



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

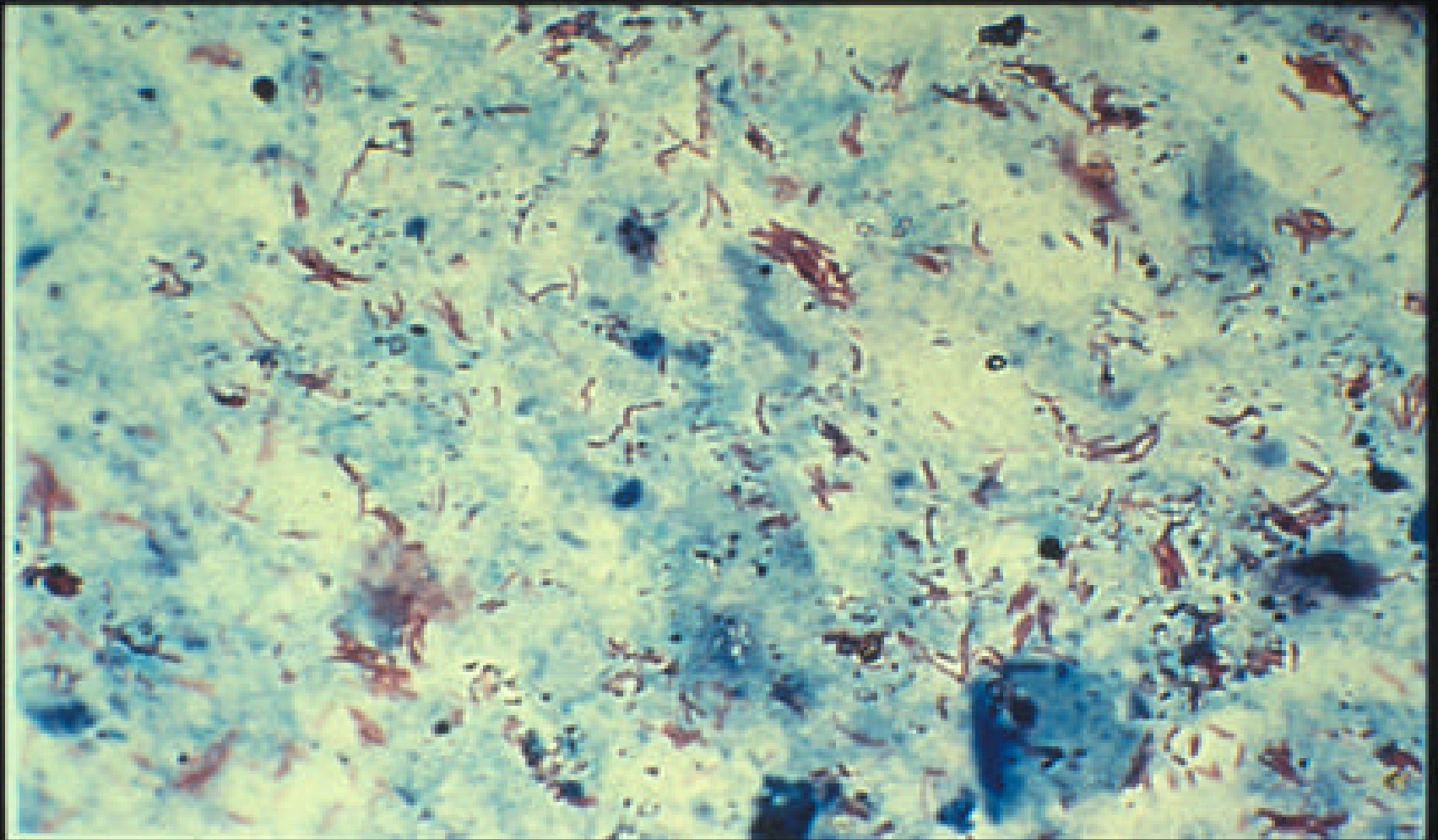
World Health Organization (WHO)

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)²⁴



Proposed Guidelines EQA

AFB Microscopy

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Proposed International Guidelines

EQA – AFB Microscopy

- **External Quality Assessment (EQA) Components**
 - onsite evaluation
 - rechecking
 - proficiency testing

EQA -- AFB Microscopy

On-site Evaluation -- Background

- **Most countries lack the resources for annual visits of peripheral laboratory by central laboratory staff**
- **Laboratories in most countries are visited by a non-laboratory District supervisor**
- **Optimum evaluation is performed by trained laboratory staff in supervisory role**

EQA - AFB Microscopy

Proficiency Testing - Background

- **Uncommon in resource-limited countries**
- **Prepared smears (South Africa) or patient slides (Senegal) sent from central laboratory**
- **Consistent challenge of laboratory test performance**
- **PT test performance may be different from testing routine patient specimens**

PT Implementation in Mexico

- Inspected 587 of 637 laboratories
- 604 microscopists given a 2 hour, 10 slide test
 - 52% had score ≥ 80
 - 33% had score 60-79
 - 15% had score < 60
- 536/604 (88.7%) finished all 10 slides
- 216 persons with score < 80 received training followed by second PT: average scores improved from 61 to 90 (P-value < 0.0001).

