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| Name |  |  |  | Code: | Code: |
| Date |  |  |  |  |  |
| Signature |  |  |  |  |  |
| Laboratory area: | No of copies: | Reason for change: |

1. Scope

This SOP describes external quality assessment of acid-fast bacilli smears stained by the Ziehl–Neelsen or fluorescent dye technique by rechecking of a random and representative sample of the routine work. This method is considered to be the most efficient since it allows monitoring of daily performance and, if well performed, is motivating for staff. Through continuous screening for substandard performance followed by identification and solving of problems, it aims to achieve optimal results in all laboratories of a network performing AFB smear microscopy.

1. Definitions and abbreviations

AFB: acid-fast bacilli

EQA: external quality assessment

FM: fluorescence microscopy staining technique

HFN: high false-negative errors

HFP: high false-positive errors

LFN: low false-negative errors

LFP: low false-positive errors

LQAS: lot quality assurance sampling

NTP: national tuberculosis programme

QE: quantification errors

ZN: Ziehl–Neelsen staining technique

1. Personnel qualifications

NA

1. Procedure

*4.1 Principle*

To guarantee representativeness, all examined smears are kept by each laboratory. Regardless of results, a sample of smears is periodically selected at random by a visiting supervisor. The samples are given to a first-level controller, for blinded re-reading; the list of results stays with the supervisor.

The supervisor compares the results obtained by the laboratory and by the first-level controller, and makes a list of smears for which results are discordant.

The list and the discordant smears are given to a second-level controller for more careful examination and correct results.

The EQA coordinator then uses the correct results to identify errors and determine whether they were made by the original laboratory or by the first-level controller.

Routine feedback is given as frequently as possible during this process by returning results and any slides with serious errors.

Final analysis must wait until a full year’s sample have been processed. Except in extreme cases, only then is it possible to identify the laboratories that have failed this screening process.

Further investigation of failing laboratories is needed for confirmation and to identify and solve the underlying problems. In the worst cases this will be done during a supervisory visit by a laboratory expert.

The performance of second-level controllers must also be evaluated to ensure that the system works reliably.

*4.2 Background and rationale*

The sample size of negative smears is based on a statistical system called LQAS (lot quality assurance sampling). The sampling method is designed to sample the lowest number of slides that will provide an indication of whether a laboratory is meeting a predetermined performance goal.

No statistical system is used for the significance of false-positives, since any high false-positive is in principle significant. While an occasional false-positive can occur anywhere due to administrative errors, only systematic false-positives caused by serious process errors are important. If these occur, they will make up a large proportion of positives/scanty positives, and will be easily detected by proportional sampling of positive and scanty positive smears (i.e. sampling in proportion to their recorded frequency in laboratory registers).

Small samples are an absolute requirement for accurate rechecking results, but they make interpretation necessary:

* A rare HFP will not trigger action, since a cause can rarely be found (registration error).
* Infrequent LFP, comparable to other laboratories and the controllers, are ignored as a limitation of rechecking (may be due to false-negative results of the controllers).
* QE are of secondary importance but they may point to faulty staining solutions or technique.
* Relatively frequent HFP indicate a serious problem, such as an unusable microscope, unreliable registration or lack of proper training, and must be further investigated.
* LFN occur even in the best laboratories and may occasionally be detected in a sample. The total number of false-negatives (HFN + LFN) is therefore considered and the proportion of HFN is used as the basis for prioritizing laboratories where false-negative problems require investigation.
* Excessive false-negatives are usually due to superficial reading. In some areas or countries, the main causes are poor carbol-fuchsin or auramine staining solutions and/or staining technique, plus poor microscopes. It is not unusual to find a combination of poor smearing and staining, poor microscopes and superficial reading (sometimes because of work overload), resulting in very low sensitivity of AFB smears.

