foundation
for innovative new diagnostics

STANDARD OPERATING PROCEDURE

Demonstration Project iLED

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Partnering for better diagnosis for all

Demonstration Project iLED

SOP

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DEMONSTRATION PROJECT
ILED STUDY PHASE 2
STANDARD OPERATING PROCEDURE

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Abbreviations

AFB	Acid fast bacilli
ANSV	Annualized negative slide volume
EQA	External quality assessment
FIND	Foundation for Innovative New Diagnostics
FM	Conventional fluorescence microscopy
HFN	High false negative
HFP	High false positive
iLED	Primo Star iLED microscope
IUATLD	International Union against Tuberculosis and Lung Disease
LED	Light emitting diode
LED-FM	LED-based fluorescence microscopy
LFN	Low false negative
LFP	Low false positive
LM	Conventional light microscopy
LQAS	Lot quality assurance sampling
QC	Quality control
QE	Quantification error
SPR	Slide positivity rate
SNRC	Supra National Reference Center
TB	Tuberculosis
WHO	World Health Organization
ZN	Ziehl-Neelsen staining

RATIONALE

These Standard Operating Procedures are meant to evaluate the effectiveness of a new LED based fluorescence microscope (Primo Star iLED, henceforth referred to as iLED) for the detection of acid fast bacilli in sputum specimens submitted for tuberculosis case finding under routine conditions.

Background

Lack of trained laboratory technologists, combined with increasing numbers of requests for sputum microscopy, results in unmanageable workloads with negative effects on TB case finding. The currently available ZN light microscopy is slow and demanding, with poor sensitivity even under optimal conditions, and further aggravates the situation.

This necessitates simple yet rapid approaches to reduce the laboratory workload and to increase the sensitivity of direct smear microscopy.

Replacing conventional light microscopy (LM) with fluorescence microscopy (FM) would be one of the options available to improve the situation in high burden countries.

A systematic review by WHO/TDR and FIND has shown that:

a) FM is on average 10% more sensitive than LM. The increased sensitivity is especially greatest in low grade positives.
b) The specificity is comparable.
c) Reading a fluorochrome stained smear takes only about 25% of the time it takes to read a ZN stained smear.

To date, the major constraints to the broader implementation of FM are the high price for fluorescence microscopes and the lack of robustness and sustainability. Furthermore, darkrooms are sometimes not tolerated due to local beliefs.

Zeiss, in a joint development agreement with FIND, has developed a fluorescence microscope with excellent performance characteristics at a most competitive price. One of the major innovations is the use of ultra bright LED (light emitting diode) technology as a reflected light source. Conventional fluorescence microscopes use expensive and very fragile gas discharge lamps (such as Xenon- or Mercury-lamps) with high power consumption and a short lifespan of only 100-200 hours. The use of a LED as a light source enables Zeiss to lower the cost of the microscope and at the same time to improve the instrument's power consumption (lifespan ≈ 20 000 hours) with a system that permits easy regulation of light intensity. The new microscope will be as robust as the popular Primo Star microscope (e.g. complete antifungal coating), will allow effortless switching from bright light to fluorescence light and can be operated on battery as well.

These innovations, in combination with the affordable price, could allow wide introduction of fluorescence microscopy and gradual replacement of conventional microscopy in the public health sector of resource-limited countries.

The first prototype version of this microscope has undergone an initial equivalence performance evaluation using high quality standard fluorescence microscopes and LJ culture as a comparator. The results of this feasibility study are summarized in the Protocol, which may be referred to by the investigator.

Meanwhile, the development phase has been completed and, based on the promising results, Primo Star iLED is ready to enter demonstration phase.

Demonstration study

The Primo Star iLED will be implemented in routine microscopy centers with and without prior experience in fluorescence microscopy to determine operational and clinical performance in the intended settings of use as well as acceptability for the laboratory staff.