Ref: Balandrano et al, National QC of AFB Microscopy in Mexico 1999 IUATLD, Budapest

EQA - AFB Microscopy

Rechecking - Background

- **Recommended by IUATLD and WHO**
- **Usually 100% of positive and 10% of negative smears**
- **Usually un-blinded — adds bias***
- **Reviews patient testing; including smear preparation, staining, and interpretation**

***Lan N.T.N. et al, 1999 Int J Tuberc Lung Dis 3(1): 55-61**

Rechecking Slides in Mexico 1998

- States provided data for 438 of 637 laboratories
- Only 303 laboratories had complete and consistent data
- Only 109/303 (36%) had any FN or FP error
- 194 (64%) laboratories with no errors had 55% of total test volume — so presence of errors was not dependent on volume

Ref: Balandrano et al, National QC of AFB Microscopy in Mexico 1999
IUATLD, Budapest

Proposed International Guidelines

EQA - AFB Microscopy Components

- Resource analysis to determine appropriate EQA
- Checklists for onsite evaluation by non-laboratory district supervisor or supervising laboratory staff
- Blinded rechecking using a random statistical sample from each laboratory
- Procedures to develop PT slides
- Sample forms

Proposed EQA - AFB Microscopy Guidelines

Key Features

Resource Analysis

- **Inventory available resources (actual/projected)**
 - **Manpower, supplies, communication, administrative, financial**
- **Examine effectiveness of current EQA activities**
- **Gather laboratory service information**
- **Planning—options for the evolution of EQA**
- **Pilot test and document changes**
- **Expansion based on availability of resources**

Proposed EQA – AFB Microscopy Guidelines

Key Features

On-site Evaluation

- **Develop a standard checklist of questions and indicators**
- **Include minimal evaluation that can be performed by non-laboratory trained personnel (e.g., inventory supplies, reagents, equipment)**
- **Include detailed evaluation that can be performed by supervisory laboratory staff**
- **Train laboratory and non laboratory staff to assure consistent application**

On-site Evaluation Performed by Non-laboratory Staff Examples:

- Are all staining reagents available and within expiration dates?
- How are wire loops cleaned?
- Is the laboratory register present and all columns completed properly?
- How is maintenance on the microscope performed?



On-site Evaluation Performed by Laboratory Staff Examples:

- Does the technician verify that the container is properly labeled?
- How are slides labeled?
- How often is the carbol fuchsin filtered?
- How many fields are examined to report a negative smear?

Proposed EQA – AFB Microscopy Guidelines

Key Features

Proficiency Testing

- **Laboratory may re-use patient slides but a procedure is provided to produce test slides for consistent slide sets**
- **Recommended slide set is 10 slides: 5 stained and 5 unstained**
- **Simple forms for slide production and collection of test results**

Semi-quantitative Reporting Ziehl Neelsen

- No AFB are found in 100 fields:
“No acid-fast bacilli observed.”
- 1-9 /100 fields: Report the exact figure.
- 10-99 AFB /100 fields, 1+
- 1-10AFB /field, 2+
- Greater than 10 AFB/ field, 3+

Result of peripheral technician	Original Result				
	Negative	1-9 AFB /100 f	1+	2+	3+
Negative	-	LFN	HFN	HFN	HFN
1-9 AFB/100 f	LFP	-	-	QE	QE
1+	HFP	-	-	-	QE
2+	HFP	QE	-	-	-
3+	HFP	QE	QE	-	-

Correct	No errors	
QE	Quantification 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

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Key Features

Rechecking

- Emphasizes “blinding” and random sample using the laboratory register
- Sample size is based on Lot Quality Assurance Sampling (LQAS) with parameters selected for test volume and desired sensitivity
- Positives and negatives sampled
- Minor errors (FP or FN with 1-9 AFB/ 100 f) are used as a surrogate

Rechecking LQAS example -Mexico

Annual Volume	Positive	Negative	Current Rechecking	LQAS Rechecking
501	76	425	119	61
2219	44	2175	262	194
6650	138	6512	789	214

Prevalence of positive slides

Negative slides/year	5%	10%	15%	20%	25%	30%
200	108	72	54	42	35	30
500	155	88	61	46	37	31
1000	184	96	65	47	37	31
5000	214	104	68	48	37	31
50000	222	105	68	48	37	31

Sample size example

Increased proportional to prevalence
of positives - 10%

$$104 = 94 + 10$$

N = 4500 negatives (5000 – 500 positives)
Sensitivity = 80%
Corresponding CV = 2.20

Interpretation of rechecking errors

- If there are no errors then lab is meeting sensitivity of 80% (95% confidence level)
- Many/most labs will have at least one error and each laboratory should be evaluated based on the number and types of errors
- Some low false negatives (LFN) are to be expected, but HFN may signal a problem
- Any false positives may indicate a systematic problem

Proposed EQA – AFB Microscopy Guidelines

Resource Analysis

Phased Approach

- 1. Assure the five elements of DOTS**
- 2. Develop a central reference and intermediate laboratories to carry out EQA**
- 3. Determine the existing capacity for EQA**
- 4. Train district health officials to evaluate the minimal functions of microscopy laboratories**

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Resource Analysis

Phased Approach (cont)

- 5. Proficiency testing to evaluate performance**
- 6. Pilot rechecking program**
- 7. Determine resources: additional PT or phased implementation of rechecking**



Acid-Fast Direct Smear Microscopy

**World Health Organization
International Union Against Tuberculosis & Lung Disease**

**Centers for Disease Control & Prevention, USA
Pan American Health Organization
Instituto Nacional Diagnóstico y Referencia Epidemiológicos, Mexico
Association of Public Health Laboratories, USA**