*4.3 Materials and personnel*

The following personnel are required:

* Supervisors for (quarterly) sample collection and routine feedback. Ideally these are the regular NTP (district) supervisors.
* A rechecking coordinator at intermediate level(s). This can be the district-level supervisor and/or the regional NTP coordinator.
* First-level controllers. There is usually one first-level controller for 10–20 laboratories. These should be individuals with good experienced – but it is the availability of their time for rechecking that is the main requirement.
* Second-level controllers. There should be one second-level controller for about 50 laboratories. These individuals need to have sufficient time available to achieve conclusive results for even the most difficult smears. Sincerity and tenacity are more important than seniority, and busy heads of laboratories are rarely the best choice.
* Field laboratory supervisors (who may themselves be first- or second-level controllers). The number needed will depend on the level of supervision required; after the initial stages of implementation, one supervisor may be able to cover up to 50 laboratories

All microscopy laboratories to be checked need:

* sufficient slide boxes to store all slides for at least one quarter;
* tools for permanent identification of slides: either pencils (for slides with frosted ends) or a diamond marker (for plain slides).

Supervisors collecting slides need:

* small slide boxes (20 slots) for slide transport, one per laboratory supervised;
* forms for sampling;
* transport.

Controllers/expert laboratory supervisors need

* a good microscope;
* sufficient time;
* 80/20 ethylether/ethanol mixture;
* staining facilities and equipment;
* transport.

Coordinators need

* forms for listing discordant results;
* forms for routine feedback downwards (if not the same as sampling form);
* forms for reporting upwards;
* ideally, a computer analysis tool in a spreadsheet format.

*4.4 Detailed instructions*

*4.4.1 Sample size and collection; keeping slides*

All laboratories performing AFB smear microscopy under the NTP must be sampled. Sampling – as well as routine feedback in the apparent absence of problems – must be confided to NTP supervisors, according to a system defined at the national level.

Keeping slides at microscopy laboratories

* Keep all slides from all patients, regardless of results, until the sample has been taken. Thereafter, discard the remaining slides and start a new collection.
* Label the slides with the laboratory register number including line and column; use a pencil (frosted-end slides) or diamond marker (plain slides). Do not write result on slides.
* After reading the slides, let the oil soak into absorbent paper (newspaper, toilet paper) until the end of the day or the following morning. Do not use xylene or any other solvent to remove the remaining oil and never rub the smears.
* Arrange the slides in boxes according to their numbering, leaving empty slots for those expected to arrive over the coming few days. If there is a shortage of slide boxes, roll slides in toilet paper taking care that they cannot touch each other, and store them in their original packaging.

Setting sample size (national level)

* Collect data on the total number of negative and positive results recorded over the past year (slides; mixed suspects and follow-up) from all AFB smear laboratories (or as many as possible). Calculate the average number of negatives examined and individual and average prevalence of positives. If fewer than 25% of laboratories have less than half the average frequency of positives, use a single standard sample size based on the averages; otherwise, use two standard sample sizes, or construct a small table of choices for individualized sample size.
* Using these averages for the smear-negative volume and the prevalence of positives, determine the required sample size from LQAS tables (Aziz et al., 2002). Choose the table for minimum relative sensitivity of 80%; in this table the lines for acceptance number = zero. Look up the sample size needed, at the intersection of the line for annual number of negatives examined and the column for positives prevalence (for both parameters, use the values closest to the calculated averages). Alternatively, use the line “80% sensitivity” in the simplified table –Table 1 – below and the positives prevalence value closest to the average calculated.
* Determine the number of smears that can be processed annually by the first-level controllers without overload. This is a maximum 5–10 ZN smears per controller and per working day, depending on the number of routine ZN smears and other work the controllers may already have. Dividing this number by the number of laboratories to be checked gives the maximum annual sample size per laboratory
* If this maximum sample size is smaller than that obtained from the LQAS tables, reduce target sensitivity to 75% or lower, until the value obtained from the tables is lower than what can be accurately processed by the controllers currently available. If this is impossible, even at the lowest minimum target sensitivity of 65% shown in the tables, rechecking cannot be implemented without additional first-level controllers. Panel testing should then be used until there are sufficient personnel.
* Divide the annual sample size thus determined to laboratories by the interval of sampling periodicity to calculate the number of slides to be collected at every interval (i.e. divide the annual sample by 4 in case of quarterly supervision).

