparation date	Volume ml	Lot	Preparation date	Volume ml

SIGNATURE: ..... Clarification: .....

Annex D.6.Preparation of aliquots for the inoculum

1- Take the reference strain of M. tuberculosis and M. bovis pansensible to drugs.

2- Label with the name and / or number of the strain or isolation the bottle or tube with glass beads.

3- With a disposable bacteriological handle, scrape the entire surface with bacterial development, avoiding taking culture medium. In case of using non-disposable handle, cool it before raising the bacillary mass (hot handle kills all microorganisms).

4- Download all the bacillary mass inside the tube with glass beads, making revolving movements of the handle on the pearls.

5- Add 1 or 2 drops of sterile distilled water, cover and vortex for 1 minute; let stand 5 minutes so that possibly formed aerosols fall.

6- Add approximately 1 or 2 ml of sterile water and vortex again for 1 minute.

7- Leave the suspension at rest for 15 minutes.

8- With a Pasteur pipette, transfer the supernatant from the tube with beads to a new tube with a screw cap, taking care not to stir the sediment.

9- Add sterile distilled water little by little until the turbidity of a McFarland 1 suspension is equalized.

10- Distribute 0.5 ml of this suspension in 2ml plastic tubes with external thread.

11- Freeze at -70°C until a new inoculum needs to be prepared.

Annex D.7.Preparation of inoculum for control

1- Thaw one of the *M. tuberculosis* and / or *M. bovis* aliquots prepared as described in Annex D.6.

2- Carry out a subculture with the strain *M. tuberculosis* in LJ and / or the *M. bovis* strain in Stonebrink according to the medium to be evaluated.

3- Incubate at 37 ° C and harvest in exponential stage of development (approximately 15 - 20 days). Very young or old cultures can give variable results.

4- Label a vial or tube with glass beads with the name and / or number of the strain or isolate.

5- With a bacteriological handle scrape the entire surface with bacterial development, avoiding taking culture medium. In case of using a non-disposable handle, cool it before lifting the bacillary mass (the hot handle kills the mycobacteria).

6- Download all the bacillary mass inside the tube with glass beads, making rotational movements of the handle on the pearls.

7- Add 1 or 2 drops of sterile water, cover and vortex for 1 minute; let stand 2 or 3 minutes to lower the possible aerosols formed.

8- Add approximately 1 ml of sterile water and vortex again for 1 minute.

9- Leave the suspension at rest for 15 minutes.

10- With a disposable Pasteur pipette, transfer the supernatant from the tube with beads to a new tube with a screw cap, taking care not to stir the sediment.

11- Add little by little the sterile distilled water until the turbidity of a McFarland 1 suspension or the one of 1mg / ml of M. bovis BCG measure the optical density. You can also use the spectrophotometer, reading at 400 nm, the OD of the suspension.

12- With micropipette or pipette, transfer 100 µl of the suspension to a tube with 9.9 ml of distilled water (dilution 1:100 10^{-2}).

13- With micropipette or calibrated pipette, transfer 1 ml of suspension 10^{-2} to a tube with 9 ml of sterile distilled water (dilution 1: 1000-10^{-3})

14- With micropipette or calibrated pipette, transfer 1 ml of suspension 10-3 to a tube with 9 ml of sterile distilled water (dilution 1: 10000-10^{-4})

15- With micropipette or pipette transfer 1ml of suspension 10^{-4} into a tube with 9 ml of distilled water (dilution 1: 100000-10^{-5}).

16- Seed with calibrated pipette 100 µl per tube in at least 2 tubes for each dilution, of the dil 10^{-3}, 10^{-4} y 10^{-5}.

17- Incubate for 60 days at 37°C and perform the CFU count at 20 and 60 days.

18- Register in the form presented in Annex 8.

19- Define the optimal dilution for the experience. It should allow 20-50 CFU in the volume to be sown per tube. A dilution close to 1/5000 is usually required, starting from a suspension with turbidity equal to N° 1 of McFarland.

Annex D.8. Inoculum test. Colony count record

	Reading 20 days											
	LJ (M Tuberculosis)						ST (M bovis)					
	TUBE					Average of colonies	TUBE					Average of colonies
Dilutions	1	2	3	4	5		1	2	3	4	5	
-2												
-3	250	>200	>250	>250	>200	>200	123	156	140	220	214	170,6
-4	18	36	22	45	30	30,2	37	24	43	35	31	34
-5	6	0	1	1	3	2,2	1	4	5	5	8	4,6
	Reading 60 days											
	LJ (M Tuberculosis)						ST (M bovis)					
	TUBE					Average of colonies	TUBE					Average of colonies
Dilutions	1	2	3	4	5		1	2	3	4	5	
-2												
-3	>200	>200	>200	>200	220	>200	216	153	142	233	170	182,8
-4	19	37	43	46	32	35,4	44	24	45	35	31	35,8
-5	6	0	0	1	1	1,6	1	6	5	4	8	4,8

Annex D.9. Quantification of the inoculum, monitoring of results

		Inoculum						Dilution end of the inoculum	Development UFC (average)	
		Reading (days)								
Year of the experience	Medium	H37Rv or isolation sensitive	AN5 or isolation sensitive	Initial	1a dil	2da dil	3a dil		1st control	2nd control
2015	LJ	20		MF#1	1/1000	1:5		1:5000	72	57
	ST		20	MF#1	1/1000	1:3		1:3000	65	79
2016	LJ	22		DO 0.24 (MF#1 400nm)	1/1000	1:4		1:4000	86	90
	ST		21	DO 0.24 (MF#1 400nm)	1/1000	1:4		1:4000	80	85
2017	LJ	15- 19 (1st, 2nd control)		DO 0.24 (MF#1 400nm)	1/1000	1:4		1:4000	125	97
	ST	15- 19 (1st, 2nd control)	15	DO 0.24 (MF#1 400nm)	1/1000	1:4		1:4000	82	75

LJ: Lowenstein Jensen, ST: Stonebrink

Annex D.10. Data registration form

This registry is only a guide that can be modified according to the characteristics of each network. It is recommended to use Excel and place both parts in the same tab in order to visualize all the fields in the same spreadsheet.

Part 1. Information from the laboratory that sends the medium

Laboratory to be evaluated							Invitation		Type of establishment		
Code assigned	Professional in charge	Institution	Service	City	Province	e-mail	Shipping e-mail	Recieved e-mail	Public	Private	Company

Part 2. Information of the mediums sent for the control

Arrival date medium and return	LJ	ST	7H11	Medium Base Commercial	Medium ready for use	Brand	Lot and Expiration	Medium Lowenstein-Jensen			Medium Stonebrink			Medium Middlebrook 7H11			Observations
								Lot Nº	Date Preparation	Result	Lot Nº	Date Preparation	Result	Lot Nº	Date Preparation	Result	

Complete with the information received from each laboratory.

Annex D.11. Form for assigning random numbers to the tubes received for quality control

Code		Contaminated		
		LJ	ST	7H11
Lab / Mid				
5				
13				
265				
35				
7				
70				
23				
1				
600				
345				
44	**ph**			
12	reserve			
Lab / Mid				
11				
9				
117				
295				
45				
367				
452				
78				
98				
52				
25	**ph**			
557	reserve			

Annex D.12: Form with the list of tubes to control of LJ medium

List of Numbers Medium LJ				
1	**181**			
5	**182**			
8	**183**			
9	**184**			
11	**189**			
13	**190**			
24	**193**			
27	**194**			
31	**195**			
32	**197**			
35	**217**			
37	**222**			
42	**224**			
49	**236**			
59	**238**			
60	**241**			
65	**253**			
66	**254**			
70	**258**			
74	**262**			
77	**265**			
79	**267**			
81	**268**			
85	**272**			
86	**275**			
89	**277**			
91	**285**			

Annex D.13. Characteristics of the medium received from participating laboratories

LABORATORY			CHARACTERISTICS OF THE ENVIRONMENT				TUBE	
NAME	CODE	Lot N°	pH	Color	Homogeneity	Observations	Size	Top
XXXXX	**A**	**1122**	**7,7**	**Vc**	Homogeneous color	very clear	16 * 150	Thread

Annex D.14.Preparation of inoculum for control

Remember that you must work with recommended biosafety conditions for handling high bacillary loads

1- Thaw one of the *M. tuberculosis* and / or *M. bovis* aliquots prepared in Annex D. 6.

2- Carry out a subculture with the strain *M. tuberculosis* in LJ and / or the *M. bovis* strain in Stonebrink according to the medium to be evaluated.

3- Incubate at 37°C and harvest in exponential stage of development (approximately 15 - 20 days). Very young or old cultures can give variable results.

4- Label a bottle or tube with glass beads with the name and / or number of the strain or isolates.

5- With a bacteriological handle scrape the entire surface with bacterial development, avoiding taking culture medium. In case of using non-disposable handle, cool it before lifting the bacillary mass (the hot handle kills the mycobacteria).

6- Download all the bacillary mass inside the tube with glass beads, making revolving movements of the handle on the pearls.

7- Add 1 or 2 drops of sterile water, cover and vortex for 1 minute; let stand 2 or 3 minutes to lower the possible aerosols formed.

8- Add approximately 1 ml of sterile water and vortex again for 1 minute.

9- Leave the suspension at rest for 15 minutes.

10- With a disposable Pasteur pipette, transfer the supernatant from the tube with beads to a new tube with a screw cap, taking care not to stir the sediment.

11- Add little by little the sterile distilled water until the turbidity of a McFarland 1 suspension or the one of 1 mg / ml of *M. bovis* BCG measure the optical density. You can also use the spectrophotometer, reading at 400 nm, the OD of the suspension.

12- With micropipette or pipette, transfer 100 µl of the suspension to a tube with 9.9 ml of distilled water (dilution 1:100 10^{-2}).

13- With micropipette or calibrated pipette, transfer 1 ml of the suspension 10^{-2} to a tube with 9 ml of sterile distilled water (dilution 1: 1000-10^{-3})

14- With micropipette or calibrated pipette, transfer 1 ml of the suspension 10^{-3} to a tube with 9 ml of sterile distilled water (dilution 1: 10000-10^{-4})

15- With micropipette or pipette transfer 1ml of the suspension 10^{-4} to a tube with 9 ml of distilled water (dilution 1: 100000-10^{-5}).

16- Choose the optimal dilution of previous experiences in order to obtain 20-50 CFU in the volume to be sown per tube.