Primary Objectives

1. To assess the feasibility of implementing Primo Star iLED for TB diagnosis at microscopy centers without prior experience of fluorescence microscopy in low to moderate-income settings and to identify barriers to implementation.
2. To determine the false positivity and negativity rate of LED fluorescence reading compared to a ZN baseline.
3. To assess the impact of this implementation on daily workload and case detection rates for low, middle and high-volume settings.
4. To determine lab technicians' appraisal of Primo Star iLED.
5. To evaluate detailed costs associated with LED-based fluorescence microscopy in comparison with conventional methods.
6. To identify minimal training needs and develop training modules accordingly.
7. To assess effects of fading speed on external quality assurance by rechecking.

STUDY FLOW

Responsibilities of supervisory sites during the study

- Supply of study forms with pre-printed Supervisory Site and Microscopy Center ID #s.
- Distribute slide boxes to microscopy centers.
- Prepare and supply staining solutions (at least once a month for Auramine solution) and other materials required for the study.
- Retrieve slide boxes and completed forms for re-checking according to SOP.
- Re-checking of slides according to SOP.
- Perform supervisory visits according to SOP
- Make photocopies of all completed forms. Original forms will be kept at the supervisory site.
- Organize shipment of completed forms (copies) by courier to FIND Data Management Unit (India) for electronic data capture.

STUDY PHASE OVERVIEW

Study phase	Duration	% slides re-checked	Staining reagents	Microscope for reading	Microscope for re-checking	Patient management	Frequency of retrieving slides /forms	Supervisory visits with checklist	Forms	Data transfer by courier
ZN Baseline	1 month	100%	Routine Zn stain	Conventional Brightfield (1000X)	Conventional Brightfield (1000X)	Based on ZN result of microscopy center	Once every 2nd week	Monthly	1. Result Form: ZN Baseline 2. Rechecking Form: ZN Baseline	At the end of phase
Training	5 days									
Proficiency testing & User appraisal	1 day	100%	For 10 Au and 10 ZN slides	Primo Star iLED (400X) Conventional Brightfield (1000X)	Only for discrepants: Primo Star iLED (400X) Conventional Brightfield (1000X)	-	-	-	1. Proficiency Testing Result Form; 2. User appraisal questionnaire	Scanned by e-mail following day
Validation	Minimum 1 month. Until targets met.	100%	Au staining reagents provided by supervisory site once per month	Primo Star iLED (400X)	Conventional FM (200-250X) (where not available Brightfield after restaining (1000X))	Based on conventional FM result from supervisory site (Brightlight if not available) *! Daily provision of results!*	Daily	Every 2nd week	1. Result Form: Validation 2. Rechecking Form: Validation	Every 2nd week
Proficiency testing & User appraisal	See above									
Implementation	3 months	As per LQAS	Au staining reagents provided by supervisory site once per month	Primo Star iLED (400X)	Primo Star iLED (400X)	Based on iLED result from microscopy center	Once every 2nd week	Monthly	1. Result Form: Implementation 2. Rechecking Form: Implementation	Monthly
Proficiency testing & User appraisal	See above									
Continuation	6 months	As per NTP	Au staining reagents by supervisory site	Primo Star iLED (400X)	Primo Star iLED (400X)	Based on iLED result from microscopy center	Monthly	Monthly	Same as implementation	Monthly

ZN Baseline Phase
iLED Demonstration Project

1. General Information

- The aim of this phase is to establish a baseline under study conditions in order to be able to compare false positivity and negativity rates for ZN with the rates for LED fluorescence during the implementation phase.
- Duration: 1 month.
- TB treatment decisions throughout this phase will be based on the local algorithm using the results of the microscopy center.
- Supervision during this phase will be as intense as during the implementation phase (supervisory visit once every 2nd week).
- All slides will be re-checked by the supervisory site and discrepant cases will be sent and resolved by the Supra National Reference Center in Germany.