**Table 1. Simplified table of LQAS sample sizes**

|  |  |
| --- | --- |
|  | **Average positivity rate in the laboratory registers** |
| **2.5%** | **5.0%** | **7.5%** | **10.0%** | **12.5%** | **15.0%** | **17.5%** |
| **Target minimum relative sensitivity** | **65%****70%****75%****80%****85%** | 165201236318360 | 91109136180242 | 617696128174 | 46577396131 | 34455676107 | 3138496693 | 2432415577 |

|  |  |
| --- | --- |
|  | **Average positivity rate in the laboratory registers** |
| **20.0%** | **22.5%** | **25.0%** | **27.5%** | **30.0%** | **32.5%** | **35.0%** |
| **Target minimum relative sensitivity** | **65%****70%****75%****80%****85%** | 2329384970 | 1825314361 | 1721294056 | 1519253550 | 1317233346 | 1216212842 | 1114202840 |

Collection of samples (by well trained programme supervisors)

* Sample slides during each supervision visit according to instructions from national level. In principle, one-quarter of the number needed per year is collected every 3 months; however, if the previous quarterly supervision visit did not take place for any reason, twice as many slides should be collected.
* Record the period covered by the sample in the sputum smear register. From the register, or from a quarterly laboratory performance report, note the total number of smears examined during this period. Divide this total by the sample size required for the current visit (rounding down to the next whole number); this yields the *sampling step*.
* Fill in a rechecking sampling form with details of laboratory identity, the period rechecked, etc. If possible, use carbon paper *under the slide identification numbers only* to obtain a second copy of the form without the results.
* Use a random number (e.g. last digit of a bank note serial number) to select the first slide of the sample, counting from the first result in the register (slides are selected using the laboratory register and must not be selected from the slide box). Identify the first slide to be collected, and note its identification number and result on the sampling form.
* From there, start counting results (without differentiating between positive/negative/scanty or suspect/follow-up) using the sampling step to find the second sample slide, noting its identification and result on the form.
* Continue in this way until the required number of slides is reached; in case the end of the period is reached before the required number of slides has been listed, return to the start of the sampling period and continue counting from there.
* Ask the technicians to retrieve the selected slides from the boxes; check these against the list. Note any slides that are missing and replace each with the next slide in the register (even if the result was different), adding its identification and result at the bottom of the form. Ensure that each selected slide is marked with the laboratory identification code; if it does not, replace it with the next slide that does bear the unique code
* If none of the selected slides has a positive or scanty AFB result, add the first one or two found in the register searching from the start of the period.
* Ensure that the remaining slides are properly discarded, and instruct the technicians to start a new collection.
* Arrange the slides in a transport box in the order listed on the form (*not* all positives together) and deliver them as soon as possible to the first-level controller, together with the form *without* *results*. Give the form *with results* to the coordinator (if different from the supervisor him/herself).

*4.4.2 Rechecking process*

First-level controls

* Ensure that reading of slides by the first-level controller is completely blinded. The controller should have *no access to the results*, only a list of slide identification numbers.
* Clean all oil-immersion examined slides with 80/20 ethylether/ethanol mixture and allow to dry.
* For each slide, make notes on the form (annex 1) about the size, thickness, evenness and background staining of the smear. For smears that are not re-stained, add notes on AFB colour and artefacts after microscopic examination.
* Restain all FM-examined smears.
* Restain all ZN slides if a staining or fading problem is suspected. This may be the case when first-level controllers miss too many scanty and 1+ results (identified as positive by the second controller after restaining); or when low quantifications without restaining turn out to be systematically higher after restaining.
* For restaining, use the routine staining cycle and a certified quality auramine (FM) or carbol-fuchsin solution (ZN). Do not decolourize first; make a note on the form stating that restaining was done.
* Check the smears using the original microscopy system (brightfield for ZN, fluorescence for FM) and standard magnification. Check the number of fields prescribed for routine work in the NTP guidelines; do not check more fields unless there is doubt about the quantification after one length.
* Note the first control result on the sampling form in the first controls column.
* Add overall notes on smearing and staining at the bottom of the forms, and send them to the local coordinator.
* Do not recheck slides that lack clear identification or that have severely damaged smears. Report them as “excluded/ID” or “excluded/damaged”.
* After rechecking, let the oil on the slides soak into absorbent paper as for routine work, then replace the slides in their original box.
* Rechecked FM slides – and ZN slides if fading is a problem – should be kept in a closed box, and in a cool place (refrigerator) if possible, until discordants have been taken out.