Annex D.15. Form for colony counting

Lowenstein Jensen neutral (reading days)					
Sowing date 06/29/2010		Reading date			
Reading made by:					
Tube n°	Reading	Observations	Tube n°	Reading	Observations
1			181		
5			182		
8			183		
9			184		
11			189		
13			190		
24			193		
27			194		
31			195		
32			197		
35			217		
37			222		
42			224		
49			236		
59			238		
60			241		
65			253		
66			254		
70			258		
74			262		
77			265		
79			267		
81			268		
85			272		
86			275		

Annex D.16. Form for the transcription of the characteristics of the medium, colony counting and analysis of results

	A	B	C	D	E	F	G	H	I	J	K	L	M
7										Mid: LOWENSTEIN-JENSEN			
8													Mid: LO
9													
10													
11													
12	LABORATORY			CHARACTERISTICS OF THE ENVIRONMENT				TUBE					NUMBE
13	NAME	CODE	Lot N°	pH	Color	Homogeneity	Observations	Size	Top	Tube n°	Tube n°	Tube n°	Tube n°
14													
15	Hospital XX	A	1122	7,7	Vc	They are not observed color inequalities	very clear	16*150	Rosca	11	42	267	979
16										59	62	88	87
17										64	68	95	95
18										66	65	90	92
19										63	65	91	91
20										62	64	88	89
21										64	74	97	96
22										66	66	93	93
23										64	68	93	93
24													
25													
26													
27													
28										Average (J25:J27)	Average (K25:K27)	Average (L25:L27)	Average (M25:M27)
29													
30													
31													
32										Average (J29:J31)	Average (K29:K31)	Average (L29:L31)	Average (M29:M31)
33													
34													
35													
36													
37													

Readings of the three readers at 20 days days

Average at 20 days

Readings of the three readers al 60 days

Average at 60 days

This form tends to simplify the calculations. In it, the formulas that must be entered for each calculation are expressed, taking into account the columns and rows that must be involved and combined. (Do not forget that the formula in Excel carries the sign =). It is recommended to introduce all the formulas and once the form is completed, incorporate the data from the colony count readings.

QUALITY CONTROL OF CULTURE MEDIA XXXX
INSTITUTE XXXXXXXXXXXXXXXXXXX

N	O	P	Q	R	S	T	U	V	W	X	Y
Inoculum: Aqueous suspension of mycobacterium tuberculosis H37 Rv											
WENSTEIN-JENSEN											
						DEVELOPMENT					
						AVERAGE OF COLONIES BY PIPE		DS 60 days	SENSITIVITY	PERCENTAGE OF DEVELOPMENT TO THE 20 DAYS	CHARACTERISTICS PECULIARS OF THE COLONIES
ER OF COLONIES READING 20/60 DAYS						20 days	60 days				
Tube n°	Tube n°	Tube n°	Tube n°	Tube n°	Tube n°	A	B		(*)	A/B x100	
511	60	972	1089	265	491	77,8	79,8		Very Good	97,5	
76	77	65	82	75	75						
85	85	76	82	75	84						
81	86	77	85	73	63						
81	83	73	83	74	74						
77	77	69	82	78	79						
88	87	80	82	79	88						
82	88	79	88	73	65						
82	84	76	84	77	77						
						Average (J28:S28)	Average (J32:S32)	DESVEST (J32:S32)	Good	T24/U24*100	
Average (N25:N27)	Average (O25:O27)	Average (P25:P27)	Average (Q25:Q27)	Average (R25:R27)	Average (S25:S27)						
Average (N29:N31)	Average (O29:O31)	Average (P29:P31)	Average (Q29:Q31)	Average (R29:R31)	Average (S29:S31)						
			Average			Average (T15:T24)	Average (U15:U24)				
			Standard deviation			Desvest (T15:T24)	Desvest (U15:U24)				
			Average +1DS			T33+T34	U33+U34				
			Average - 1DS			T33-T34	U33-U34				
						#¡DIV/0!	#¡DIV/0!			#¡DIV/0!	

With respect to batch in particular

About all the batches

Annex D.17.Preliminary report of medium results

City, xxxx of xxxxx of xxxx

«Name »
«Position »
«Institution»
«Address»
«City»"Postal Code»
«Province »
« Labor e-mail »

Of our most consideration:

We anticipate the sensitivity achieved by the batches sent by your laboratory for the Quality Control of Culture Medium made on xxxxx.

Mediums	Lot	Sensitivity
Löwenstein Jensen	«LLJ»	«RLJ»
Stonebrink	«LST»	«RST»

As in previous experiences, we have categorized sensitivity as follows

Very good: number of colonies above the average plus a standard deviation,
Good: number of colonies between the medium plus / minus a standard deviation
Not acceptable: number of colonies lower than the average plus a standard deviation

Later, we will send by mail the complete information corresponding to this experience of quality control, with detail and comparison of the development produced by the group of controlled lots and the observations that arise from the information received from the participating laboratories. We are completing the analysis.

We want to thank you for the effort to participate and greet it affectionately.

Signature _____

Sheet 1/1

Annex D.18. Final report on medium results

City, XX of XXXXX of XXXX

«Name»
«Position»
«Institution»
«Address»
«Postal Code»«City»
«Province»

Of our most consideration:

Completing the report that we sent on xx / xx / xx, we attach the complete analysis of the xx experience of the Quality Control of Culture Medium for the Diagnosis of Tuberculosis.

Your laboratory is assigned the code: «Code_of_laboratory»

We appreciate your participation and we are at your disposal to provide more information or resolve concerns that arise from the analysis of the attached results.

Signature _____

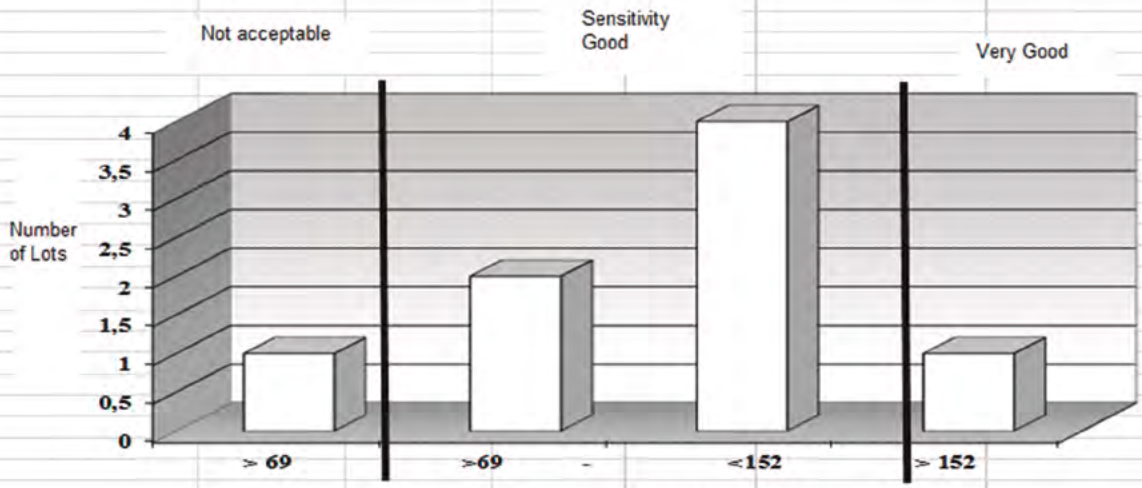
Annex D.19. Data to report next to the final report

QUALITY CONTROL OF CULTURE MEDIA
COOPERATIVE STUDY INTERLABORATORIES 8th Control
NATIONAL NETWORK OF TUBERCULOSIS LABORATORIES
NATIONAL INSTITUTE XXXXXXX

MID : Lowenstein Jensen — **Inoculum: Suspension of mycobacterium tuberculosis H37 Rv**

CODE FROM LABORATORY	Characteristics medium		TUBE		DEVELOPMENT AVERAGE OF COLONIES BY TUBE	SENSITIVITY (*)
	Lot N°	pH	Size	Cover		
A	1122	7.7	17x180	Rosca	172.9	Very Good
C	8	7.5	15X150	Rosca	122.8	Good
E	38	7.5	15x150	Rosca	135.2	Good
F	19	7,8	17x180	Algodón	138,6	Good
G	13	7,7	15x150	Algodón	108,7	Good
H	01/10	7,9	17x110	Rosca	89,9	Good
R	no informado	8,1	16x115	Rosca	41,5	Not acceptable
S	5	7,2	16x110	Rosca	75,0	Good

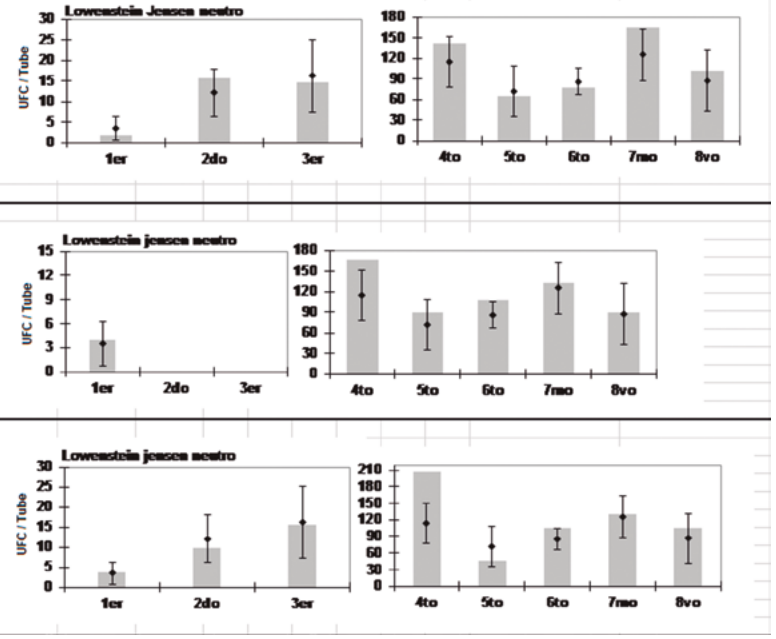
Average	**110,6**
Standard deviation	**41,2**
Media + 1DS	**151,8**
Media - 1DS	**69,4**



(*) Good = Average +/- standard deviation
Very good> medium + standard
Not Acceptable <average - standard deviation

Annex D.20. Monitoring the quality of the culture medium

Laboratory	Code	LOWENSTEIN JENSEN NEUTRO
Hospital XXXX	AF	1er 2do 3er 4to 5to 6to 7mo 8vo 1,9 15,6 14,5 141 64,1 76,8 164,8 101,1 3,5 12,1 16,2 114 71,6 85,8 125 86,7 2,8 5,8 8,9 36,6 36,7 19,3 37,8 45
Hospital XXXXXX	H	1er 2do 3er 4to 5to 6to 7mo 8vo 4 166 89,4 108 132,7 89,9 3,5 114 71,6 85,8 125 86,7 2,8 36,6 36,7 19,3 37,8 45
Hospital XXXXXX	I	1er 2do 3er 4to 5to 6to 7mo 8vo 3,8 9,7 15,6 207 45,4 104,4 129,8 105,9 3,5 12,1 16,2 114 71,57 85,8 125 86,7 2,8 5,8 8,9 36,6 36,73 19,3 37,8 45