Study phase	Duration	% slides re-checked	Staining reagents	Microscope for reading	Microscope for re-checking	Frequency of supervisory visits
ZN baseline	1 month	100%	Routine Zn	Conventional Brightfield (1000X)	Conventional Brightfield (1000X)	Once every 2nd week

2. Staining of slides

- All incoming sputum samples slides will be stained for ZN examination according to NTP and laboratory guidelines.
- Staining of slides will be done with the same staining solutions used under routine conditions.

3. Reading of slides

- Slides will be read using the available brightfield microscope.
- Semiquantitative results for ZN will follow the scale used by the local NTP.

4. Storage of slides at microscopy centers

- After reading, all slides will be kept in slide boxes provided by supervisory sites.
- Slide boxes will be labeled as follows:

ZN Baseline

Microscopy Site ID
Box Number
Slide ID – Slide ID

- Study personnel will collect all boxes during the 2 monitoring visits and transport them to the supervisory site for re-checking.

5. Re-checking of slides

- 100% of slides will be re-checked by the supervisory site.
- Results will be entered in the Rechecking Form.
- Once re-checking of all slides is finished, comparison of results and false positivity and negativity rates will be determined by FIND.
- Discordant slides (i.e. ZN negative at study site and ZN positive at supervisory site) will be sent to the Supra National Reference Center in Germany for resolution.
- Re-checked results will be provided to study sites and local authorities when required (to provide information for local QC).

6. Data entry

- A Result Form – ZN Baseline will be provided by FIND (see below).
- The supervisory site will retrieve all completed forms from microscopy sites every second week and make a back-up photocopy of all forms to be kept on-site.
- A courier service will be arranged to retrieve all completed forms from the supervisory sites at the end of ZN baseline phase; and to be sent to FIND data management unit in India (A - 32, Mohan Co-operative Industrial Estate. New Delhi -110044, India) for electronic data capture. (Exception: Some sites will carry out first data entry on site and will only dispatch forms after completion of first data entry).
- Please tick “Completed form sent to FIND” once it has been sent to FIND-India.

7. Instructions to microscopy centers

Prior to starting the ZN baseline phase, all microscopy sites will be informed that they are participating in an implementation project for a new generation of fluorescence microscopes and receive the following instructions:

In collaboration with National TB programs and international organizations, this demonstration project aims at a programmatic implementation and evaluation of the Primo Star iLED fluorescence microscope system in comparison to the existing microscope standard in low-income settings. The implementation will be carried out in 5 phases:

1. *ZN baseline (1 month; establish a baseline status for ZN prior to switching to iLED)*
2. *iLED training (5 days)*

3. *Validation (1 month; Microscopy centers use iLED only, but patient management is based on conventional fluorescence result of the supervisory site; most work-intensive and logistically complex phase, since slides must be transported to supervisory site on a daily basis and results provided to microscopy centers the next day).*
4. *Implementation (3 months; patient management on the basis of the iLED result at microscopy centers; intensive rechecking and supervision by supervisory site).*
5. *Continuation (6 months; patient management on the basis of the iLED result at microscopy centers; rechecking and supervision by supervisory site reduced to what is being done under Program conditions).*

Design of ZN baseline phase:

a) The only difference for the microscopy centers compared to routine conditions is that all slides must be kept for rechecking and that an additional RESULT FORM will be provided to record results. The completion of the form will be explained to the microscopists and completion will be practiced at least once.

b) TB treatment decisions throughout this phase will be based on ZN results from the microscopy centers and follow the local algorithm.

c) Slide boxes will be provided by the supervisory sites. Labeling of slide boxes will be explained to the microscopists: Microscopy site ID number, 1st slide ID – last slide ID.

d) All stored slides and result forms should be collected once weekly by the supervisory site. 100% of slides will be re-checked by the supervisory site in a blinded fashion.

e) The supervisory site will sent RESULT FORMS and RECHECKING FORMS to the central data entry unit in India by a pre-arranged courier service.

f) Labeling, staining of slides and reporting of results will follow NTP guidelines and will not deviate from routine procedures.

