Setting reference standards for Microscopy Evaluations

C. N. Paramasivan

Head of TB laboratory Support

Partnering for better diagnosis for all

QC & EQA Documents

- **WHO**
  - **LABORATORY SERVICES IN TUBERCULOSIS CONTROL**
    - **ORGANIZATION AND MANAGEMENT**
    - **Smear microscopy**
    - **Culture**
  - **Global DRS Report III**
  - **WHO IUATLD DRS Guidelines**
  - **Generic DRS protocol of India**
  - **APHLS, CDC, WHO etc EQA Guidelines**
  - **RNTCP EQA Guidelines**

Organization of Mycobacteriology Services

- Pyramidal structure recommended
  - To maintain proficiency
  - Smear, Culture, DST, ID, Storage, Molecular typing
- To maintain efficiency and cost-effectiveness
  - Depending on the economical situation
- Three main levels
  - National: Reference lab, Specialized
  - Intermediary: District, Region, Province
  - Peripheral: Microscopic Centers
- Additional: Peripheral Treatment Centers, Supra-National Ref. Labs

Organization of Mycobacteriology Services with reference to Smear Microscopy

- Decisions on technical issues, with NTP
  - Eg. Equipments, supplies
  - Eg. Appropriate threshold for smear-positivity
- Organization - Participation in:
  - Training
  - Supervision
  - QC of smear microscopy
- Training, supervision, quality control
- Carry out Operational Research

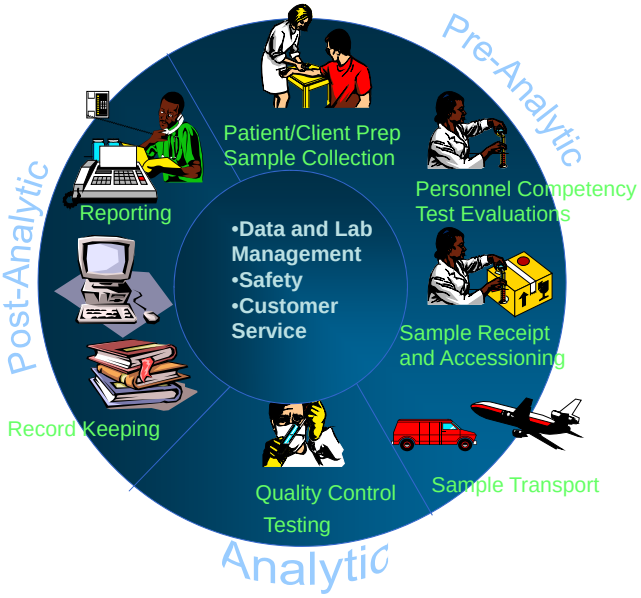
QAP in Sputum Smear Microscopy

5

Components of QAP

- Quality Control
- Quality Improvement
- Proficiency Testing

The Quality Assurance Cycle



The Quality System

QAP

QA: A system designed to continuously improve the reliability and efficiency of laboratory service

EQA: A Process to assess lab performance

- Onsite evaluation to review QC
- Allows participant labs to assess their capabilities by comparing their results with those obtained in other laboratories

QI: Components of diagnostic services analyzed (Data collection, Data analysis, Problem solving, Identifying defects) often relies on on-site evaluation visits

Quality Control

- A process of effective & systematic monitoring of laboratory performance
- Ensures that the laboratory results are accurate, reliable and reproducible
- Ensures the competency of diagnostic services

Quality Control applied to:

- Lab. arrangement & administration, specifically to TB
- Equipment
- Collection & transport of specimens
- Handling of specimens
- Stains & reagents
- Bacteriological procedures
- Reporting of results

QAP

- Ensures
  - Personnel with adequate training and experience.
  - Proper specimen collection.
  - Proper performance of tests.
  - Efficient processing of results.
  - Reagents and equipment are of good quality