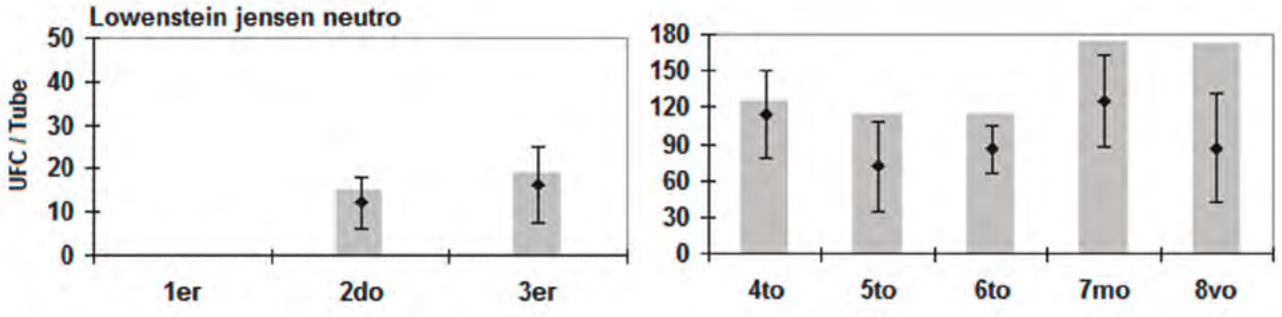
Annex D.21. Monitoring report on the quality of the medium

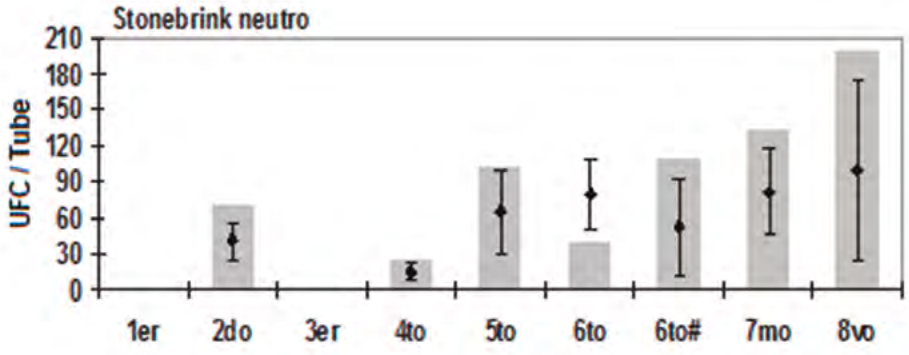
MONITORING THE QUALITY OF THE CULTURE MEDIUM

(2nd to 8th Control of the Quality Assurance Program of the National Network of Tuberculosis Laboratories, years …… to ……)

Laboratory name

Quality of solid culture medium





Did not participate in the 1st control

REFERENCE

average standard deviation of all batches tested

value reached by the laboratory

*** did not send sample**

#2ndreturn

CFU/tube colony forming units/tube

ANNEX E - PHENOTYPIC SUSCEPTIBILITY TESTS

Annex E.1. Preliminary letter

Ref: Quality control of susceptibility tests
to anti-tuberculosis drugs

Place, xx of xxxxxx of xxxx

Dear

We are organizing the quality control of antituberculosis drug susceptibility tests this year. In order to coordinate the delivery of the appropriate number of panels we need to please communicate us by signed note, via email or fax the following:

1- the method(s) currently employed from samples or isolates to produce clinical reports in your laboratory.

2- drugs tested in the respective methods.

3- the method (s) you are currently validating.

4- date of the last verification of the operation of the safety cabinet in which it carries out the susceptibility tests, and the periodicity with which it is carried out.

5- model and classification (class and type) of the safety cabinet used to perform susceptibility tests in your laboratory.

If your laboratory for any reason can not participate in this interlaboratory experience, please inform us to try together to solve the problem and avoid the unnecessary sending of biological material of high risk.

Without another particular, we greet you very attentively.

Annex E.2. Form with the coding of isolates by panel

Isolation Code		Laboratory XXX							Laboratory XXX				PANEL N°						Laboratory XXX						Contaminated	Not viable
OMS	LSN o LRN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
1A	161	1	115	215	342	404	670	669	943	534	58	3	63	547	861	177	384	685	721	873	936	479	724	878	981		
1B	297	314	775	892	68	294	917	834	638	119	137	546	1058	895	1048	591	101	23	2	979	9	886	1079	176	272		
2A	318	198	258	370	371	372	438	467	730	889	1089	662	980	381	653	432	103	41	924	966	729	535	190	596	230		
2B	336	461	800	903	321	817	336	244	296	456	69	140	376	379	687	840	1025	1028	1069	557	1043	32	902	124	827		
3A	396	602	319	270	507	941	167	921	420	134	85	264	304	912	354	842	219	300	302	951	353	600	645	953	166		
3B	752	180	867	676	536	553	389	770	616	720	874	148	659	660	24	1063	98	152	274	640	624	464	162	899	982		
4A	960	183	358	359	284	815	816	798	178	56	174	1003	1004	185	615	809	920	213	201	714	1057	612	480	663	20		
4B	1084	136	182	288	343	375	523	865	962	991	1006	365	323	802	803	970	900	993	83	236	366	831	1026	728	871		
5A	1271	227	114	256	499	1046	250	5	47	309	298	59	120	286	340	779	541	764	36	38	470	92	193	506	228	1	
5B	1273	1082	232	1014	617	105	172	413	574	594	229	76	1064	253	262	402	424	118	186	436	870	1018	1065	517	634		1
6A	1371	100	110	415	618	235	708	838	630	718	704	411	494	851	945	691	66	31	559	614	998	67	224	341	938		
6B	1525	760	893	910	918	950	858	793	952	210	290	516	768	531	558	628	786	949	715	417	442	398	514	160	248	1	
7A	2015	629	475	632	315	39	556	1005	1100	410	554	555	837	709	710	657	212	647	14	37	60	181	444	455	855		
7B	2197	586	246	688	95	233	1038	719	331	996	109	223	386	648	679	1002	1031	1088	1091	267	269	930	1062	414	707		
8A	2669	77	542	405	940	142	422	433	268	508	567	771	868	773	758	759	828	135	983	830	459	195	988	78	238		
8B	5682	113	1036	774	560	62	43	750	931	1013	761	735	841	188	25	672	332	418	577	1040	394	29	489	588	880		
9A	5879	668	35	1032	825	8	490	592	905	907	923	999	396	132	328	1007	1008	453	528	214	545	835	16	72	678		
9B	7066	582	733	778	790	584	1054	807	564	485	133	231	156	237	665	578	677	848	904	888	570	664	667	784	785		
10A	7313	1074	82	967	312	530	122	539	580	55	844	984	887	1021	987	1012	562	563	7	922	963	603	87	202	407	1	
10B	7623	301	139	824	891	906	241	265	1086	525	652	876	1023	605	75	747	748	1030	498	121	864	510	989	361	171		
11	8448	597	1041	726	477	345	346	357	971	839	303	452	731	1072	755	1017	15	573	80	400	428	697	866	287	385		
12	8563	495	382	610	28	161	337	151	373	928	266	601	959	316	969	1070	734	780	199	973	974	944	1083	964	933		
13	8936	307	751	1095	397	1085	12	458	504	692	808	810	934	1059	568	483	111	631	533	972	1035	421	976	1045	651	1	
14	8976	81	468	609	749	79	843	846	776	13	104	194	702	278	445	625	957	656	644	295	538	293	392	524	1024		
15	9263	860	954	548	769	654	242	116	11	123	61	703	585	717	727	1099	217	511	799	44	1096	519	65	239	317		
16	9386	1019	1092	1094	71	205	518	737	738	739	740	741	742	743	744	745	746	814	566	869	1090	88	255	383	619	1	1
17	9608	10	292	329	348	349	350	351	352	509	990	52	543	863	753	425	403	207	772	791	1037	689	992	74	64		
18	9727	537	1071	259	606	225	797	1087	680	681	700	1011	216	551	448	125	1093	757	463	427	901	437	633	434	716		
19	9743	325	150	762	804	699	598	622	1009	157	472	247	285	55	471	968	320	859	393	590	243	639	796	705	46		1
20	9815	997	305	935	829	289	549	412	189	419	766	795	937	1010	48	222	324	505	725	792	896	1001	1027	1049	915		

Annex E.3. Information requested for susceptibility test control

Quality Control Suceptibility Testing *Mycobacterium tuberculosis*
Interlaboratory Cooperative Study - Latin American Network of Tuberculosis Laboratories.

• As soon as they are received, the strains are subcultured in two Löwenstein Jensen tubes (LJ), each planting 0.3 ml of the suspension sent.

• Incubate until abundant development is detected. Verify that there are at least 20 colonies. If it was necessary to gather 20 colonies, work with the two tubes subcultured As an exception, it is always possible to subcultured from the suspension sent. Do not repeat the LJ tubes again (to avoid working with a selection of clones).

• Perform the susceptibility test from the subcultured in LJ according to the routine method used in your laboratory.

• Complete the attached form with the results and information requested and send it by postal mail to:

Institution, E-mail / tel: email to:

Participating laboratory:

Method used:
Results of the 1st line drug sensitivity tests (mark with a cross):

Isolation	Isoniazid		Rifampicin		Ethambutol		Pyrazinamide Method: Conc.:	
	S	R	S	R	S	R	S	R
24								
32								
61								
135								
164								
198								
239								
240								
264								
266								
342								
348								
358								
417								
522								
541								
543								
571								
647								
655								
677								
738								
771								
778								
845								
870								
880								
1021								
1027								
1088								

S: Sensitive **R:** Resistant

If you did not perform any test or could not interpret any result, indicate here the cause:

Method used:

Results of drug sensitivity tests of 2nd line (mark with a cross)

Isolation	Quinolone (*)		Kanamycin		Amikacin		Capreomycin	
	S	R	S	R	S	R	S	R
24								
32								
61								
135								
164								
198								
239								
240								
264								
266								
342								
348								
358								
417								
522								
541								
543								
571								
647								
655								
677								
738								
771								
778								
845								
870								
880								
1021								
1027								
1088								

S: Sensitive **R:** Resistant
(*) Clarify the drug that is being evaluated

If you did not perform any test or could not interpret any result, indicate here the cause:

WORK ROUTINE

1. Taking into account **all the methods used to originate clinical reports,** complete the following table:

N° of CASES whose isolate was tested for susceptibility	**Year**
	XXXX
without **total** treatment	
without multiresistant pretreatment	
without extremely resistant treatment	
with **total** pretreatment	
with multi-resistant pretreatment	
with extremely resistant pretreatment	
totals no information regarding treatment	
multiresistant without information regarding treatment	
N° of total SUSCEPTIBILITY TESTS performed	

• QUALITY CONTROL OF RECORDS AND REPORTS.

We request to send along with the survey, and in order to evaluate the performance of the laboratory in the work routine, the following documentation:

- The results report of bacteriological studies corresponding to isolations XXX, XX and XXX. Consider that they developed from sputum samples grown in your laboratory. Prepare the reports as you would in your work routine, using the forms in use in your laboratory.