The following materials will be provided by supervisory sites:

1. Slide boxes in sufficient numbers to allow storage of all slides (these should be labeled by the microscopy site with microscopy site ID number and first and last slide ID stored)
2. Result Forms
3. Two binders (1 for empty result forms and 1 for completed result forms to avoid loss of completed forms)

RESULT FORM: ZN BASELINE PHASE

(User: Microscopy Center)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°*	Slide ID (where applicable)**	Date of sample reception	Date of slide reading	Results***		Lab Tech ID****	Date of result provided to clinic*****
					Neg	Pos		

* Specimen N°: for diagnosis assign “a, b, c” or “1, 2, 3, etc”; for treatment follow-up or other group assign “F1, F2, etc”.
** Register slide ID where available if different than Patient ID-Specimen N°.
*** If negative, tick “neg”; if positive register “scanty”, “1+”, “2+” or “3+” according to IUATLD/WHO scale.
**** Number assigned by the supervisory site.
***** If result is provided by microscopy center directly to patient instead of clinic, enter this date.

RECHECKING FORM: ZN BASELINE PHASE

(User: Supervisory Site)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°*	Slide ID (where applicable)**	Results***	
			Neg	Pos

* Specimen N°: for diagnosis assign “a, b, c” or “1, 2, 3, etc”; for treatment follow-up or other group assign “F1, F2, etc”.

** Register slide ID where available if different than Patient ID-Specimen N°.

*** If negative, tick “neg”; if positive register “scanty”, “1+”, “2+” or “3+” according to IUATLD/WHO scale.

Training – Outline
Primo Star *iLED* Demonstration Project

1. General Information

The training will be standardized for all countries and is intended for microscopists and supervisors participating in the LED demonstration projects. Training participants will already have experience in ZN microscopy and/or conventional fluorescence microscopy. Participants will gain theoretical skills to perform fluorescence-based smear microscopy using the Primo Star iLED. Participants will understand and be able to follow the study flow.

2. Training Curriculum

- Focus on hands-on training: Minimum number of slides to be read per day per participant:
  - Day 1: 10 slides
  - Day 2: 10 slides (staining and reading)
  - Day 3: 20 slides (staining and reading)
  - Day 4: 20 slides
  - Day 5: 15 slides + proficiency testing
- Training modules:

Module	Topic	Comment
1	Introduction: The Possible Role of LED-based Fluorescence Microscopy in Improving the Global Tuberculosis Situation	Introductory comment
2	Demonstration Project Primo Star iLED – Study Outline	Lecture
3	Use and maintenance of the Primo Star iLED	Practical session
4	Safety Precautions for TB Microscopy Including Collection and Transport of Sputum Samples from TB suspects	Read only
5	Managing Supplies for Fluorescence-based AFB Microscopy	Only for supervisory sites
6	Preparation of Reagents for Fluorescence-based AFB Microscopy	Only for supervisory sites
7	Smear Preparation and Fluorescence-based Staining Methods	Lecture & practical session
8	Reading, Recording and Reporting of fluorescent smears	Lecture & practical session
9	Assuring Quality of Fluorescence-based AFB Microscopy	Lecture & practical session
-	Study-specific SOP training	Practical focus on forms, storage of slides

Validation Phase
Primo Star *iLED* Demonstration Project

1. General Information

- Duration: validation phase 1 month.
- Patient management based on conventional fluorescence result provided by supervisory site.
- All slides will be re-checked by the supervisory site and discrepant cases will be sent and resolved by the Supra National Reference Center in Germany.

Study phase	Duration	% slides re-checked	Staining reagents	Microscope for reading	Microscope for re-checking	Patient management	Frequency of supervisory visits
Validation	1 month or until targets are met	100%	Prepared by supervisory sites using high quality reagents	Primo Star iLED (400X)	Conventional Fluorescence (200-250X) or where not available Brightfield (1000X)	Based on conventional FM result from supervisory site	Visit once every 2nd week. Daily pick up of forms.