QAP

Ensures

- Detection of errors
- Prompt and corrective measures
- Preventive maintenance
- Continuous training of staff
- Documentation
- Coordination
- Timely feedback

Quality Assurance :

IQC + EQA

IQC :

Set of lab procedures undertaken to

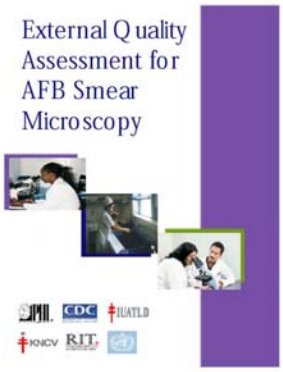
- Assess continuously laboratory network
- Reliability of results
- Consistency of results
- Control of laboratory out put

QA : IQC + EQA

EQA: (PROFICIENCY TESTING)

- **Assess laboratory performance by an External agency**
- **Retrospective and periodic.**
- **Establish Inter- laboratory consistency**
- **Credibility of laboratory**

15



International Guidelines for External Quality Assessment (EQA) of AFB Smear microscopy

World Health Organization (WHO)

International Union Against TB and Lung Disease (IUATLD)

Royal Netherlands Tuberculosis Association (KNCV)

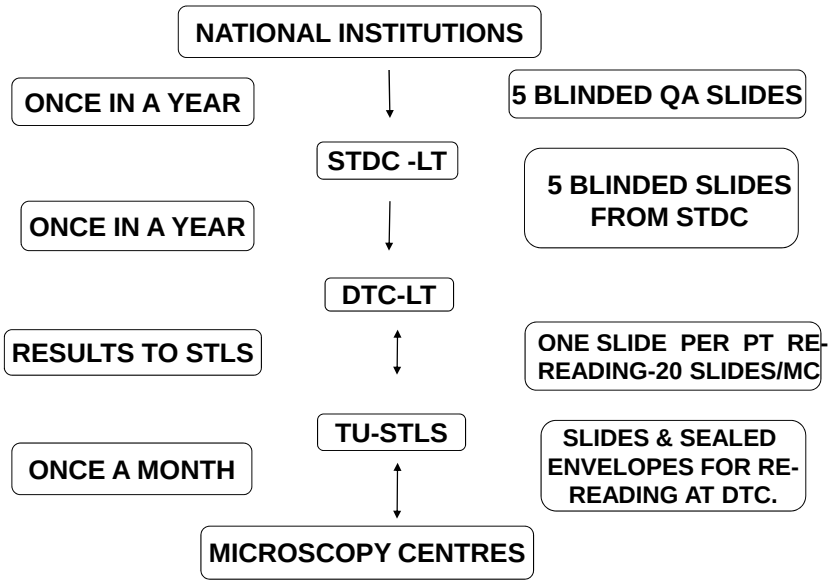
Association of Public Health Laboratories (APHL)

Centers for Disease Control and Prevention (CDC)

Japan Anti-Tuberculosis Association (JATA)

16

QA Network in Microscopy Under RNTCP - India



Selection of Slides for QC

- **Selection of the sample (from register)**
  - Estimate smears examined over period
  - Divide by sampling step = Z
  - Choose random number to start
- **LQAS method: from industry**
  - For smallest possible samples
  - One-sided test: confidence limits??
  - Outcome: not more vs. More than x% error
  - Samples size needed: from LOAS tables
  - Parameters to be set by NTP management
  - Confidence level (95%)
  - Acceptance number "D" (specificity)
  - Critical value false Negative - calculate
  - Prevalence Positive smears, Desired sensitivity
  - Definition & size of lot: Annual turn-over

18

Control Reading of Routine Smears

First screening at District/Regional level

Blind checking absolutely necessary
No results on the slides!!
Coordinator keeps lists with results
Do not overload controllers: 10 smears per day?
Essentially same technique as centers

Re-staining prior to cross-checks is best

Fading of Fuchsine colour
Not to miss gross errors of stain/staining

Specificity and Sensitivity

- **Specificity**
  - **Set at 100%**
  - **Any false positive should trigger action.**
- **Sensitivity**
  - **Ability of LTs to detect AFB relative to the Controllers**
  - **Recommended Sensitivity = 75-90%**

False Positive and False Negative

- **False positive**
  - **Permissible ERROR rate of close to '0'**
- **False Negative**
  - **Are to be expected with 'Scanty smears'**.