Responsible signature _____

Annex E.4. Template for the recording of results of control isolates

Quality control N° 20 Isolates N° 1371 X/XX/20XX
MICROSCOPIC EXAM: cords

REPIQUES: LJ y ST 23/01	DECONTAMINATION:	DECONTAMINACION:

Observations:

IDENTIFICATION TESTS
Lateral immunochromatography *(M. tuberculosis complex)*
Result
Positive ☐ **Negative** ☐
Biochemical tests:

Niacin:	HT	CFA
Photochromogenicity:	Urease	Arylsulfatase
Nitrate: + (3/2)	ß-glucosidase	Pyrazinamidase (-) 07/02/2014
Catalase TA 68°C	ß-galactosidase	

TH: tween hydrolysis, CFA: iron and ammonium citrate

SUSCEPTIBILITY TESTS
MGIT960
Lot: Planting date: 27-01 de Lj

Reading day	Witness	S	H	R	E	K	AK	Cp	O	Lev	Mox 0,5	Mox 2,5
07-02	Oka	R	R	R	S	S	S	R	R	R	R	R

PROPORTION METHOD
Lot Nº Planting date :

Preliminar Reading	
Date	Resistant to

Final reading			Informed:							
Date	Dil	Witnesses	S	H	R	E	K	AK	Cp	O
	-3									
	-5									
	-6									

It is identified as	Date Report

Observations in report:

Annex E.5. General information of control participants

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Laboratorio			Participa	Si no participa ¿cuál es el motivo?	Método utilizado para diagnóstico clínicos	Drogas utilizadas								Fecha de última validación de la cabina de bioseguridad	Periodicidad de la validación de la cabina de seguridad	Panel nº	Fecha de recepción de cepas	Fecha de remisón de resultados	Tiempo transcurrido de informe del centro evaluado (dias)	Fecha de envío del informe del centro evaluador
2																					
3	Institución	Profesional a cargo	Respondió				H	R	E	Z	K	Q*	AK	CP							
4	Hospital XXXXX	Lic. XXXXX																		DIAS(R4;S4)	
5	Hospital XXXXXX	Dra.XXXXXXXX																		DIAS(R5;S5)	
6																				DIAS(R6;S6)	
7																				DIAS(R7;S7)	
8																				DIAS(R8;S8)	

These data allow to obtain general information about coverage, methodologies used by laboratories, tested drugs and biosafety conditions. Remember that for the formula in Excel to be carried out they must bear the = sign in front of it (=DIAS (R4;S4)

Annex E.6. Isolation results by drug

Drug Rifampicin

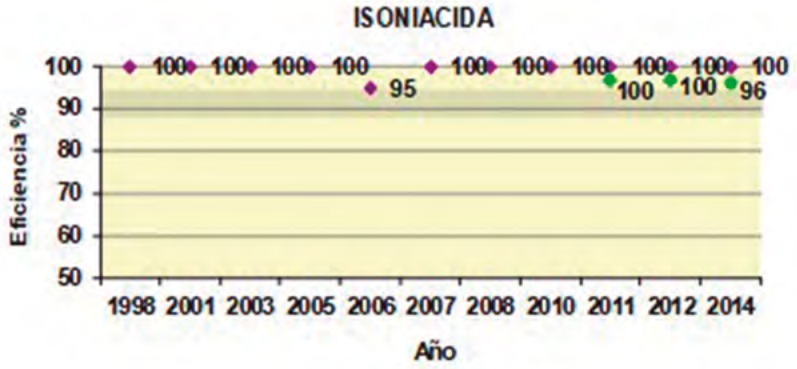
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1			Source		WHO	Hospital XXX (MGIT 960)									Hospital XXXX (LJ)								
2	YEAR XXXX		Round		Concordance in result of 31 laboratories	Code		Success		Success SC	D SC	Not accomplished		couple ok	Code		Success		Success SC	D SC	Not accomplished		couple ok
3			QQ	1			2	R	S			R	S			2	R	S			R	S	
4	493	1A	19	R	100%	895	1	1							10	1	1						
5	4634	1B	19	R		874	1	1						1	499	1	1						1
6	1185	2A	19	R	100%	670	1	1							326	1	1						
7	2161	2B	19	R		173	1	1						1	201	1	1						1
8	242	3A	19	R	100%	592	1	1							126	1	1						
9	657	3B	19	R		291	1	1						1	334	1	1						1
10	283	4A	19	S	97%	969	1		1						286	FR							
11	3521	4B	19	S		67	1		1					1	57	FR							1
12	715	5A	19	R	100%	17	1	1							105	1	1						
13	1407	5B	19	R		661	1	1						1	311	1	1						1
14	5013	6A	19	S	97%	513	1		1						1060	1		1					
15	9177	6B	19	S		476	1		1					1	832	1		1					1
16	9268	7A	19	R	100%	345	1	1							551	1	1						
17	9094	7B	19	R		773	1	1						1	860	1	1						1
18	6080	8A	19	R	100%	193	1	1							1080	1	1						
19	8004	8B	19	R		1062	1	1						1	302	1	1						1
20	5025	9A	19	R	100%	226	1	1							939	1	1						
21	9553	9B	19	R		709	FS							0	915	1	1						1
22	6605	10A	19	S	100%	328	1		1						968	1		1					
23	9290	10B	19	S		866	1		1					1	445	FR							1
24	375	11	19	S	100%	176	1		1						922	FR							
25	2510	12	19	R	100%	908	1	1							848	1	1						
26	3290	13	19	R	100%	78	1	1							637	1	1						
27	2904	14	19	S	97%	490	1		1						381	1		1					
28	2174	15	19	R	100%	233	1	1							316	1	1						
29	5757	16	19	S	100%	2	1		1						1076	FR							
30	7391	17	19	S	100%	120	1		1						418	1		1					
31	8841	18	19	R	100%	625	1	1							40	1	1						
32	6520	19	19	S	97%	982	1		1						790	1		1					
33	5376	20	19	R	100%	433	1	1							468	1	1						
34	R							18	11	0	0	0	0	10									
35	S								29				0	9								0	
36	SC																						
37	Total																						

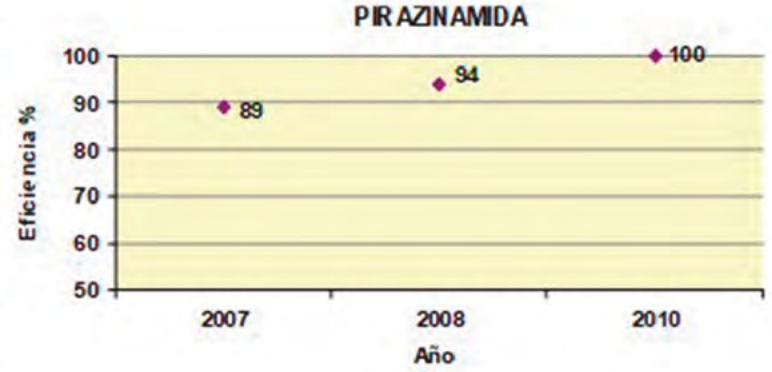
38	results of participating laboratories	Total correct results	29
39		True resistant	18
40		FR	0
41		True sensitive	11
42		FS	0
43		Sensitivity (%)	
44		Specificity (%)	
45		Efficiency (%)	
46		In-laboratory reproducibility (%)	

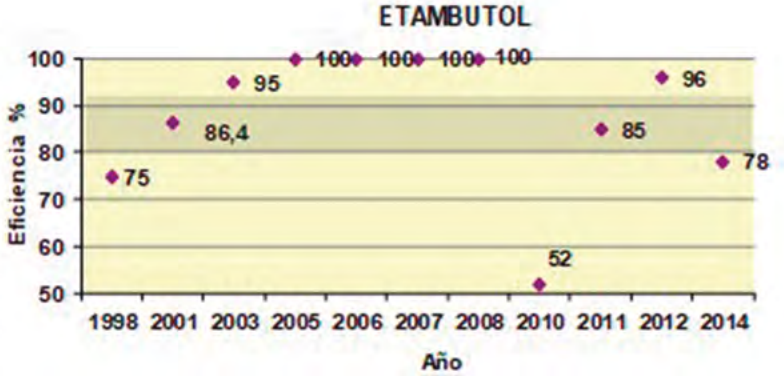
47	SC	without considering for the calculation (agreement <80%)
48	FR	false resistant
49	FS	false sensitive
50	*******	No result (contaminated, not viable, doubtful)
51	D SC	inability to isolate without considering
52	Unrealized	Complete only for isolations with agreement greater than 80%
53	Couple OK	Duplicate results
	Each color represents an isolation	

This form tends to simplify the calculations. In it, the formulas that must be entered for each calculation are expressed taking into account the columns and rows that must be involved and combined (remember to place the sign = in front of each formula). It is recommended to introduce all the formulas and once the form is completed, incorporate the data of the test results. One flap per drug should be generated and the formulas for each laboratory and for each method that applies should be introduced.

Eficiencia Isoniacida%											
	1998	2001	2003	2005	2006	2007	2008	2010	2011	2012	2014
Eficiencia %	100	100	100	100	95	100	100	100	100	100	100
									97	97	96
Eficiencia Rifampicina%											
	1998	2001	2003	2005	2006	2007	2008	2010	2011	2012	2014
Eficiencia %	100	100	100	100	100	100	100	100	100	100	100
									96	96	96
Eficiencia Etambutol%											
	1998	2001	2003	2005	2006	2007	2008	2010	2011	2012	2014
Eficiencia %	75	86,4	95	100	100	100	100	52	85	96	78
Eficiencia Pirazinamida%											
	2007	2008	2010								
Eficiencia %	89	94	100								







Annex E.8. Results report

Quality Control of Susceptibility Tests *Mycobacterium tuberculosis.*

Laboratory: Hospital XXXX

Panel No. 19 submitted by the World Health Organization (WHO)

Strains code		Results reported by the laboratory R = resistant NI= No informed S = sensitive ND = no development, or contaminated				Results according to the consensus of the Supranational Laboratories (WHO / International Union for the Fight against Tuberculosis, Global Projecton Anti-tuberculosis DrugResistanceSurveillance) 1 = Right NC = No considered (reproducibility) FR = false resistant less than 80%) FS = false sensitive			
		Investigated drugs				Investigated drugs			
OMS	XXX	Pza	H	R	E	Pza	H	R	E
1A	895	R	R	S	S	1	1	1	NC
1B	874	R	R	S	R	1	1	1	NC
2A	670	S	R	R	S	NC	1	1	NC
2B	173	S	R	R	S	NC	1	1	NC
3A	592	S	R	R	S	FS	1	1	FS
3B	291	R	R	R	R	1	1	1	1
4A	969	S	S	R	S	1	1	1	1
4B	67	S	S	R	R	1	1	1	FR
5A	17	S	R	R	S	1	1	1	1
5B	661	S	R	R	S	1	1	1	1
6A	513	R	S	S	S	1	1	1	1
6B	476	S	S	S	S	FS	1	1	1
7A	345	R	R	R	R	1	1	1	NC
7B	773	R	R	S	S	1	1	FS	NC
8A	193	S	R	R	S	FS	1	1	FS
8B	1062	R	R	R	R	1	1	1	1
9A	226	S	R	S	S	NC	1	FS	FS
9B	709	S	S	S	S	NC	FS	FS	FS
10A	328	R	S	S	S	FR	1	1	1
10B	866	S	S	S	S	1	1	1	1
11	176	R	S	S	S	1	1	1	1
12	908	S	R	R	R	1	1	1	1
13	78	R	R	S	S	1	1	1	1
14	490	R	S	R	S	1	1	1	1
15	233	S	R	R	S	FS	1	1	1
16	2	S	S	S	S	1	1	1	1
17	120	S	S	S	S	1	1	1	1
18	625	R	R	R	S	1	1	1	FS
19	982	R	S	R	S	FR	1	1	1
20	433	S	R	S	R	1	1	1	1

Method used: 3		Total correct results				**20**	**29**	**27**	**18**
1- Proportions in LJ		True resistant				**11**	**18**	**16**	**4**
2- BACTEC 460		False resistant				**2**	**0**	**0**	**1**
3- MGIT 960		True sensitive				**9**	**11**	**11**	**14**
4- Nitrate reductase		False sensitive				**4**	**1**	**3**	**5**
Arrival		Sensitivity (%)				**73**	**95**	**84**	**44**
Strains: 05/20/2014		Specificity (%)				**82**	**100**	**100**	**93**
Results: 09/22/2014		Efficiency (%)				**77**	**97**	**90**	**75**
Time spent: 122 days		Intralaboratory reproducibility (%)				**50**	**90**	**90**	**57**

The following tubes are duplicates of the same strain and were used to evaluate the intralaboratory reproducibility:
(874 and 895); (173 and 670); (291 and 592); (67 and 969); (17 and 661); (476 and 513);(345 and 773); (193 and 1062); (226 and 709);(328 and 866).