2. Staining of slides

- An Auramine slide will be prepared for all incoming sputum samples.
- Fluorescence staining should be done no more than 48 hrs in advance before reading results.
- Staining solutions will be prepared by supervisory sites using preferably Merck staining reagents. However, other brands can be used if continuous supply and high quality is ensured.
- Staining solution will be provided to microscopy centers once per month (limited shelf life for Auramine!).
- Instructions to perform fluorescent stain:
  - Place slides on a staining rack and flood with TB Auramine O for 20 min
  - Wash **gently** in running water
  - Decolorize with 0.5% acid-alcohol for 3 min
  - Wash slides **gently** in running water
  - Counterstain with TB Potassium Permanganate for 1 min
  - Wash **gently** in running water
  - Air dry

3. Reading of slides by microscopy centers

- Record results using the following magnification:

Table: Magnification

Microscope	Magnification
iLED	400x (no dark room, no direct sunlight)

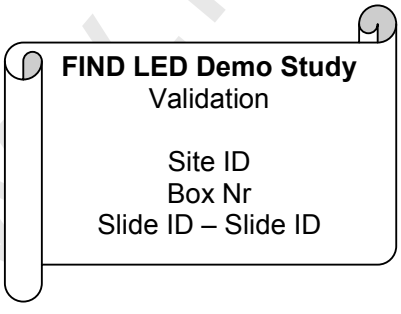
- Semiquantitative results will be documented using the IUATLD/WHO scale:

Table: Semi quantitative scale for reading with iLED

IUATLD/WHO SCALE (1000x field=HPF) Result	READING
	iLED FLUORESCENCE (400x magnification; 1 length = 40 fields = 200 HPF
Negative	Zero AFB/1 length
Scanty	1-19 AFB/1 length
1+	20-199 AFB/1 length
2+	5-50 AFB/1 field on average
3+	>50 AFB/1 field on average

4. Storage of slides

- After reading all slides will be kept in slide boxes.
- Slide boxes will be labeled as follows:



- Study personnel will collect boxes on a daily basis during validation phase and transport them to the supervisory site for re-checking.

5. Re-checking of slides

- 100% of slides will be re-checked by the supervisory site using a conventional fluorescence microscope (only where not available: use Brightfield microscope).
- Only if staining is considered of low quality, the slide will be re-stained before re-checking and results will be registered accordingly.
- Results will be entered in the Rechecking Form – Validation Phase.
- Results of re-checking will be provided to the microscopy sites immediately, since patient management will be based on these results.
- Once re-checking of all slides is finished at the end of validation phase, comparison of results and false positivity and negativity rates will be determined by FIND.
- Discordant slides (i.e. iLED negative at study site and iLED positive at supervisory site) will be sent to the Supra National Reference Center in Germany for resolution.
- Re-checked results will be provided to study sites and local authorities when required (to provide information for local QC).

Table: Semi quantitative scale for rechecking with conventional FM

IUATLD/WHO SCALE (1000x field=HPF)	CONVENTIONAL FLUORESCENCE (200-250x magnification; 1 length = 30 fields = 300 HPF
Negative	Zero AFB/1 length
Scanty	1-29 AFB/1 length
1+	30-299 AFB/1 length
2+	10-100 AFB/1 field on average
3+	≥100 AFB/1 field on average

6. Data entry

- Data will be entered in the laboratory log book and then transferred to the Result Form; Validation Phase provided by FIND (see below).
- The supervisory site will retrieve completed forms from microscopy sites every day together with the slides of this day.
- A courier service will be arranged to retrieve all completed Result forms and Rechecking forms from the supervisory sites every 2nd week; and to be sent to FIND data management unit in India (A - 32, Mohan Co-operative Industrial Estate. New Delhi -110044, India) for electronic data capture.

- Please tick “Completed form sent to FIND” once it has been sent to FIND-India.

7. 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.

