MODS Quality assurance

What is quality assurance?

The field of quality assurance is large and at times the terminology can be confusing. However the key elements of interest here are quality control (QC), external quality assessment (EQA) and quality improvement (QI).

Quality Control (QC)

QC is the process of systematic monitoring of the work performed in the laboratory. This includes evaluating the precision (repeatability) and accuracy (trueness) of the test results, the performance of test reagents, and how well the laboratory staff carry out the test.

Indicators and required standards (thresholds) are identified and monitored routinely (daily, weekly, monthly, etc. as appropriate). If pre-established standards are not met corrective actions are mandated. These are pre-defined in the QC plan and designed to address underlying causes of problems or deficiencies. QC activities must be documented, and documentation should record the results of on-going monitoring and corrective actions taken if standards are not met.

External Quality Assessment (EQA)

EQA is a program organized by an external agency (national or supranational lab, or other reference laboratory) to objectively and systematically check agreement of laboratory results with a reference standard. **Proficiency panel testing** is the most common EQA mechanism, though for methodologies like MODS which use sputum samples rather than strains of *M tuberculosis* there is no currently agreed format; **split sample checking**, **blinded rechecking** and **on-site evaluation** are other approaches. Detected deficiencies will identify laboratories needing **quality improvement**.

Quality improvement (QI)

Laboratories that persistently fail to meet QC and EQA standards should host site visits and reviews of standard operating procedures and equipment checks as indicated.

In addition, as part of routine EQA, all participating laboratories should host a comprehensive site review annually to review test procedures, QC monitoring results and documentation, and equipment maintenance. A standard check list such as that provided in Annex 5 of Laboratory Services in Tuberculosis Control. Part I; WHO, Geneva, 1998 should be developed for this purpose.

This document outlines proposed QC and EQA activities for TB laboratories implementing MODS

Quality Control

1. Sputum Sample Quality

"The result of any laboratory test is only as good as the sample received in the laboratory". Reliable TB culture and DST requires sputum samples of acceptable quality. The quality of these samples should be routinely monitored and analysed to identify systematic deficiencies in sputum sample acquisition and handling. Analysis by referring healthcare establishment should permit targeted corrective actions where needed.

Indicator 1: sputum samples should be transported appropriately to the receiving laboratory (in accordance with WHO guidelines)

Standard: 100% properly packaged

Monitoring: all sample shipments should be evaluated; the correctness of packaging, and presence or absence of spills recorded; immediate feedback of improper packaging, and presence of spills requiring resubmission of specimens should be sent to the submitting health center; data should be analysed monthly

Indicator 2: all samples should be correctly labeled and arrive with properly completed request forms

Standard: a) 100% of samples must be labeled with patient identifier;
b) ≥ 95% of forms should contain all data required

Monitoring: all samples and forms should be evaluated on receipt and deficiencies recorded; data should be analysed monthly

Indicator 3: sputum samples should arrive at the reference laboratories within 72 hours of collection from patients.

Standard: ≥ 90% of samples arrive within 72 hours of collection (≤ 72 hours)

Monitoring: the interval between the date/time of sample collection (as recorded on the request form) and date/time of arrival (as registered at the receiving laboratory) should be noted for every sample received; data should be reviewed monthly

Indicator 4: sputum samples should be of adequate quality

Standard: >80% of samples should be of sufficient quantity (at least 2ml) and quality (local classifications may be used)

Monitoring: quarterly

Corrective Actions (for all 4 indicators):

On-site visits to review pattern of deficiencies with responsible persons at the healthcare establishment in order to pinpoint the source(s) of problems (need for staff training, need for easier access to refrigeration, problems with the transport system, etc.) and to develop a plan to resolve them.

Increase frequency of monitoring or revise type of monitoring until deficiencies resolved (6 successive months of acceptable performance).

2. Decontamination

Decontamination must balance the need to destroy respiratory flora that can contaminate and overgrow mycobacterial culture without excessive destruction of mycobacteria that would produce false negative culture results. Contamination rates of less than 2% suggest excessively harsh decontamination; rates of greater than 5-8% suggest insufficient decontamination leading to unacceptable costs, excess work and delays from the need to repeat cultures.

Indicator: culture contamination (bacterial overgrowth) rates should fall within an acceptable range.