The standardized and validated methods to study the sensitivity of Mycobacterium tuberculosis to antituberculosis drugs are very accurate and, the experiences of the WHO Supranational Laboratories network have established that it is possible to expect an efficiency greater than 90% for ethambutol and greater than 95%. % for isoniazid and rifampicin. Efficiency acceptability limits have been proposed (average efficiency - 1 standard deviation of the results obtained in this international network during 5 years of work). Thus, efficiencies consistently lower than 90% for ethambutol and 95% for isoniazid and rifampicin were classified as unacceptable.

CONCLUSIONS

According to the preceding considerations, your laboratory has demonstrated, **by the proportions method in MGIT 960, good quality to evaluate the activity to isoniazid and not acceptable quality to evaluate the activity to Rifampicin and ethambutol.**
It is worrisome the appearance of false sensitive in Rifampicin, a key drug for the treatment of tuberculosis. We recommend to evaluate again the panel isolations with erroneous results for rifampicin, in order to rule out some error in the sowing process.
In the case of the drug ethambutol, the results of both laboratories of all multi-resistant isolates are monitored.

OBSERVATIONS

Attached is the monitoring of the efficiency demonstrated by the laboratory during the course of this control.

Signature_____

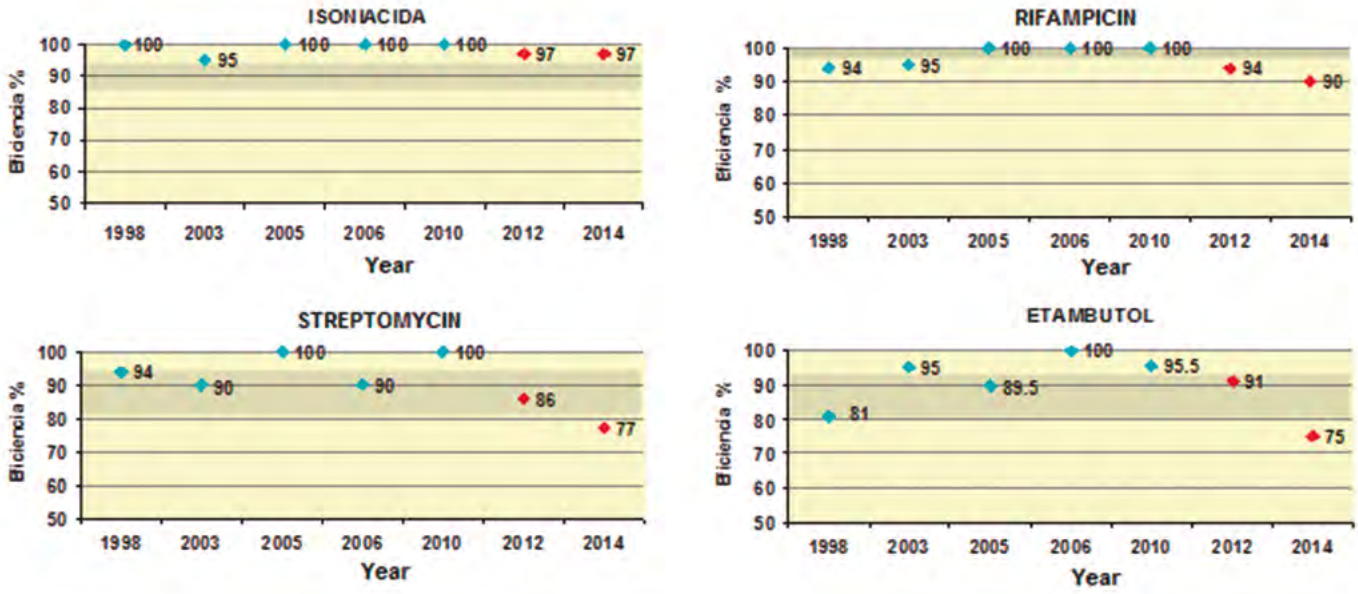
Annex E.9. Monitoring the quality of PS

Monitoring the quality of susceptibility tests of *Mycobacterium tuberculosis*

Interlaboratory Cooperative Study - National Network of Tuberculosis Laboratories

Institute XXXXXXXX

Hospital XXXX



Did not participate in the years 2001, 2008 and 2011

- Result by method of proportions in LJ
- Result by proportions method in BACTEC MGIT 960

Values achieved in the Hospital XXXXXX

- Range of values between the averages reached internationally in laboratories with good quality and minimum acceptable

ANNEX F. GENOTIPIC SUSCEPTIBILITY TESTS

Xpert MTB / RIF system

Annex F.1. Preparation of suspensions of inactivated mycobacteria

Procedure

• Confirm the panel with the strains that make up the panels sent to the SRL by the Institute of Tropical Medicine of Belgium for the quality control of susceptibility tests to antituberculous drugs. Include those selected by consensus of SRL that assist Latin American countries taking into account the test to be evaluated (Xpert, FL-LPA or SL-LPA). Exclude strains with results with agreement less than 80% and/or those that have results obtained by phenotypic and discrepant genotypic methods.

• Carry out the tests with Xpert or LPA with suspensions with an approximate concentration of 5000 bacilli/ml.

• Prepare the suspensions that make up the panel for each NRL, following the following protocol and respecting the biosafety conditions to handle suspensions of *M. tuberculosis*:

- With a bacteriological handle scrape the entire surface with bacterial development, avoiding taking culture medium.

- Download all the bacillary mass inside the tube with glass beads, making rotary movements of the handle on the pearls.

- Add 1 or 2 drops of sterile water, cover, and vortex for 1 minute.

- Add approximately 1 ml of sterile water and vortex again 1 minute.

- Leave the suspension at rest for 15 minutes.

- With a Pasteur pipette, transfer the supernatant from the tube with beads to a new tube with a screw cap, taking care not to remove the sediment.

- Add water to the contents of this tube with thread, until achieving the turbidity of a McFarland suspension 1.

- With micropipette or pipette, transfer 1 ml of the suspension to a tube with 9 ml of distilled water (dilution 1:10, 10^{-1}).

- With micropipette or pipette, transfer 100 µl of suspension 10^{-1} to a tube with 9.9 ml of distilled water (dilution 1: 100 10^{-3}).

- With micropipette or calibrated pipette, transfer 1 ml of suspension 10^{-3} to a tube with 9 ml of distilled water (dilution 1:10, 10^{-4}).

- With micropipette or calibrated pipette, transfer 1 ml of suspension 10^{-4} to a tube with 9 ml of distilled water (dilution 1:10, 10^{-5}).

- Inoculate 200µl of dilutions 10^{-3}, 10^{-4} and 10^{-5} in two tubes of LJ or 7H11 medium and incubate at 37°C for 30 or 40 days, to quantify colony forming units.

- Heat the remaining dilutions 10^{-1}, 10^{-3}, 10^{-4} and 10^{-5} in a water bath at 80°C for 30 minutes to kill the bacilli.

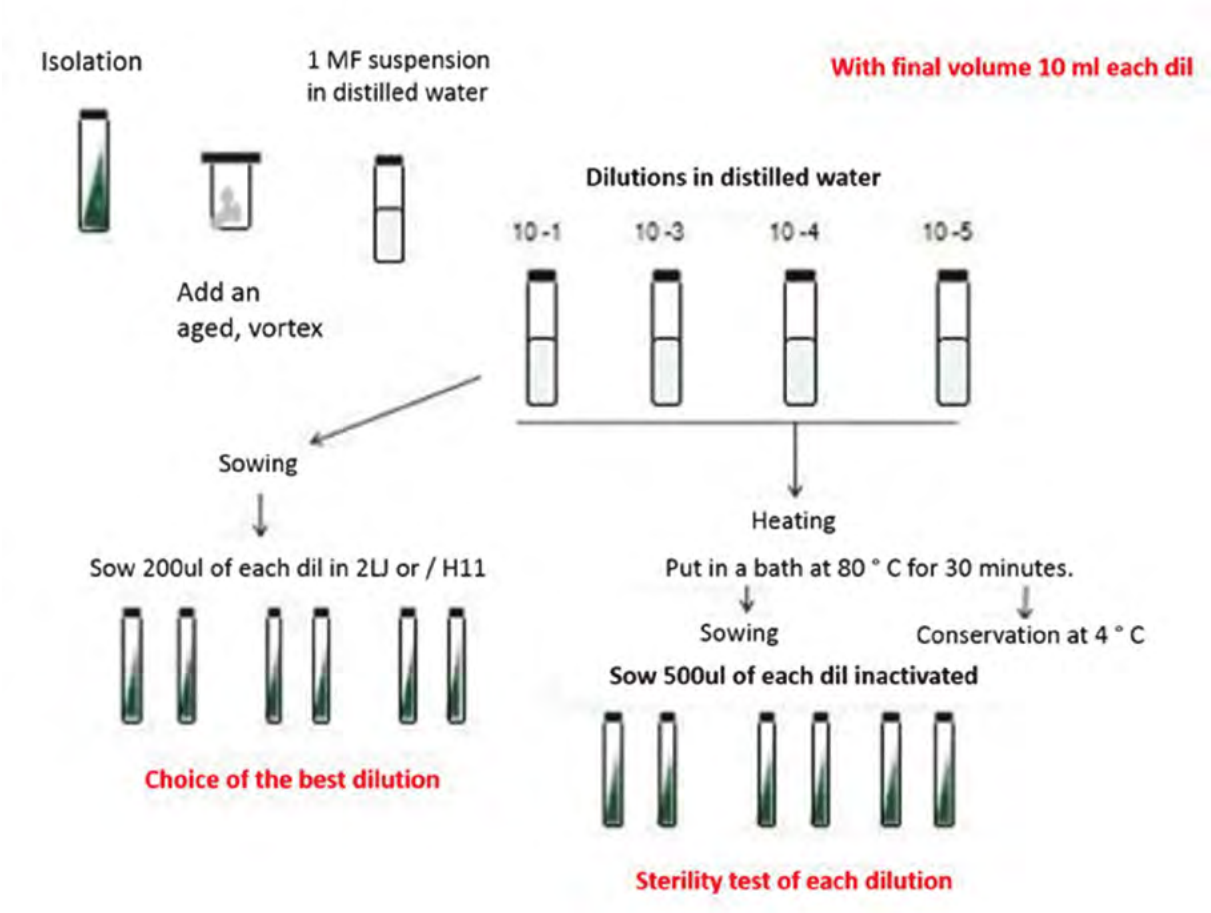
- Inoculate 500 µl of each of the inactivated suspensions in two tubes of LJ or 7H11 medium and incubate at 37°C for 40 days to verify the absence of development.