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

RESULT FORM: VALIDATION PHASE

(User: Microscopy Center)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°*	Slide ID (where applicable)**	Date of sample reception	Date of slide reading	Results***		Lab Tech ID****	Date of result provided to clinic*****
					Neg	Pos		

* Specimen N°: for diagnosis assign “a, b, c” or “1, 2, 3, etc”; for treatment follow-up or other group assign “F1, F2, etc”.
** Register slide ID where available if different than Patient ID-Specimen N°.
*** If negative, tick “neg”; if positive register “scanty”, “1+”, “2+” or “3+” according to IUATLD/WHO scale.
**** Number assigned by the supervisory site.
***** If result is directly provided to patient, enter this date.

RECHECKING FORM: VALIDATION PHASE

(User: Supervisory Site)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°	Slide ID	Staining*		Result at Supervisory Site without re-staining***		Result at Supervisory Site if re-stained***	
			Good	Poor (U/O)**	Neg	Pos	Neg	Pos

* Tick if good or poor (write "U" if under-decolorized, "O" if over-decolorized).

** Only if staining is considered of low quality, re-stain the slide and register the results under "Result at Supervisory Site if re-stained"

*** If negative, tick "neg"; if positive register "scanty", "1+", "2+" or "3+" according to IUATLD/WHO scale.

Implementation Phase
Primo Star *iLED* Demonstration Project

1. General information

- Duration: 3 months.
- Patient management based on iLED fluorescence result of microscopy center.
- Compared to validation phase, reduced mothly re-checking by supervisory site as per LQAS shown below.

Study phase	Duration	% slides re-checked	Staining reagents	Microscope for reading	Microscope for re-checking	Patient management	Frequency of supervisory visits
Implementation	3 months	As per LQAS	Prepared by supervisory sites	Primo Star iLED (400X)	Primo Star iLED (400X)	Based on iLED result from microscopy center	Monthly

2. Staining of slides (same as during Validation phase)

- An Auramine slide will be prepared for all incoming sputum samples.
- Fluorescence staining should be done no more than 48 hrs before reading results.
- Staining solutions will be provided by supervisory sites once per month.

3. Reading of slides by microscopy centers (same as during Validation phase)

Table: Magnification

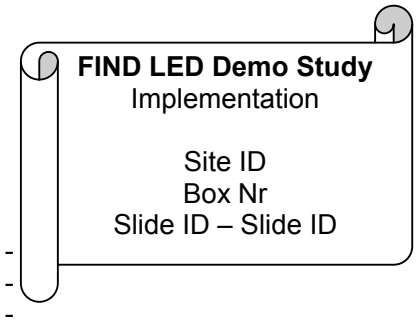
Microscope	Magnification
iLED	400x (no dark room, no direct sunlight)

Table: Semi quantitative scale for reading with iLED

IUATLD/WHO SCALE (1000x field=HPF)	READING
	iLED FLUORESCENCE (400x magnification; 1 length = 40 fields = 200 HPF
Negative	Zero AFB/1 length
Scanty	1-19 AFB/1 length
1+	20-199 AFB/1 length
2+	5-50 AFB/1 field
3+	>50 AFB/1 field

4. Storage of slides

- After reading all slides will be kept in slide boxes.
- Slide boxes will be labeled as follows:



- Study personnel will collect all boxes every second week during implementation phase and transport them to the supervisory site for re-checking.

5. Re-checking

- Slides will be re-checked on a monthly basis according to LQAS below.
- Only if staining is considered of low quality, the slide will be re-stained before re-checking and results will be register accordingly.
- Re-checking at supervisory sites will be done using the Primo Star iLED.
- Once re-checking of all slides is finished, comparison of results and false positivity and negativity rates will be determined by FIND.
- Discordant slides (i.e. iLED negative at study site and iLED positive at supervisory site) will be sent to the Supra National Reference Center in Germany for resolution.
- Re-checked results will be provided to study sites and local authorities when required (to provide information for local QC).