Standard: contamination rate of 2-8%
Monitoring: monthly

Corrective Actions:
Decontamination solution reagents should be checked
Decontamination procedures (both reagent preparation and decontamination process) should be reviewed with staff and several decontamination runs should be observed to look for errors that would lead to over- or under-decontamination.

3. Culture

TB culture QA is monitored principally through EQA procedures discussed below. However, several internal QC procedures are appropriate for monitoring the quality of culture preparation, assessing reliability of results and to help identify problems with cross contamination. In addition, in laboratories performing MODS in parallel with standard methods, comparison of results between the two methods affords the opportunity to monitor culture results on an on-going basis.

The MODS standard operating procedure (SOP) indicates the need to routinely verify media sterility by 48 hour incubation at 37°C prior to usage. Each MODS plate contains a sample-free column containing all media components which serves as an internal negative control. On a separate MODS plate daily positive control cultures should be run in parallel with sample cultures to verify the ability of prepared media to support mycobacterial growth.

Indicator 1: for smear positive patients, there should be a high correlation with culture positivity.

Standard: for samples from smear positive patients not receiving TB therapy 100% of MODS cultures should be positive
Monitoring: weekly

Corrective Actions:
Check repeat smear for discrepancy with smear results reported on test request form – if the repeat smear is negative, there may be a problem with the sample submitted, or with the results reported by the referring health center. The treatment status of the patient may need to be investigated.
If smear is confirmed as true positive, check media to ensure it supports normal growth of positive controls
Check centrifugation procedures to ensure 3000g is being used (up to 3800g).
Check decontamination procedure and contamination rate to ensure decontamination procedure is not unduly harsh.

Indicator 2: cross-contamination should be looked for on a continuous basis.
An unusually high number / clustering of positive cultures amongst smear-negative samples processed on the same day should raise concerns that cross-contamination has taken place. (or, more generally, a sudden increase in positive cultures, particularly amongst smear-negative samples with consecutive laboratory code numbers signals possible cross-contamination).

With MODS testing, *M. tuberculosis* growth in negative control wells is a clear sign that cross-contamination has occurred.

Standard: No growth of *M tuberculosis* in any negative control well in MODS plates
Whenever unusual clustering of positive cultures processed on the same day is observed or if a positive culture result is inconsistent with other results from the same patient, cross-contamination should be considered and action taken.

Monitoring: daily; quarterly review of number of suspected episodes of cross-contamination

Corrective Actions
MODS plate with TB growth in negative control wells should be discarded and MODS cultures with original samples repeated
All other MODS plates prepared on the same day as that in which cross-contamination was observed should be screened for cross-contamination.
Review laboratory logbook for other culture-positive specimens processed at same time

Review smears (original & repeat) and patient histories for all samples in any unusual culture-positive cluster; consider requesting new samples from involved patients
Review laboratory procedures

Indicator 3: In laboratories performing MODS in parallel with another standard culture method, MODS results should be similar (taking greater MODS sensitivity into account).

Standard: ≥97% MODS positivity when standard culture is positive
≥97% standard culture negativity when MODS negative
Monitoring: monthly, taking account of time lag in availability of standard culture results

Corrective Actions:
Procedures to investigate the ability of prepared 7H9-OADC media to support growth (including review of positive control wells), assess decontamination, and look for potential cross-contamination as outlined in previous sections should be followed (depending on the type of discordance identified).

4. Drug susceptibility testing (DST)

Laboratories performing DST with more than one method should compare results of DST from the same samples (or patients) whenever both are performed. EQA, described later, is also important for DST.

According to the MODS SOP, daily positive control samples (1 pan-susceptible; 1 MDR) should be run in parallel with sample cultures to verify that media (with and without antibiotics) were prepared properly and that the test is functioning as expected.

Indicator 1: Pan-susceptible and MDR control samples should exhibit the expected pattern of growth in culture wells with and without antibiotics.

Standard: 100% conformance with expected results
Monitoring: daily; quarterly review of problem frequency

Corrective Action:
If control growth deviates from the expected pattern, all plates prepared on the same day as the positive control plate should be discarded; MODS should be repeated with the original samples and fresh reagents
Evaluate reagents, stock solutions and storage conditions
Review procedures for preparation of stock solutions and media with antibiotics

Indicator 2 (for labs performing 2 types of DST): Identification of isoniazid resistance, rifampicin resistance and both should correlate highly when more than one method is used.