- Keep the dilutions 10^{-3}, 10^{-4} and 10^{-5} at 4°C until the number of colony-forming units in each dilution is determined, in order to select the dilution to be used.

After incubations:

- Verify that no colony has grown in inactivated suspensions.

- Quantify the concentration of viable bacilli that existed in the different dilutions before inactivating.



Depending on the use (support for verification or quality control) given to the panel, panels with different numbers of strains must be prepared to send.

Annex F.2. Information requested for susceptibility test control

Support of the SRL to the NRL for the verification of an automated closed system of extraction and amplification of DNA in real time for the detection of rifampicin resistance in *Mycobacterium tuberculosis* (Xpert MTB/RIF)

Take into account the following recommendations:

• Work at least with the same biosafety standards as for smear sputum samples http://www1.paho.org/Spanish/AD/DPC/CD/tb-labs-baciloscopia.pdf

• Process each tube as if it contained a sputum sample

• Follow the protocol usually applied in your laboratory

• Each procedure must be performed by the operator (s) who routinely perform it in the routine

• Complete the attached form with the results and information requested and send it by mail to your reference laboratory.

Participating laboratory:
PANEL Nº
Uses common cartridge ❒ ultra ❒
Test scores:

Sample	Result		Amount of DNA from *M. tuberculosis* ***	Mutated probe ****	Observations
	Equipment *	Laboratory **			

* Record the result that the equipment issues: N (MTB Not Detected); T (MTB Detected Rif Resistance Not Detected); RR (MTB Detected Rif Resistance Detected); TT (MTB Detected by traces Rif Indeterminate); I (Invalid, enter the Error 5006/5007/5008, 5011, 2008, 2127, 2037 or 2014/3074/3075/1011)

** TB ND (M. tuberculosis not detected); TB RR (M. tuberculosis resistant to rifampicin); TB SR (M. tuberculosis susceptible to rifampicin); TB (M. tuberculosis, could not determine the resistance to rifampin)

*** HIGH (positive in less than 16 cycles), HALF (positive between 16 to 22 cycles), LOW (positive between 23 to 28 cycles), VERY LOW (positive after 28 cycles), TRACES (less than 37 cycles for the ULTRA cartridge)

**** Mutated probe: record as identified on the curve

Some validation experience of the Xpert MTB / RIF method has been carried out in your country
YES ☐ NO ☐

If the answer was yes, complete the following information
Use ☐ Pulmonary samples
☐ Extrapulmonary samples
☐ Isolates (positive cultures)

To evaluate the results he took as reference
☐ The result of sputum smear
☐ The result of culture
☐ Other (describe)

Summarize the results in the table according to the type of samples used and the result of smear microscopy

Pulmonary samples (smear positive)

Result Method Xpert MTB/RIF	A result obtained by the reference method		Total
	TB	No TB was detected	
Positive			
Negative			
Total			

Pulmonary samples (smear negative)

Result Method Xpert MTB/RIF	Result obtained by the reference method (culture)		Total
	TB	No TB was detected	
Positive			
Negative			
Total			

Pulmonary samples (smear positive and negative)

Method Xpert MTB/RIF	Reference method: ..... Used		Total
	Resistant to R	No resistant to R	
Mutation			
No mutation detected			
Total			

1. For what kind of patients does the method apply in the work routine?

.....

.....

.....

In your country has a working algorithm been agreed upon to use the Xpert?

YES ☐ NO ☐

In case your answer is YES, attach a copy

2. Have there been problems with the equipment? Mark the problems

Problems with the equipment		**Cartridges with errors**	
The modules are not detected		Failure of the control probe with stopping of the amplification. (Error 5006, 5007, 5008)	
The barcode scanner does not work		Signal loss of the amplification curve (Error 5011)	
Supposed module failure (flashing red light)		Pressure exceeds the maximum pressure allowed (Error 2008)	
Clogging of the cartridge		Loss of communication with the module (Error 2127)	

3. Do you track the frequency of errors, invalid results, lack of results per module and user?
YES ☐ NO ☐

4. Indicate the percentage of tests for each marked problem, placing as numerator the number of cartridges invalidated according to the different problems and as the denominator the total number of cartridges used

5. Do you report recurring errors to Cepheid? YES ☐ NO ☐

6. Date on which you have performed the last calibration of each GeneXpert equipment for the investigation of *M. tuberculosis* ____ /____ /____

7. If there is more than one team, who coordinates the calibration?

8. Have you had problems with the supplies or spare parts? YES ☐ NO ☐

If your answer is YES, mark the type of inconvenience

- ☐ high number of defective cartridges
- ☐ lack of stock of cartridges (list the number of months)
- ☐ discarding cartridges for lack of use before expiration
- ☐ bad conditions for storage
- ☐ lack of replacement of defective modules (record the number of months)
- ☐ other

Send a copy of the report with the results of strains XX and XX using the format (form) used in the work routine

.....
Responsible signature

Annex F.3. Results report

Quality Control Susceptibility Testing *Mycobacterium tuberculosis.*

External Control Interlaboratorios 20XX - National Network of Tuberculosis Laboratories.

Institution that carries out the control

Laboratory:				
Panel N° 18 submitted by the World Health Organization (WHO)				
Strains code			**Results reported by the laboratory**	**Results according to the consensus of the Supranational Laboratories** (WHO / International Union for the Fight against Tuberculosis, Global Project on Anti-tuberculosis DrugResistancSurveillance)
WHO	**Laboratory X**	**sequencing result rpoB**	**P** = presence of mutation **A** = absence of mutation **NI** = not interpretable **ND** = not detected	**1** = Right **Fp** = false presence of mutation **Fa** = false absence of mutation
1A	**826**	**His526Asn**	P	**1**
1B	**758**	**His526Asn**	ND	***************
3A	**539**	**His526Try**	P	**1**
3B	**879**	**His526Try**	P	**1**
4A	**354**	**Ser531Leu**	P	**1**
4B	**406**	**Ser531Leu**	P	**1**
5A	**527**	**Ser531Leu**	P	**1**
5B	**145**	**Ser531Leu**	NI	***************
6A	**32**	**Wtype**	A	**1**
6B	**97**	**Wtype**	A	**1**
7A	**572**	**Wtype**	A	**1**
7B	**124**	**Wtype**	A	**1**
9A	**726**	**Wtype**	A	**1**
9B	**65**	**Wtype**	A	**1**
11	**1092**	**Wtype**	A	**1**
12	**1039**	**Wtype**	A	**1**
14	**353**	**asp516Val**	P	**1**
17	**844**	**Ser531Leu**	P	**1**
18	**985**	**Wtype**	A	**1**
**********	**1020 (*)**	*************	ND	***************
Method used: 5 1- Proportions in LJ 2- BACTEC 460 3- MGIT 960 4- Nitrate reductase 5-Xpert MTB/R			**Total correct results**	**17**
			True resistant	**8**
			False resistant	**0**
			True susceptible	**9**
			False susceptible	**0**
			Sensitivity (%)	**80**
			Specificity (%)	**100**
			Efficiency (%)	**89**
			Intralaboratory reproducibility (%)	**100**

(*) isolation identified as *M. kansasii*

The following tubes are duplicates of the same strain and were used to evaluate the intra-laboratory reproducibility:
(758 and826); (539 and879); (354 and406); (145 and527); (32 and97); (124 and572); (65 and726).

Conclusions

No discrepancies of results were verified with the Xpert MTB / RIF molecular method. However, the laboratory could not interpret the result of one panel sample. While, it did not detect DNA, in another sample corresponding to *Mycobacterium tuberculosis.*

Observations

Signature _____

Annex F.4. Example list of inactivated bacillary suspensions forwarded to the NRL of country XX for the preparation of panels for aptitude tests.

Panel PS 12 of QQ18			Panel N°		
	WHO strain	Strain N	RIF mutation	Country XX	Country XXX
1A	2426	1092	His526Asn	831	826
4A	3268	121	Ser531Leu	338	354
14	4646	812	asp516Val	991	353
6B	7601	1168	Wtype	963	97
	7881		M. kansasii	895	32

Annex F.5 Example for the preparation of panels for quality control for an NRL that needs to evaluate a number of laboratories ≤ to 15.

Selection of the suspension

This material will be useful for the NRL to prepare the necessary panels to control the quality of the diagnosis made by Xpert MTB / Rif in the network of tuberculosis laboratories in your country.

The NRL will receive from the SRL vials containing approximately 50 000 bacilli/ml labeled as follows:

(1) *Mycobacterium tuberculosis* resistant to RIF (2 tubes of 1,8 ml each)
(2) *Mycobacterium tuberculosis* resistant to RIF (2 tubes of 1,8 ml each)
(3) *Mycobacterium tuberculosis* susceptible to RIF (2 tubes of 1,8 ml each)
(4) *Mycobacterium tuberculosis* susceptible to RIF (2 tubes of 1,8 ml each)
(5) MOTT (2 tubes of 1,8 ml each)

These strains have been characterized as reported by the SRL of the Institute of Tropical Medicine of Belgium in relation to:

i) the identification of *M. tuberculosis;*
ii) presence and type of mutation in the region of the *rpoB* gene explored or absence of mutation.

Since each tube contains a suspension of bacilli with an approximate concentration of 50,000 bacilli/ml, it will be necessary to perform a 1/10 dilution to achieve an approximate concentration of 5000 bacilli/ml, a concentration that has been established as the optimum for the performance of this aptitude test. With these dilutions, panels will be prepared, consisting of the 5 bacillary suspensions diluted by the NRL.

To calculate the number of panels to prepare, consider that the LRN should test a panel to confirm that the results are as expected and that, in case you need to replace a vial or repeat a shipment, it is advisable to have at least 2 reservation panels.

To prepare the panels, choose cryotubes or tubes with a screw cap that allow safe shipping without the risk of spillage. Follow the rules of transport of samples that apply in your country.

Preparation of the panels in the LRN

Number of laboratories to be controlled in the laboratory network of your country: **15**
Number of panels to prepare: **18**
Total volume of suspension of 5,000 bacilli / ml to be prepared with each strain: **18 ml**

Proceed as follows

• Prepare 1 tube that can contain up to 20 ml, dispense 16.2 ml of sterile distilled water and label with the number on the tube sent by the SNL.

• Take the two cryotubes labeled with the same number (N° 1) that were sent by the SRL.

• Transfer and collect the contents of both in a single empty tube and mix with a vortex.