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

6. Data entry

- Data will be entered in the laboratory log book and then transferred to the Result Form: Implementation Phase provided by FIND (see below).

- The supervisory site will retrieve completed forms from microscopy sites every 2nd week together with all slides.

- A courier service will be arranged to retrieve all completed Result forms and Rechecking forms from the supervisory sites once per month; and to be sent to FIND data management unit in India (A - 32, Mohan Co-operative Industrial Estate. New Delhi -110044, India) for electronic data capture. (Exception: Sites carrying out electronic first data entry internally will only send forms after completion).

- Please tick “Completed form sent to FIND” once it has been sent to FIND-India.

RESULT FORM: IMPLEMENTATION PHASE

(User: Microscopy Center)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°*	Slide ID (where applicable)**	Date of sample reception	Date of slide reading	Results***		Lab Tech ID****	Date of result provided to clinic*****
					Neg	Pos		

* Specimen N°: for diagnosis assign “a, b, c” or “1, 2, 3, etc”; for treatment follow-up or other group assign “F1, F2, etc”.

** Register slide ID where available if different than Patient ID-Specimen N°.

*** If negative, tick “neg”; if positive register “scanty”, “1+”, “2+” or “3+” according to IUATLD/WHO scale.

**** Number assigned by the supervisory site.

RECHECKING FORM: IMPLEMENTATION PHASE

(User: Supervisory Site)

iLED Demonstration Project

Completed form sent to FIND ☐

Microscopy Center ID # _____

Supervisory Site ID # _____

Patient ID	Specimen N°	Slide ID	Staining*		Result at Supervisory Site without re-staining***		Result at Supervisory Site if re-stained***	
			Good	Poor (U/O)**	Neg	Pos	Neg	Pos

* Tick if good or poor (write "U" if under-decolorized, "O" if over-decolorized).

** Only if staining is considered of low quality, re-stain the slide and register the results under "Result at Supervisory Site if re-stained"

*** If negative, tick "neg"; if positive register "scanty", "1+", "2+" or "3+" according to IUATLD/WHO scale.

PROCEDURES FOR MICROSCOPY

1. Reagents preparation

1.1. Equipment and materials

- Balance, with a sensitivity of 0.1 g
- Brushes to clean bottles before reuse
- Containers for the newly prepared stains (dark amber glass bottles or plastic bottles)
- Distilled or purified water
- Filter paper, large, adapted to funnels
- Flasks (conical or flat-bottomed balloons), capacity at least one liter
- Funnels, large ones to fill bottles
- Labels for bottles
- Stirring plate with heating and magnetic stirrers
- Chemicals (see below), preferably from Merck. However, other brands could be used if continuous supply by the same manufacturer can be ensured.

1.2. Reagents for Ziehl-Neelsen staining

1.2.1. Carbol-fuchsin staining solution

1% Carbol-fuchsin

	Ingredients	Quantity
1	Basic fuchsin, certified grade	10.0 g
2	Alcohol (denaturated ethanol or methanol), technical grade	100.0 ml
3	Phenol, analytical grade	50.0 g
4	Distilled or purified water	1000.0 ml

Add the alcohol to the phenol in a 1 liter conical flask and mix.

Add the basic fuchsin powder and mix until completely dissolved. If necessary, you can add about 50 ml of the water, and mix again. Once the fuchsin is dissolved, add the remaining water to arrive at a total volume of 1 liter. If available, leave the complete mixture on a magnetic stirrer for a few hours.

Label the bottle: **"1% carbol-fuchsin"**, record date of preparation and sign your initials. Also write the date when bottle is first opened. These solutions should be kept in a cupboard or in a dark-colored bottle, and should not be used **beyond** 12 months.

1.2.2. Acid decolorizing solution

25% Sulfuric Acid

	Ingredients	Quantity per liter
1	Sulfuric acid (conc.), technical grade	250.0 ml
2	Distilled water	750.0 ml

Prepare in a conical, 2-3 liter volume flask of Pyrex quality.