EQA Method Considerations

"The focus of **EQA** is on the identification of laboratories where there may be serious problems resulting in poor performance, not on the identification of individual slide errors or the validation of individual patient diagnoses"

Three methods to evaluate laboratory performance:

- On-site evaluation
- Panel Testing
- Blinded rechecking

On site Evaluation

Observation of Worker performance under actual conditions

- Condition of Equipment
- Laboratory Safety
- Adequacy of supplies
- Processing
  - Smearing
  - Staining
  - Reading
  - Reporting
- Problem Solving

23

On-site Visit by Personnel Should Make Sure

- **Availability of:**
  - **Written standard operating procedure**
  - **An adequate supply of reagents within expiration dates**
  - **Proper, well functioning equipment and an adequate supply of consumables such as a functional Microscope, slides etc.**
- **Internal QC is performed at the required intervals.**
- **Laboratory Safety practices are observed.**
- **Record keeping is accurate and consistent with requirements of NTP**
- **Results are promptly reported to treatment centers or physicians**
- **Staff have received adequate training with refresher courses.**

24

On-site visit by personnel should make sure that

- A functional Microscope is available
- Patient slides are available & properly stored for EQA
- Staff have received adequate training with refresher courses

25

Panel Testing

- A system for sending stained and/ or unstained slides from the central laboratory to the peripheral sites for reading and interpretation at regular intervals is recommended as the minimum requirement to assess proficiency
- Least expensive and resource intensive of the 3 methods of EQA
- Limitation: Checks only Technicians ability to stain and/ or read smears not a useful means to assess routine laboratory performance

Panel Testing

- **Panel Testing is useful to:**
  - **Supplement rechecking program**
  - **Provide some preliminary data on peripheral laboratory capabilities prior to implementing a rechecking program**
  - **Assess current status of performance or to quickly detect problems associated with very poor performance**
  - **Evaluate proficiency of laboratory technicians following training**
  - **Monitor performance of individuals when adequate resources are not available to implement a rechecking program.**

27

Panel Testing

Issues Regarding to Panel Testing:

Proper preparation of test smears

Number of slides to be included in the test panel set

Types of smears to include (stained and unstained, low positive, smears that are too thick or thin, poorly stained smears)

Mechanism for sending slides to the peripheral laboratories (Post, Courier, District Supervisor)

Form for test laboratories to record results

Time allowed for technicians in the test laboratories to complete panel and report result

Evaluation criteria for acceptable performance

Plan for reporting results to the test laboratory and implementing corrective action if needed

Mechanism to resolve discrepant results.

28

Blinded Rechecking

Blinded rechecking / rereading- Best Method

Peripherals Sites- Controller High level lab.

A country wide program for blinded rechecking of slides at regular intervals should be the long term goal for optimal EQA

29

Classification of Errors

		Reference Lab Results				
		Neg	Sc.	1+	2+	3+
Center Lab Results	Neg	Correct	Correct SFN	HFN	HFN	HFN
	SC	Correct SFP	Correct	Correct	QE	QE
	1+	HFP	Correct	Correct	Correct	QE
	2+	HFP	QE	Correct	Correct	Correct
	3+	HFP	QE	QE	Correct	Correct

Correct	No Errors	
QE	Qualification Error	Minor Error
LFN	Low False Negative	Minor Error
LFP	Low False Positive	Minor Error
HFN	High False Negative	Major Error
HFP	High False Positive	Major Error