Local (district) coordinator

* Copy the first-level controller’s results to the original form with the microscopy laboratory results, and the microscopy laboratory results to the first-level controller’s copy.
* Identify slides for which the laboratory results and the first-level controller’s results are discordant; these will be either positive (or scanty) versus negative, or a quantification difference of at least 2 steps (scanty versus 2+ or 3+; 1+ versus 3+).
* Discordant results for each microscopy laboratory sampled should be listed on the discordant rechecking form [annex 2]. Record the name of the laboratory, the identification number of each slide concerned, and the two discordant results. For some laboratories, record the laboratory result as “Result 1” and the controller result as “Result 2”; for other laboratories, record the controller result as “Result 1” and the laboratory result as “Result 2”.
* Request all the slides on the discordant list from the first controller. Keep them in the closed box – in the refrigerator if there is the possibility of fading (FM, restained ZN) – until they can be sent, together with the discordant form, to the second controller.
* *Do not provide feedback* for samples with discordants at this stage, since errors have not yet been identified.

Second-level controls

* Restain all ZN slides with discordant results unless they were restained at the first control level and have not faded again. Make a note if restaining is done.
* Recheck these smears, assuming that AFB are present. Use both laboratory result and first-controller result (results 1 and 2) as a guide to the number of fields to be checked. For negative/clear positive discordance, two lengths are sufficient before concluding that the result is negative; for scanty/negative discordance, examine five lengths before declaring a negative result; for quantification differences examine as many fields as necessary to yield a confident result.
* Note the quantified result in the column for the second controller; add qualitative remarks (annex 2).
* Make a copy of the form for the record and send the original to the local coordinator, together with all the slides.

Local (district) coordinator

* Copy the second control results to the appropriate column on both the original forms.
* Using this second controller result as the “gold standard”, determine who was responsible for the error and, from Table 2 below, the nature of the error. *Remember that errors may be made by the first controller as well as by the laboratory.*
* For results that were not discordant, consider that both the microscopy laboratory and the first controller were correct in their conclusions.
* Fill in the tables at the bottom of the form (annex 1) with the numbers of slides rechecked and the errors, by both the microscopy laboratory and the first controller, identified.

**Table 2. Classification and definition of errors found in rechecking**

|  |  |
| --- | --- |
| **Registered result being controlled** | **Final result** |
| **negative** | **scanty** | **1+** | **2+** | **3+** |
| **negative** | correct | LFN | HFN | HFN | HFN |
| **scanty** | LFP | correct | correct | QE | QE |
| **1+** | HFP | correct | correct | correct | QE |
| **2+** | HFP | QE | correct | correct | correct |
| **3+** | HFP | QE | QE | correct | correct |

HFP: high false-positive, negative versus clearly positive result

HFN: high false-negative, positive versus totally negative result

LFP: low false-positive, negative versus scanty result

LFN: low false-negative, scanty versus totally negative result

QE: quantification error, at least two steps difference in quantification

*4.4.3 Interpretation, recording and reporting*

Final interpretation is possible only after all the annual samples have been processed, including second control on discordants. However, very poor results may already be evident after one quarter, and these need immediate action.

Local (district) coordinator

* After feedback has been provided to microscopy laboratories and first controllers, and corrections have been made, report to the higher intermediate level (regional, provincial) EQA coordinator by means of the summary quarterly report in Annex 3.
* Keep a copy of each completed sampling/feedback form on file.
* Request an immediate visit by a laboratory expert to microscopy laboratories with consistently bad results: these are the centres where most 1+ – 3+ results are false-positive (assuming that more than one was rechecked); and those where the percentage of false-negative results approaches the prevalence of positives.

