Ensuring quality in diagnostic trials: Microscopy Studies

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Partnering for better diagnosis for all

Evaluation Study design

Evaluation of Clinical Performance*:

Sensitivity:

Direct & conc. smears in culture pos. cases compared to LM & conventional FM

Specificity:

In culture neg. cases compared to LM & conventional FM

- **Average field or time to positivity was compared to LM & conventional FM**

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Evaluation Study design

Operational Performance:

- **Inter-reader reproducibility of results**
- **Assessment of technicians' appraisal in terms of:**
  - **ease of use**
  - **maintenance**
  - **design and comfort**
  - **robustness**
  - **contrast, brightness etc..**
- **Assess necessity of dark room**
- **Assess suitability for:**
  - **Auramine-Rhodamine stain**
  - **Methylene blue counterstain**
- **Assess:**
  - **speed of fading for different stains**
  - **effect of fading on result interpretation**

Assessment of lab personnel appraisal at study sites

- **Acceptance of product design: ++**
- **Switch between bright field and fluorescence: +++**
- **Comfort of using Auramine O: ++**
- **Recognizing the advantage of LEDs: +++**
- **No waiting time period unlike regular FM: +++**
- **Body size and posture: ++**
- **Focus mechanism: ++**
- **Objectives and magnification quality: ++**
- **Contrast and colour impression of Plan-Achromat objectives: +++**
- **Homogeneity of fluorescence illumination: +++**

Scores: 0 to +++

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Demonstration studies

- **Study phases**
  - **Baseline**
  - **Validation**
  - **Implementation**
  - **Continuation**
- **Baseline phase**
  - **QC and EQA as per national guidelines**

Validation Phase

Completed form sent to FIND ☐

iLED Demonstration Project – Validation Phase

Evaluation of Performance

Performance target	Description	Result
A	# of correct readings (demonstration site compared to supervisory site)*	/total slides rechecked *(to be calculated by FIND)*
B	# acceptable quality of Auramine stains**	/total slides rechecked
C	# of major errors in the panel***	/10

* 100% of slides during validation phase
** At least 10% of slides during validation phase, better 100%
*** Positive result vs. negative result or vice versa

A > 95%
B = 100%
C ≤ 30%
} Targets met: continue with implementation phase

Any other combination
} Targets not met: additional training and proficiency testing

Performance Targets for Validation Phase & Proficiency Panel

Performance targets for validation phase and proficiency panel

Microscopy centers will only move to next phase if the following performance targets are met:

- 95% accordance between validation results of microscopy center and supervisory site.
- Quality of Auramine stains acceptable in 100% of slides examined.
- < 2 false results in the proficiency testing panel.
- For evaluation of proficiency performance targets complete the respective form (see below).

Sites that meet these performance targets are ready to enter the implementation phase. Sites that fail to continue validation phase undergo proficiency testing until targets are met.

Implementation Phase: Re-checking

Frequency: Eg LED FM study; on a monthly basis according to LQAS

Table: Laboratory quality assurance system (LQAS) for implementation phase

Annualized number of negative slides at microscopy center (ANSV)	Slide positivity rate (SPR %)				
	2.5-4.9	5.0-7.49	7.5-9.9	10-14.9	≥15
	Monthly number 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

Quality control

- **Internal quality control of newly prepared batches of reagents for microscopy as per approved SOP**
  - ***Quality controls have to be performed by microscopists***
- **Internal QC:**
  - **Using unstained panel slides prepared from an external reference site**
- **Re-reading (to be carried out by Supervisory sites);**
  - **All or**
  - **For high volume sites only - a percentage of all slides**

Quality control…

External QC

Table: Frequency of re-checking per study phase

Study phase	Nr of slides to be rechecked	Frequency of retrieving slides	Microscope for re-checking	Monitoring visits
ZN baseline	100%	Once every second week	Bright field (1000X)	Monthly
Validation	100%	Daily	Conventional fluorescence (200 – 250X)	Once every second week
Implementation	As per LQAS	Once every second week	Primo Star iLED (400X)	Monthly
Continuation	As per NTP	Once per month	Primo Star iLED (400X)	Monthly

External QC…

Re-checking will be carried-out at two different levels.

The supervisory site responsible, with only experienced rereading slides Discordant slides to be read by an SNRL for resolution.

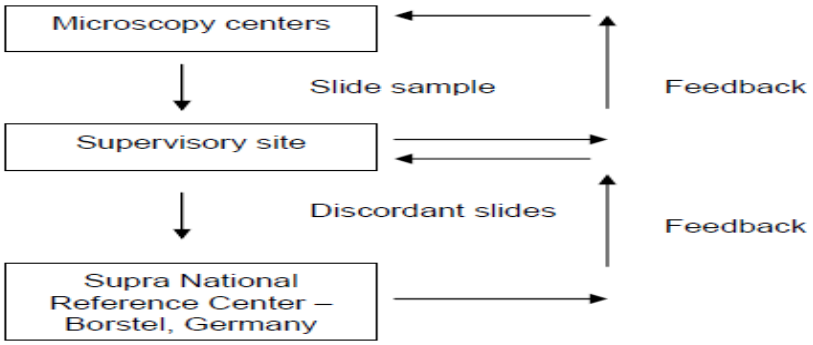


Figure: Organization of re-checking process

Error Identification and Corrective Actions

Table 3: Classification of Errors

Result by MC LT	Result of Controllers				
	Neg	Scanty	1+	2+	3+
Neg	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