Classification of Errors

- **Major errors:**
  - **HFN : 1+ 2+, 3+ reported as negative**
  - **HFP : Negative reported as 1+ or higher grade**
- **Minor errors:**
  - **SFP : Negative reported as scanty**
  - **SFN: Scanty reported as negative**
  - **QE : Positives off by more than ± 1 grade**

31

Quality Improvement

- **Process by which laboratory services are analyzed continuously to improve reliability, efficiency & utilization**
- **Achieved by anticipating & preventing problems rather than by identifying & correcting problems after their occurrence**
- **Most efficient during on-site visits**

32

LQAS

- **Lot Quality Assurance Sampling**
  - **Optimal sample size**
    - **Statistically acceptable samples**
    - **Assesses work quality of Laboratory technicians**

33

Variables Used in Determining Sample Size

- **LOT**
  - **Results in a economical & statistically valid sample**
  - **Total number of negative slides per year, per quarter, per month**
  - **importance of choosing time interva.**

34

Critical Value

- An upper threshold of the proportion of false negatives
- To be chosen from an estimate
  - Historical false negative rates
- Calculation based on;
  - Prevalence of positives
  - Expected parameters of sensitivity and specificity comparing to controller.

Instructions to Centers

- Administrative heads of centers informed of dispatch of slides
- Request to get slides read independently by as many readers as possible
- Instructed to send results and slides within one month
- Reminders sent in case of non-receipt of results within stipulated time.

Table 2: Lot quality assurance sampling (LQAS) for implementation phase (80% sensitivity, 100% Specificity and '0' Acceptance number)

Annualized no. of negative slides (ANSV) at the demonstration site	Slide positivity rate (SPR%)				
	2.5-4.9	5.0-7.49	7.5-9.9	10-14.9	≥15
	Monthly sample size[1] of randomly selected slides to be re-checked				
301-500	22	14	12	10	8
501-1000	28	18	12	10	8
>1000	40	20	14	10	8

Slide positivity rate and annualized negative slide volumes are calculated based on the data available for one month from each of the microscopy centers during 'ZN baseline' phase.

The sample size for each microscopy center is selected based on SPR and ANSV, as per Table 2 given above.

Follow-up Action

- **Decoded results sent to respective centers indicating:**
  - **Positive consistency**
  - **Negative consistency**
  - **Overall agreement**
  - **Major errors**
  - **Minor errors**
- **Recommendations for improvement in performance, if needed**
- **Copies of communications sent to Central level**

38

Rewards of a QAP

- Ensures a quality product or generation of a reliable service
- Helps physicians in establishing proper & rapid diagnosis, thus generating confidence & better health care for the patient
- Creates of a good reputation for the lab.
- Motivates factor for staff to work better
- Satisfies mandate requirements for Accreditation
- Prevents of legal suits and associated complications.

39

Good Laboratory Practices

- Proper collection of samples
- identification of specimens with special labels on hazardous specimens collection, storage & transportation under conditions to prevent deterioration of samples
- accurate performance of test
- Release of reports after proper scrutiny
- Delivery of reports to the correct destination on time
- Cordial relationship with the users

40

Setting Reference Standards for Microscopy Evaluations

- No. of Centers using LQAS for rechecking
- No. of onsite visits conducted against mandated by the project, at each level
- No. of Microscopy Centers with High False results after using LQAS at least once during the project
- No. of Supervisors with High False results on Panel testing

Summary

- Maintaining the structure of a Laboratory network is an integral part of an Evaluation Project
- Internal Quality Control and External Quality Assurance are components which are built in Evaluation Projects
- Setting of reference standards must include all the above mentioned parameters

Maximum grading of DMC & TRC Smear Results (DRS : Gujarat)

DMC Max. Smear	TRC Max. SMEAR				Total
	1+	2+	3+	Neg	
Not available	1	2	2	1	6
1+	476	194	30	21	721
2+	375	278	73	8	734
3+	380	580	245	8	1213
SC	68	16	1	5	90
Total	1300	1070	351	43	2764