• Transfer 1.8 ml of this suspension to the tube containing 16.2 ml of sterile distilled water (total 18 ml) (1:10 dilution).

• Mix with a vortex.

• Distribute 1 ml of the homogenized suspension in 2 ml cryotubes with the lid with an external thread.

• Label each of the 18 tubes of the strain with numbers selected at random. You can use the following generated list with the computer consecutively:

List of random numbers

1	168	253	77	658	278	757
115	362	262	542	327	445	463
215	377	402	405	732	625	427
342	440	424	940	875	957	901
404	736	118	142	297	656	437
670	836	186	422	1081	644	633
669	929	436	433	299	295	434
943	913	870	268	683	538	716
534	84	1018	508	108	293	898
58	723	1065	567	112	392	318
3	884	517	771	473	524	4
63	783	634	868	1076	1024	671
547	180	408	773	301	487	767
861	867	387	758	139	106	1052
177	676	429	759	824	1098	54
384	536	263	828	891	852	1055
685	553	338	135	906	322	406
721	389	416	983	241	474	117
873	770	649	830	265	813	399

• Take the first 18 numbers and label each of the tubes of strain 1.

• Record the numbers of that strain that has corresponded to each panel that is being prepared. You can use an Excel spreadsheet.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Cepa 1	1	115	215	342	404	670	669	943	534	58	3	63	547	861	177	384	685	721

• Repeat the procedure with the rest of strains 2, 3, 4 and 5 and incorporate the numbers in the excel spreadsheet.

	Paneles Nº																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Cepa 1	1	115	215	342	404	670	669	943	534	58	3	63	547	861	177	384	685	721
Cepa 2	873	168	362	377	440	736	836	929	913	84	723	884	783	180	867	676	536	553
Cepa 3	389	770	253	262	402	424	118	186	436	870	1018	1065	517	634	408	387	429	263
Cepa 4	338	416	649	77	542	405	940	142	422	433	268	508	567	771	868	773	758	759
Cepa 5	828	135	983	830	658	327	732	875	297	1081	299	683	108	112	473	1076	301	139

• Group, taking into account the table, the five vials of each panel 1. Example: Panel 1.

Panel 1	Panel 2	Panel 3
1	115	215
873	168	362
389	770	253
338	416	649
828	135	983

• Cover the screw cap of each vial with parafilm paper to secure and protect its closure.

• Individually pack each vial.

• Place the vials in a single bag labeled with the corresponding panel number.

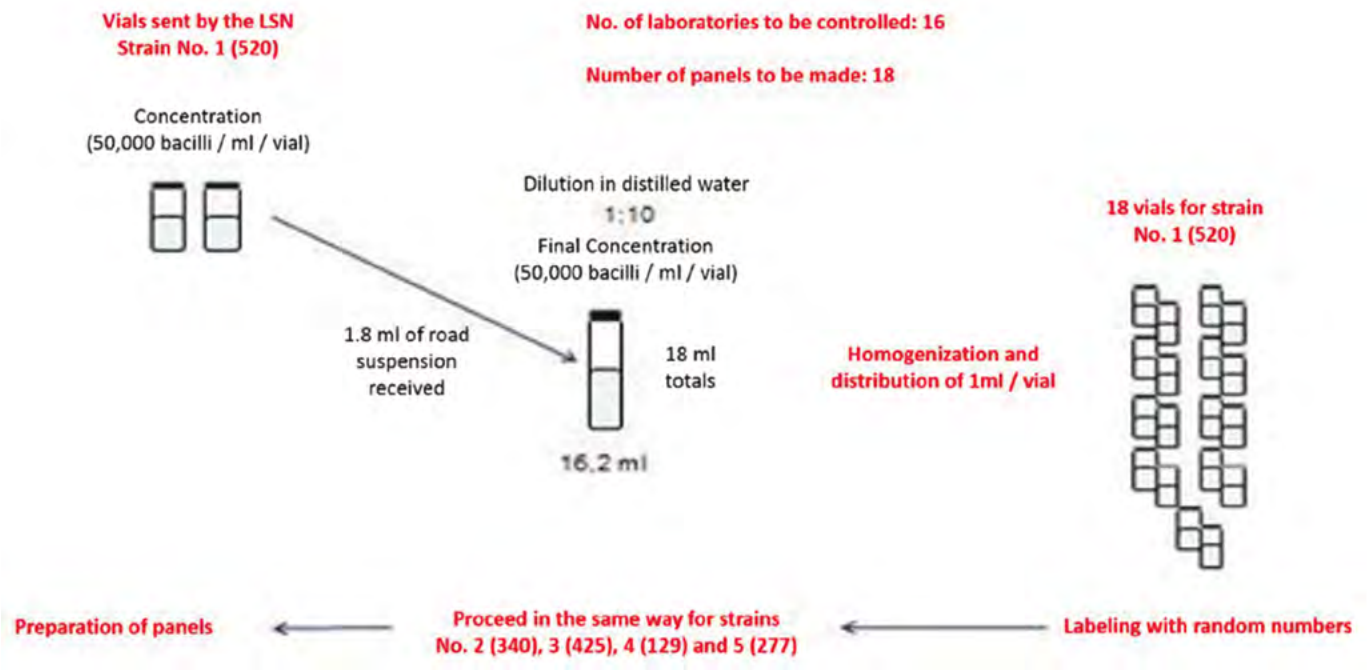
• Keep the panels refrigerated until the shipment is made, preferably at -20ºC.

• Condition the shipment according to the regulation of transport of biological material that applies to each country. Place the bag in a first plastic container which will go in other cardboard containers.

• Attach instructions for rehearsal with each panel.

• Register the panel number to be sent to each laboratory to be controlled and the one to be tested by the NRL.

• Coordinate with each laboratory the date of sending and receiving the corresponding panel.



Annex F.6. Form for external assessment control

External assessment control of peripheral laboratories users of an automated closed system for the extraction and amplification of DNA in real time for the detection of rifampicin resistance in *Mycobacterium tuberculosis* (Xpert MTB/RIF)

Take into account the following recommendations:

• Work at least with the same biosafety standards as for smear sputum samples http://www1.paho.org/Spanish/AD/DPC/CD/tb-labs-baciloscopia.pdf

• Process each tube as if it contained a sputum sample

• Follow the protocol usually applied in your laboratory

• Each procedure must be performed by the operator (s) who routinely perform it in the routine

• Complete the attached form with the results and information requested and send it by mail to:

Laboratory or participating Health Center:
PANEL Nº
Test scores:

Sample	Result		Amount of DNA *M. tuberculosis* ***	Mutated probe ****	Observations
	Equipment *	Laboratory **			

* Record the result that the equipment issues: N (MTB Not Detected); T (MTB Detected Rif Resistance Not Detected); RR (MTB Detected Rif Resistance Detected); I (Invalid; enter the Error 5006/5007/5008, 5011, 2008, 2127, 2037 o 2014/3074/3075/1011)

** TB ND (*M. tuberculosis* not detected); TB RR (*M. tuberculosis* resistance to rifampicin); TB SR (*M. tuberculosis* sensitive to rifampicin); TB (*M. tuberculosis,* could not determine the resistance to rifampicin)

***HIGH (positive in less than 16 cycles), HALF (positive between 16 to 22 cycles), LOW (positive between 23 to 28 cycles), VERY LOW (positive after 28 cycles)

**** mutated probe: record as identified on the curve

1- For what type of patients is the method applied in the work routine?
.....
.....
.....

2- Have there been problems with the equipment? Mark the problems

Problems with the equipment		**Cartridges with errors**	
The modules are not detected		Failure of the control probe with stopping of the amplification. (Error 5006, 5007, 5008)	
The barcode scanner does not work		Signal loss of the amplification curve (Error 5011)	
Supposed module failure (flashing red light)		Pressure exceeds the maximum pressure allowed (Error 2008)	

Clogging of the cartridge		Loss of communication with the module (Error 2127)	
The determination with invalid results		Cartridge integrity test failed (Error 2037)	
Failure in sample processing control		Heating failure or temperature. (Error 2014, 3074, 3075, 1001)	
Determination without results		**Other.....**	

3- Do you track the frequency of errors, invalid results, lack of results per module and user?
YES ☐ NO ☐

4- Indicate the percentage of runs for each marked problem, placing as numerator the number of cartridges invalidated according to the different problems and as the denominator the total number of cartridges used

5- Do you report recurring errors to the NRL? YES ☐ NO ☐

6- Have you received supervision visits from the NRL?

7- Date on which you have performed the last calibration of each GeneXpert equipment for the investigation of *M. tuberculosis* ____ /____ /____

8- Have you had problems with the supplies? YES ☐ NO ☐

If your answer is YES, mark the type of inconvenience

- ☐ high number of defective cartridges
- ☐ lack of stock of cartridges (list the number of months)
- ☐ discarding cartridges for lack of use before expiration
- ☐ bad conditions for storage
- ☐ lack of replacement of defective modules (record the number of months)
- ☐ other

Send a copy of the report with the results of strains XX and XX using the format (form) used in the work routine

.....
Signature

Annex F.7. Analysis of results

20XX		Source	WHO		Laboratory XXX		Laboratory XXX	
		Round	1	RopB	N° Code	Informed result	N° Code	Informed result
		QA						
2426	1A	18	M	His526sn	513	20	831	
3268	4A	18	W	Ser531Leu	535	0	338	
4646	14	18	W	wildtype	360	20	407	
7601	6B	18	M	wildtype	284	10	963	
21454				M. KansasII	679	20	20	
					70			SUM

ANNEX G. GENOTIPIC SUSCEPTIBILITY TESTS

LPA system (FL-LPA or SL-LPA)

Annex G.1. Information requested for susceptibility test control

Support for the verification of an open system of amplification and reverse hybridization (LPA) for the detection of resistance to rifampicin and isoniazid (FL-LPA) or to second-line antituberculosis drugs (SL-LPA)

Take into account the following recommendations:

• Work, for the extraction and loading of DNA, at least with the same biosafety standards as for the handling of samples (Laboratories of moderate risk). For the rest of the process take into account the two separate spaces that are used in any molecular practice that use open amplification systems (preparation of amplification mixture and an opening of amplified products)

• Process each tube as if it contained a sputum sample

• Follow the protocol that is usually applied in your laboratory

• Each procedure must be performed by the operator (s) who usually perform it in the routine

• Complete the attached form with the results and information requested and send it by email to your reference laboratory.

Participating laboratory:

PANEL Nº
Results of the tests to R and H:

	TUB band P: present A: absent	Result M: mutated No M: not mutated			Locus control band P: present A: absent			Mutated probe *	Observations
Sample	*M tuberculosis*	RIF	INH		RIF	INH			
		*rpo*B	*kat*G	*inh*A	*rpo*B	*kat*G	*inh*A		

*the detected mutation that is indicated by the absence of wild-type band and/or the presence of a mutant band for each group of genes should be reported.