Regional coordinator

The system of reporting from this level must be determined by each country and depends on the means available (e.g. electronic).

* If possible, record information from the incoming rechecking reports, including data on routine performance of the microscopy laboratories, in a rechecking analysis and database workbook (see Annex 4.1 to 4.6)
* Provided that all required data have been entered (including routine performance data), the proportion of true positives detected by the first controllers, and the relative detection fraction of these by the laboratories, will appear in the annual analysis sheet of the workbook. These values will be updated once the results for subsequent quarters have been entered. In addition, the percentage true high positives detected will appear, both per laboratory and for the area as a whole.
* The centres that need closer monitoring of performance, and possibly problem-solving supervisory visits, will be indicated in the annual analysis sheet. This can be done automatically by the computer, provided that flagging criteria are set appropriately to pick up all centres with relative detection fraction below 0.75, and all centres with percentage true positives below 90% (provided that this represents more than 1 HFP). A list of these laboratories can then be generated automatically.
* Report to the higher (national) level, sending either the complete workbooks in electronic format or a print-out of the annual analysis and summary sheets, together with the list of possible problem centres.

National level

* From electronic or paper reports received, enter the totals (annex 3) per district/region – for microscopy centres as well as first controllers – in a national workbook. This will yield the overall performance per administrative area compared with the overall detection capacity by the controllers

*4.4.4 Validation*

Local coordinators

* Local coordinators will ensure that the performance of first-level controllers is also analysed, and that the slides considered to represent HFP are returned along with the feedback, so that individuals responsible for errors get the chance to point out serious mistakes by first and second controllers.

Regional coordinator (annual complete sample)

* From time to time, the results obtained by second controllers discordant slides should be checked, to see whether the second controllers most often confirm presence of AFB (as should be the case).
* Discard all rechecking results as unreliable if relative detection by the microscopy laboratories compared with the controller is higher than 1.00 across all the laboratories for which that controller is responsible. Another first-level controller should be designated for that area or the workload of the original controller should be considerably reduced.
* Verify the number of HFP erroneously declared by the second controller and reported by the lower level coordinator when providing the routine feedback. Investigate the matter with the second controller and appoint a replacement controller if the problem is particularly serious.

*4.4.5 Feedback and problem-solving*

Regional coordinator (annual complete sample)

* Provide feedback to the local coordinator sending the analysis sheet and list of substandard laboratories for the area. Give advice on appropriate action, e.g. indicate priority centres for visit by an expert, or centres where an additional sample should be rechecked or a panel test taken for further investigation of a suspected problem.
* Provide feedback to the second controller in case of problems (e.g. high positives missed too frequently, see above).

Local coordinator

* Always return one completed copy of the form (annex 1) to the microscopy laboratory (can be delivered by the supervisor who collects the samples, during his/her next supervision visit, giving only general comments/recommendations made by the controllers.
* For any slide on which a serious error was identified (HFP, HFN) on supervision, check that the laboratory results on the form match with those in the register; correct both copies of the form if necessary. Ask the local technician to check the smear again, if a high false-positive was found, to show the AFB. Make a note on both copies of the form if true AFB can be shown; if artefacts were confused with AFB, start the retraining process.
* Once all samples for the quarter have been processed, discuss the overall results with the first controller, particularly if he/she made too many errors. Return any slides on which a serious error by this first controller is suspected, providing the opportunity for reassessment (e.g. AFB missed by the second controller).
* If a microscopy laboratory is apparently producing consistently very poor results (almost all 1–3+ false; sensitivity ration below 0.6), arrange for an immediate problem visit by a laboratory expert – even before the annual sample size is reached – to investigate and solve the problems.
* Arrange for the same visits for laboratories on the list of unsatisfactory performance centres. Set priorities according to degree of deficiency and available resources; foresee close monitoring by repeated visits.
* Take further samples or use a panel test for other centres on the list with borderline performance (i.e. those not in need of an immediate visit by an expert).