Standard: 95% concordance
Monitoring: weekly (as results of standard methods become available)

Corrective Action:
Evaluate reagents, stock solutions and storage conditions
Review procedures for preparation of stock solutions and media with antibiotics
An independent 3rd test (e.g. molecular testing) should be undertaken to identify which of the discordant results is most likely incorrect and in need of review

5. Results Reporting

Timely reporting of TB culture and DST results that affect patient management is essential. Failure to report results quickly undermines the benefit of the technical rapidity of MODS.

Indicator: Positive TB culture results and identification of MDR-TB must be reported to the responsible health center physician or TB program staff in a timely manner

Standard: ≥90% of positive TB / MDR-TB results should be reported within 24 hours (48 hours on weekends) to the facility/providers responsible for a patient with positive results

Monitoring: interval between positive result availability and result notification monitored quarterly

Corrective Action:
Procedures for result transmission will be reviewed and causes for notification delays / failures identified (determine whether the problem is at the reference lab, the receiving facility, or both). Potential solutions will be developed by involved staff and their supervisors and evaluated monthly until the standard is met for 6 successive months, where after monitoring at quarterly intervals will resume.

External Quality Assessment

While proficiency testing (PT) using panels of well-characterized TB strains that are processed by participating laboratories is the most common EQA method for indirect drug susceptibility testing methodologies, safe and reliable procedures for PT of panels of sputa are still under development and not currently validated. At present we recommend routine blinded rechecking of MODS laboratory results by an appropriate local reference laboratory.

The tables below provide estimates of the annual quantity of EQA (re-test) samples that would be submitted to the Peruvian National Reference Laboratory for re-testing by a regional government laboratory serving a population of 2,000,000. Estimates are based on the assumption that each regional lab receives approximately 4000 samples per year, 3000 of which are smear-positive. With this scheme, the reference laboratory workload would be 65 sputum samples and 950 strains per MODS laboratory per year. If this is deemed an excessive burden then INH mono-resistant strains could be considered as optional (or only a selection, say 1 in 10, could be re-tested), since MDR is almost never misclassified as isoniazid mono-resistance in the MODS assay.

Confirmation of	**Samples sent**	**Frequency**	**Estimate**
Negative culture	MODS culture-negative samples	Every 50th smear-negative, culture-negative Every smear-positive culture-negative	20 20
Positive culture	MODS culture-positive samples	Every 20th smear-negative, culture-positive No smear-positive, culture-positives	25 0
MDR	MODS MDR strain	All MODS MDR strains	350
INH mono-resistance	MODS INH-resistant strain	All INH mono-resistant strains	450
RIF mono-resistance	MODS RIF-resistant strain	All RIF mono-resistant strains	50
RIF/INH susceptible	MODS RIF/INH susceptible strain	Every 20th MODS RIF/INH susceptible strain	100

Standards of acceptable EQA performance

1. **Culture sensitivity:**
    a. Any MODS culture-negative sample from a regional laboratory found to be culture-positive in the reference laboratory will prompt action.

    Action: a site visit should be conducted and additional tubes of media requested for assessment of growth promoting characteristics. Culture QC performance (Section III - indicator 1: culture positivity rate for smear positive samples) will be reviewed

    b. Any MODS culture-positive samples found to be culture-negative in the reference laboratory will prompt action (although this is to be expected periodically due to the greater detection sensitivity of MODS)

    Action: the regional laboratory will be asked to request repeat samples from the patients involved. If all the repeat samples obtained are also culture-negative in either laboratory a site visit will be conducted to identify potential areas for cross-contamination and QC procedures for detection of cross-contamination will be reviewed.

2. **DST accuracy:**
    a. the standard for detection of rifampicin and isoniazid resistance is 95% concordance of results for combined sensitivity and specificity, or "efficiency". Efficiency is the sum of true positives and true negatives divided by total results. Concordance of results between the MODS laboratory and the reference laboratory will be evaluated quarterly for efficiency.

    Action: if the efficiency for MDR detection falls below 95%, a site visit will be performed to review procedures for preparing media and processing cultures.

Whenever a regional laboratory's performance falls below the defined standards for acceptable culture and/or DST testing, the issuance of culture/DST results for clinical care should be suspended and clinicians notified accordingly. This suspension may be revoked once satisfactory performance is achieved and maintained for one month.

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