PANEL Nº

Results of the tests for injectables and quinolones:

	TUB band P: present A: absent	Result M: mutated No M: not mutated				Locus control band P: present A: absent				Mutated probe *	Observations
Sample	*M tuberculosis*	Injectables		Quinolones		Injectables		Quinolones			
		rrs	*eis*	*gyr*A	*gyr*B	*rrs*	*eis*	*gyr*A	*gyr*B		

*the detected mutation that is indicated by the absence of wild-type band and/or the presence of a mutant band for each group of genes should be reported.

1- Some validation experience of the LPA method has been carried out in your country

YES ☐ NO ☐

FL-LPA (isoniazid and rifampicin) ☐ SL-LPA (injectables, quinolones) ☐

2-If the answer was yes, complete the following information

He used ☐ pulmonary samples
☐ isolates (positive cultures)

To evaluate the results I take with reference

☐ the result of sputum smear
☐ the result of culture
☐ other (describe)

Summarize the results in the table according to the type of samples used and the result of smear microscopy. Indicate the percentage of invalid and indeterminate results (stratifying by drug if applicable) obtained during the validation stage.

Pulmonary samples (smear positive)

Result Method LPA (FL-LPA)	Result obtained by the reference method (culture)		Total
	TB	No TB was detected	
Positive			
Negative			
Total			

Pulmonary samples (smear negative)

Result Method LPA (FL-LPA)	Result obtained by the reference method (culture)		Total
	TB	No TB was detected	
Positive			
Negative			
Total			

Pulmonary samples (smear positive and negative)

Method LPA (FL-LPA)	Reference method: ..... Used		Total
	Resistant to R	No resistant to R	
Mutation			
No mutation detected			
Total			

Isolations

Method LPA (FL-LPA)	Reference method: ..... Used		Total
	Resistant to R	No resistant to R	
Mutation			
No mutation detected			
Total			

Pulmonary samples (smear positive and negative)

Method LPA (FL-LPA)	Reference method: ..... Used		Total
	Resistant to INH	Not resistant to INH	
Mutation			
No mutation detected			
Total			

Isolations

Method LPA (FL-LPA)	Reference method: ..... Used		Total
	Resistant to INH	Not resistant to INH	
Mutation			
No mutation			
Total			

In case the verification has been done for the SL-LPA, complete the tables for the respective drugs. In the case of second-line injectables and/or quinolones, it is suggested to perform the analysis for the drugs separately so that the accuracy of the system can be observed to identify the susceptibility to the different injectable drugs of the second line and to the quinolones of a different generation.

3-For what kind of patients does the method apply in the work routine?

.....

.....

.....

In your country, has a working algorithm been agreed upon to use the LPAs?

YES ❐ NO ❐

In case your answer is YES, attach a copy

How many laboratories in the network have implemented the method?

4-Do you track the frequency of invalid/indeterminate results per user?

YES ☐ NO ☐

5-Have some of the computers in the network been inactive? How many? Why?

6-Have you had problems with the supplies? YES ☐ NO ☐

If your answer is YES, mark the type of inconvenience

- ☐ lack of stock of strips (list the number of months)
- ☐ Discard strips for lack of use before expiration
- ☐ bad conditions for storage
- ☐ other

7-Send a copy of the report with the results of strains XX and XX using the format (form) used in the work routine

.....

Signature

Annex G.2. Results report

Quality Control Susceptibility Tests of *Mycobacterium tuberculosis.*

External Control Interlaboratorios 20XX - National Network of Tuberculosis Laboratories.
Institution that carries out the control

Laboratory:							
Panel N° 18 submitted by the World Health Organization (WHO)							
Strains code		**Results reported by WHO** **M: mutated gene** **No M: No mutation**		**Results reported by the laboratory**		**Results according to consensus of the Supranational Laboratories** (WHO / International Union for the Fight against tuberculosis, Global Project on Anti-tuberculosis DrugResistanSurveillance)	
WHO	**Institution XXX**	**Sequencing result *rpo*B**	**Sequencing result katG e inhA**	**P** = absence of wild band with or without presence of corresponding mutation **A** = absence of mutation **NI** = No interpretable (**)		**1 = correct** **Fp = false presence of a mutation** **Fa = false absence of a mutation**	
				R	**H**	**R**	**H**
1A	**513**	**M**	M	P	P	1	**1**
1B	**601**	**M**	M	P	P	1	**1**
3A	**607**	**M**	M	P	P	1	**1**
3B	**447**	**M**	M	P	P	1	**1**
4A	**535**	**M**	No M	P	A	1	**1**
4B	**515**	**M**	No M	P	A	1	**1**
5A	**360**	**No M**	M	A	P	1	**1**
5B	**921**	**No M**	M	A	NI	1	**********
6A	**793**	**No M**	M	A	P	1	**1**
6B	**284**	**No M**	M	A	P	1	**1**
7A	**556**	**No M**	No M	A	A	1	**1**
7B	**24**	**No M**	No M	A	A	1	**1**
9A	**913**	**M**	No M	P	A	1	**1**
9B	**1023**	**M**	No M	P	A	1	**1**
11	**485**	**No M**	No M	A	A	1	**1**
12	**261**	**No M**	M	A	P	1	**1**
14	**45**	**No M**	No M	A	A	1	**1**
17	**524**	**M**	M	P	P	1	**1**
18	**740**	**M**	M	P	P	1	**1**
********	**679(*)**	**********		**ND**		**********	
Method used: 6 1- Proportions in LJ 2- BACTEC 460 3- MGIT 960 4- Nitrate reductase 5-Xpert MTB/RIF 6-LPA FL-LPA				**Total correct results**		**19**	**19**
				Tru resistant		**10**	**11**
				False resistant		**0**	**0**
				True sensitive		**9**	**8**
				False resistant		**0**	**0**
				Sensitivity (%)		**100**	**100**
				Specificity (%)		**100**	**100**
				Efficiency (%)		**100**	**94,7**
				Intralaboratory reproducibility (%)		**100**	**86**

(*) isolation identified as *M. kansasi*

(**) the CC, AC, Tub controls, those corresponding to the locus did not appear or for the case of the negative control, bands other than AC and CC appear or when the controls indicate that the test is valid, but the bands that indicate the presence or absence of mutations do not have the intensity of control.

The following tubes are duplicates of the same strain and were used to evaluate the intra-laboratory reproducibility:
(513 and 601); (607 and 447); (535 and 515); (306 and 921); (793 and 284); (556 and 24); (913 and 1023).

Conclusions

There were no discrepancies in results with the FL-LPA molecular method for the detection of *Mycobacterium tuberculosis* complex and the resistance to rifampicin and isoniazid. However, the laboratory could not interpret the mutation result or not of a panel sample.

REMARKS

Signature _____

Annex G.3. List of panels for quality control sent to the NRL of country XX for the preparation of the panels for the aptitude tests

For quality control of isoniazid and rifampicin (FL-LPA system)

			Mutations			
Panel PS 12 of QQ18			RIF	INH		1
	WHO strain	Strain N	rpo B	Kat G	inh A	Country XXX
1A	2426	1092	His526Asn	Ser315Thr	wildtype	831
4A	3268	121	Ser531Leu	wildtype	wildtype	338
14	4646	812	wildtype	wildtype	wildtype	991
6B	7601	1168	wildtype	wildtype		963
	21454	M. kansasii				895

For quality control of injectables and quinolones (SL-LPA system)

			Mutations				
Panel PS 12 of QQ18			Km / Ak / Cap		Ofx		1
	WHO strain	Strain N	rrs S	eis	gyr A	gyr B	Pais XXX
1A	2426	1092	wildtype	wildtype	Ser91Pro		109
4A	3268	121	wildtype	G-10A	wildtype		125
7A	7132	468	Ala1401Gly	wildtype	Asn533Ser		88
9A	6020	324	wildtype	wildtype	Ala90Val&ser91Pro		389
	21454	M. kansasii					90

Annex G.4. Information requested for susceptibility test control

External assessment control of peripheral laboratories users of an open system of amplification and reverse hybridization (LIPAs) for the detection of *M. tuberculosis* and resistance to rifampicin and isoniazid (FL-LPA) or to second-line anti-tuberculosis drugs (SL-LPA)

Take into account the following recommendations:

- Work, for the extraction and loading of DNA, at least with the same biosafety regulations as for the manipulation of samples. For the rest of the process take into account the two separate spaces that are used in any molecular practice that use open amplification systems (preparation of amplification mixture and an opening of amplified products)

- Process each tube as if it contained a sputum sample

- Follow the protocol that is usually applied in your laboratory

- Each procedure must be performed by the operator (s) who routinely perform it in the routine

- Complete the attached form with the results and information requested and send it by mail to your reference laboratory.

Participating laboratory:

PANEL Nº

Results of the tests to Rif and INH:

Sample	TUB band P: present A: absent	Result M: mutated No M: not mutated			Locus control band P: present A: absent			Mutated probe *	Observations
	M tuberculosis	RIF	INH		RIF	INH			
		*rpo*B	*kat*G	*inh*A	*rpo*B	*kat*G	*inh*A		

*the detected mutation that is indicated by the absence of wild-type band and/or the presence of a mutant band for each group of genes should be reported.

PANEL Nº

Results of the tests for injectables and quinolones:

Sample	TUB band P: present A: absent	Result M: mutated No M: not mutated				Locus control band P: present A: absent				Mutated probe *	Observations
	M tuberculosis	injectables		quinolones		injectables		quinolones			
		rrs	*eis*	*gyr*A	*gyr*B	*rrs*	*eis*	*gyr*A	*gyr*B		

*the detected mutation that is indicated by the absence of wild-type band and/or the presence of a mutant band for each group of genes should be reported.

1- For what kind of patients is the method applied in the work routine?
.....
.....
.....

2- Do you track invalid/indeterminate results by a user? YES ☐ NO ☐

3- Do you report invalid / indeterminate recurrent results to the LRN?
YES ☐ NO ☐

Indicate the percentage of invalid and indeterminate results (per drug if applicable) obtained during the last year .....

4- Have you received supervision visits from the NRL?

5- Have you had problems with the supplies? YES ☐ NO ☐
If your answer is YES, mark the type of inconvenience

- ☐ lack of stock of strips (list the number of months)
- ☐ Discard strips for lack of use before expiration
- ☐ bad conditions for storage
- ☐ other

6-Send a copy of the report with the results of strains XX and XX using the format (form) used in the work routine

.....
Responsible signature

Annex G.5. Analysis of results

20XX		Source	WHO			Laboratory XXX		Laboratory XXX	
		Round	1	RESULT		N° Code	Informed result	N° Code	Informed result
		QA		Kat G	inhA				
2426	1A	18	M	Ser315Thr	wildtype	513	20	831	
3268	4A	18	W	wildtype	wildtype	535	0	338	
4646	14	18	W	wildtype	wildtype	360	20	407	
7601	6B	18	M	wildtype	-15T	284	10	963	
21454					M Kansasii	679	20	20	
						70			SUM
			Efficiency score			70			(N4: N8)N9*100/100

In it, the formula that must be entered for each calculation is expressed, taking into account the columns and rows that must be involved and combined. It is recommended to introduce all the formula and once the form is completed, incorporate the data of the results of the tests.

Since these teams detect resistance to different drugs (first and second line), they must make as many flaps as drugs are being controlled.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