Laboratory expert/supervisor

* When visiting laboratories to identify and solve problems, take with you the rechecking results for the whole year and any slides with serious errors that are still available.
* Allow the type and extent of the problems to dictate the nature of the investigation, taking account of remarks on smear and staining quality and any observed quantification error.
* Start by reviewing the error slides with the local technician:
* Use the local microscope, to check that it is functioning properly.
* To assess knowledge in case of false-positives, ask the technician to show what he or she considered to be AFB.
* For false-negatives in particular, check the staining solutions. Are the dates within limits? Is carbol-fuchsin a dark red colour when poured on a slide? Is there excess precipitate in the auramine solution? Any aberrant finding may indicate bad staining solutions as the cause of the problem.
* If there is no problem with the staining solutions, check the colour of AFB in recently stained positives. If the staining is not strong red and solid, suspect a problem with staining technique and request a demonstration.
* Look macroscopically at the recent slide collection to judge the quality of smearing, decolourization and counterstaining.
* Check the laboratory register for further clues (e.g. excessive proportion of isolated positives, or too many/too few scanty results).
* Further guidance for the investigation may be found in Annex 5. If no clear-cut problem can be identified, conclude that this was either a chance finding or careless work (e.g. too little time spent on reading slides). If the latter is the case, this will be confirmed by similar levels of error being found consistently in future samples.
* Any problem that is identified must be corrected at the very earliest opportunity, by on-the-spot training (e.g. in good smearing or staining technique), replacement, repair or adjustment of the microscope, replacement of staining solutions. etc.
1. Related documents

Aziz MA et al. *External quality assessment for AFB smear microscopy*. Washington, DC, Association of Public Health Laboratories, 2002.

Rieder HL et al. *Priorities for tuberculosis bacteriology services in low-income countries*, 2nd ed. Paris, International Union Against Tuberculosis and Lung Disease, 2007.

**Annex 1. Blinded rechecking of sputum smear examinations for acid-fast bacilli**

Microscopy laboratory\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Local technician(s)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Period in lab. register checked\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date sampled\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

First controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Laboratory\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Laboratory\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Microscopy lab.** | **Results of** | **Specimen** | **Size** | **Thickness** | **Staining** | **Comment** |  | **Microscopyl lab.** | **Results of** | **Specimen** | **Size** | **Thickness** | **Staining** | **Comment** |
| **Slide no.** | **Result** | **first control** | **second control** | **Slide no.** | **Result** | **first control** | **second control** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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Specimen, size, thickness, staining: M = marginal, P = poor

|  |  |  |
| --- | --- | --- |
| **Microscopy laboratory** |  | **First controller** |
| **Totals reported results on the samples** |  | **Totals reported results on the samples** |
| **Positive:** | **Positive:** | **Negative:** |  | **Positive:** | **Scanty:** | **Negative:** |
| **Summary of errors identified** |  | **Summary of errors identified** |
| **Major errors** | **Minor errors** |  | **Major errors** | **Minor errors** |
| **HFP** | **HFP** | **LFP** | **LFN** | **QE** |  | **HFP** | **HFN** | **LFP** | **LFN** | **QE** |
|  |  |  |  |  |  |  |  |  |  |  |
| **Total major errors:** | **Total minor errors:** |  | **Total major errors:** | **Total minor errors:** |

HFP = high false-positive, HFN = high false-negative HFP = high false-positive, HFN = high false-negative

LFP = low false-positive, LFN = low false-negative LFP = low false-positive, LFN = low false-negative

QE = quantification error QE = quantification error

**Conclusions** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Recommendations** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Annex 2. Rechecking of sputum smears for acid-fast bacilli: list of discordants**

District\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Period covered by sample\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

First controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| **Laboratory checked** | **Slide no.** | **Result 1** | **Result 2** | **Second controls** |
| **Result** | **Remarks** |
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**Annex 3. Recommended formats of forms used for rechecking (manual filling): quarterly/annual report form**

Region\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Quarter\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Year\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

First controller(s)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ QA coordination\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- |
| **Laboratory No** | **Laboratory names** | **Performance of microscopy l laboratories** | **Performance of first controller** |
| **No. of smears recheckeda** | **No. of errorsb** | **Controller IDc** | **No. of smears recheckedd** | **No. of errorse** |
| **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** | **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
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|  | **Totals** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