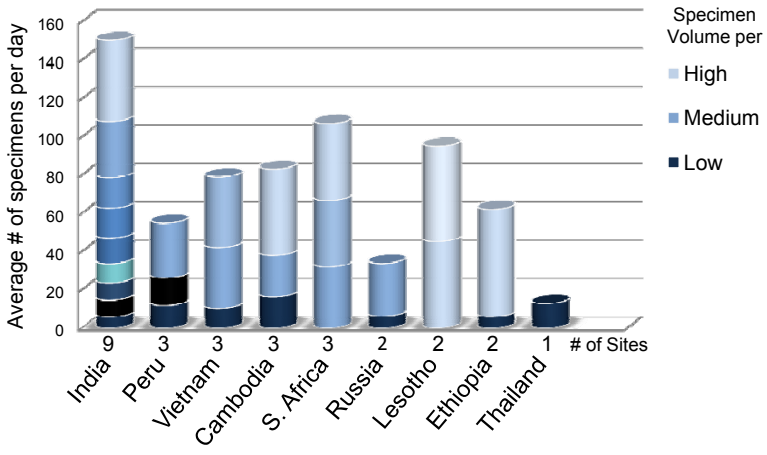
Correct: No errors
QE: Quantification error: Minor error
LFN: Low False Negative: Minor error
LFP: Low False Positive: Minor error
HFN: High False Negative: **Major error**
HFP: High False Positive: **Major error**

Continuation Phase

- **QC and EQA**
  - **As per National guidelines**
- **To integrate into the existing national TB control programme**

iLED Demonstration Study

9 Countries & 28 Sites



iLED Demonstration: Performance compared to ZN

- Direct performance comparison with ZN during validation (India, Peru, South Africa)
- 12 sites, 2 slides/patient, read with ZN/iLED; rechecking with FM
- Significantly higher relative sensitivity of iLED vs ZN
- Equivalent specificity

Sensitivity*			Specificity*		
iLED	ZN	P-Value	iLED	ZN	P-Value
93.2% (1228/1317) [90.5% - 94.1%]	77.7% (1023/1317) [73.6% - 79.4%]	<.001	98.9% (8137/8229) [98.6% - 99.1%]	98.5% (8106/8229) [98.2% - 98.8%]	0.900

*Compared to conventional FM

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iLED Demonstration: TB patient detection yield

- iLED compared to ZN resulted in an increased case detection rate
- 2-3 smears/new TB suspects (difference between iLED and ZN appears lower)
- Difference in detection of new cases was significant with a 14% increased yield of confirmed TB patients compared to ZN

A iLED / total screened	B ZN / total screened	Difference (A-B)	P-value
14.3% (435/3036) [13.1% - 15.6%]	12.5% (381/3036) [11.4% - 13.8%]	1.8% (1.3%, 2.4%)	<.001

* Considering sites with reliable information on new cases as opposed to TB treatment monitoring cases

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iLED Demonstration: Performance compared to FM

- Validation:
  - 100% daily rechecking FM; patient management based on FM
- Criteria for entering implementation phase:
  - ≥95% agreement iLED vs. FM
  - acceptable staining qty 100%
  - PPT ≥ 80%
- Met by 27/28 sites
- >95% relative specificity reached by 27/28 (>97% for majority)
- 80-100% relative sensitivity & close correlation to baseline performance
- Overall significant improvement of relative sensitivity compared to baseline

Baseline (ZN)			Validation (iLED)			p-values
Accuracy (% agreement)	Sensitivity (among ZN +)	Specificity (among ZN -)	Accuracy (% agreement)	Sensitivity (among FM +)	Specificity (among FM -)	1. Accuracy 2. Sensitivity 3. Specificity
96.8% (16333/16866) [96.5% - 97.2%]	**87.7%** (1835/2092) [84.6% - 88.4%]	**98.1%** (14498/14774) [97.8% - 98.4%]	**97.7%** (17812/18224) [97.4% - 97.9%]	**94.2%** (2567/2726) [92.2% - 94.6%]	**98.4%** (15245/15498) [97.9% - 98.5%]	0.001 <.001 0.850

iLED Demonstration: Performance compared to FM over time

- iLED performance remained strong throughout the implementation & continuation
- Excitement of using a new technology was not only a temporary effect

Validation			Implementation			Continuation		
Accuracy (% agrmnt)	Sensitivity (among FM +)	Specificity (among FM -)	Accuracy (% agrmnt)	Sensitivity (among FM +)	Specificity (among FM -)	Accuracy (% agrmnt)	Sensitivity (among FM +)	Specificity (among FM -)
97.7% (17812/18224) [97.4% - 97.9%]	**94.2%** (2567/2726) [92.2% - 94.6%]	**98.4%** (15245/15498) [97.9% - 98.5%]	**98.0%** (12230/12484) [97.4% - 98.1%]	**96.7%** (3244/3356) [95.6% - 97.2%]	**98.4%** (8986/9128) [97.8% - 98.5%]	**97.1%** (574/591) [95.4% - 98.2%]	**96.7%** (146/151) [92.2% - 98.6%]	**97.3%** (428/440) [95.2% - 98.4%]

*Based on rechecked data = fraction of the total number of slides read by iLED (>36,000 during implementation & >9,000 slides during continuation).

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Summary

- QA for Microscopy Studies should be as per existing WHO recommendations; eg. use of LQAS system for rechecking
- Panel slides for training and checking of reagents to be sourced from accredited sites
- Maintenance phase should be as per existing NTP norms, amenable to integration into the existing NTP algorithm