HFP = high false-positive, LFP = low false-positive, HFN = high false-negative, LFN = low false-negative, QE = quantification error.

a Fill in the counts of results positive, scanty 1–9 or negative as registered at the controlled laboratory for the checked smears (column laboratory results from the rechecking form).

b Fill in the numbers of errors found for the controlled laboratory after discordants were rechecked by the second controller.

c Fill in the name or identification code of the first controller who reread slides from the laboratory concerned.

d Fill in the counts of results positive, scanty 1–9 or negative as registered by the first controller (column first controller results from the rechecking form).

e Fill in the numbers of errors found for the first controller after discordants were rechecked by the second controller.

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Annex 4. Examples of sheets for compilation and analysis of rechecking results (computer tool)**

**A4.1 Rechecking of sputum smears for AFB: quarterly compilation sheet**

 Region Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Quarter 1**

Year \_\_\_\_\_\_\_\_\_\_ EQA coordinator\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Evaluation of microscopy laboratories** | **Evaluation of first controller** |
| **District code** | **Name of controlled laboratory** | **Results reported for** **rechecked smears (no.)** | **Errors detected in EQA (no.)** | **Controller ID** | **Results reported for** **rechecked smears (no,)** | **Errors detected in EQA (no.)** |
| **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** | **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
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**A4.2 Rechecking of sputum smears for AFB: annual compilation sheet**

Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Year**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

EQA coordinator\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |
| --- | --- | --- |
|  |  | **Evaluation of microscopy laboratories** |
| **District code** | **Name of rechecked laboratory** | **Results reported for** **rechecked smears (no.)** | **Errors detected in EQA (no.)** |
| **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
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| **Error rates** | **Total FP** | **Total FN** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
|  |  |  |  |  |  |  |

**A4.3 Rechecking of sputum smears for AFB: evaluation of first controllers**

Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Year**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

EQA coordinator\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |
| --- | --- | --- |
|  |  | **Evaluation of microscopy laboratories** |
| **Lab No** | **Name/code of** **first controller** | **Results reported for** **rechecked smears (no.)** | **Errors made in EQA samples (no.)** | **Percent true pos. detected** | **Percent true pos./all pos.** |
| **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
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| **Error rates** | **Total FP** | **Total FN** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
|  |  |  |  |  |  |  |

**A4.4 Rechecking of sputum smears for AFB: annual analysis of microscopy laboratory performance**

Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Year**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

EQA coordinator\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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|  |  |  |  |  |  |  |  | **Percentage of true positive/scanty overall** **detected by the controllers** |  |
| **District code** | **Name of rechecked laboratory** | **Smears examined during the year** | **Smears rechecked by EQA (no.)** | **EQA rechecking results** |
| **Total** | **% positive** | **% scanty** | **Pos.** | **Scanty** | **Neg.** | **HFP (no.)** | **HFN (no.)** | **LFN (no.)** | **Detection proportional to controllersa** | **Percent true positives/all positivesb** |
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a Each laboratory’s detection of true positives is compared with that of the controllers and expressed as a number:

 1.00 indicates exactly the same detection capacity; 0.50 indicates only half the positives detected by controllers were found;

 1.05 indicated that the laboratory may be slightly better than controllers in detecting positives.

b Indicates the reliability of a positive result.

**A4.5 Summary results of rechecking of sputum smears for AFB**

**Region** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Year**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |
| --- | --- | --- |
|  | **Number** | **Percentage** |
| No. of operational laboratories |  |  |
| No. of those rechecked  |  |  |
| No. of positive slides rechecked |  |  |
| No. of negative slides rechecked |  |  |
| Overall region % positives in the laboratories’ routine |  |  |
| Overall region % high false-positives |  |  |
| Overall region % false-negatives |  |  |
| Overall region % true positives/all positives |  |  |
| Overall region detection proportional to controllers |  |  |
|  |
| Laboratories with more than 1 HFP |  |  |
| Laboratories with 100% true positives |  |  |
| Laboratories with 95–99% true positives |  |  |
| Laboratories with 90–94% true positives |  |  |
| Laboratories with 85–89% true positives |  |  |
| Laboratories with <85% true positives |  |  |
|  |
| Laboratories with more than 1 FN |  |  |
| Laboratories as good as controllers at detecting positives (≥0.95) |  |  |
| Laboratories almost as good as controllers at detecting positives (0.85–0.94) |  |  |
| Laboratories moderately good at detecting positives (0.75–0.84) |  |  |
| Laboratories doing poorly at detecting positives (0.50–0.74) |  |  |
| Laboratories doing very poorly at detecting positives (<0.50) |  |  |

**A4.6 Rechecking of sputum smears for AFB: summary report by district**

Second controller\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ **Year**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

EQA coordinator\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |
| --- | --- | --- |
|  |  | **Evaluation by district** |
| **District code** | **Results reported for** **rechecked smears (no.)** | **Errors made in EQA samples (no.)** | **Percent true positives detected** | **Percent true positives/all positives** |
| **Pos.** | **Scanty** | **Neg.** | **HFP** | **LFP** | **HFN** | **LFN** | **QE** |
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**Annex 5. Investigation of errors detected in rechecking, by error pattern.**

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| --- | --- | --- |
| **Pattern of errors** | **Possible causes** | **Checks to be done** |
| HFP +++ and HFN +++ (nonsense results) | 1. Unusable microscope
2. Has no knowledge of AFB
3. Doesn’t look
 | 1. Examine a 3+ using that microscope.
2. Test with clear-cut positive/negative and a good microscope.
3. Exclude other causes.
 |
| A single HFP | 1. Administrative error
2. As for more frequent HFP (below)
 | 1. Compare lab. register with QC listing – correct no. and result?
2. Exclude causes of more frequent HFP.
 |
| Regularly HFP with or without LFP | 1. Poor registration routine
2. No systematic restaining before QC
3. Not quite clear on what AFB is
4. Selling positive results
 | 1. Check lab. register (up to date?), use of sputum examination form, labelling of sputum containers.
2. Restain and re-examine HFP; again positive?
3. Look for inconsistent results of suspects in lab. register: regularly isolated positive/scanty?
4. Exclude other causes.
 |
| Rare LFP | Errors of QC | Ignore if they occur at comparable frequency for controllers. |
| Many LFP, with or without low-grade HFP | 1. Poor controls and counter-checks
2. Not quite clear on what AFB is
3. Contaminated carbol-fuchsin stain
 | 1. Almost no LFN detected? Feedback of slides to laboratories – can they show the AFB?
2. Cross-check especially large sample of scanty smears
3. Test laboratory’s stain on known negative smears/central laboratory records on stain QA?
 |
| Single HFN (2–3+)  | 1. Administrative error as for single HFP
2. Very thick smear and/or poor light
3. Not looked at at all
 | 1. Exclude other causes, check register as above (up to date? complete?)
2. Look at recent smears. Thickness? Too dark blue? AFB clearly seen in thicker parts (if applicable, use mirror)? Position of condenser and diaphragm?
3. Exclude other causes
 |
| More HFN and/or many LFN,  quantification too low | 1. Bad stain and/or poor staining (and restaining done)
2. Poor smearing technique
3. Problems with microscope
4. Careless microscopy
5. Contaminated methylene blue or rinsing water (and restaining done)
 | 1. Check carbol-fuchsin stain – dark red, shiny (= well concentrated)?

Check whether AFB are well stained in fresh (not restained) positive smear – solid, strong red colour? Observe staining procedure – sufficient time, heating?1. As for single HFN above.
2. Use same microscope to examine a known positive – light, brightness? Image clear? See above.
3. Exclude other causes.
4. Aspect of the AFB typical? use methylene blue (distilled water for rinsing) on known negatives in repeat staining cycles, then check for atypical AFB.
 |
| Very high proportion of LFN | Contaminated methylene blue or rinse water (restained) | As above |
| Serious QE (too low gradings) | 1. Poor stain/staining (restained)
2. Problems with microscope
 | 1. As above
2. As above
 |