Pour in all the water (as cold as possible), then add the acid slowly along the sides of the flask.

Stop regularly and keep swirling to let it cool; if too hot, stop for some time or cool the walls of the flask under a running tap with precaution not to let tap water splashing inside the flask.

ATTENTION!!! Sulfuric acid has to be added to water **(DO NOT ADD WATER TO ACID).** In addition to protective lab coats, always wear gloves and safety glasses when handling strong acids.

Label the bottle **"25% H_2SO_4"**, record date of preparation and sign your initials. Write date when bottle is first opened. This solution can be kept indefinitely. For study purposes only, 25% H_2SO_4 will be used at all trial sites.

Some national guidelines recommend an alternative decolorizing solution consisting of hydrochloric acid and alcohol.

	Ingredients	**Quantity per liter**
1	Hydrochloric acid (conc.), technical grade	30.0 ml
2	Alcohol (e.g. 96% ethanol)	970.0 ml

Use a 1-liter flask and pour slowly Hydrochloric acid into alcohol.

Label the bottle **"3% acid-alcohol"**, record date of preparation and sign your initials. Write date when bottle is first opened. This solution can be kept indefinitely.

1.2.3. Methylene blue counterstaining solution

0.1% Methylene Blue

	Ingredients	**Quantity**
1	Methylene blue, certified grade	1.0 g
2	Distilled water	1000.0 ml

Label the bottle **"0.1% Methylene blue "**, record date of preparation and sign your initials. Write date when bottle is first opened. Solutions should be kept in a cupboard or in a dark bottle, and should not be used **beyond** 12 months.

For color-blind workers, the use of Picric acid solution (7 g/l in water), which yields a yellow background, is recommended.

1.3. Reagents for fluorescence microscopy with Auramine O staining

1.3.1. Stain solution

0.1% Auramine*

	Ingredients	Quantity
1	Auramine O, certified grade	1.0 g
2	Alcohol (denaturated 95% ethanol or methanol), technical grade	100.0 ml
3	Phenol, crystals, analytical grade	30.0 g
4	Distilled or purified water	870.0 ml

* To be used ≤ 1 month

First dissolve auramine in alcohol and phenol crystals in water and mix both solutions. Mix only amounts that can be used within a few weeks, since the working solution is less stable. Working solutions have to be kept in dark bottles, or better yet in a cupboard.

Thorough mixing for about one hour on a magnetic stirring plate is recommended, but the solution should not be heated. Filter the Auramine solution by pouring through a funnel with filter paper held over the slides while transferring to a definitive container.

Label the bottle: **"0.1% auramine"**, record date of preparation and sign your initials. Write date bottle is first opened. Stocks and solutions should not be used beyond 1 month.

1.3.2. Decolorizing solution

Decolorizing solution - **0.5% Acid Alcohol***

	Ingredients	Quantity per liter
1	Hydrochloric acid (37% fuming), technical grade	0.5 ml
2	Alcohol (denaturated 95% ethanol or methanol), technical grade	100 ml

* To be used ≤ 6 months

Add 995 mL of 95% alcohol to a two liter Pyrex conical flask.

Measure 5 mL of concentrated hydrochloric acid in a cylinder.

Pour it *slowly* into the flask containing the alcohol, directing the flow of acid gently along the inner side of the flask with constant swirling. Mix well by swirling.

Label the bottle **"0.5% ACID-ALCOHOL"**, record date of preparation and sign your initials. Write date when bottle is first opened. Stocks and solutions should not be used beyond 6 months.

1.3.3. Quenching solution

0.5% Potassium Permanganate*

	Ingredients	Quantity
1	Potassium permanganate, certified grade	5.0 g
2	Distilled or purified water	1000.0 ml

* To be used ≤ 3 months

Add the powder to 0.5 liter of distilled or purified water, which has been placed in a conical flask. Swirl the contents of the flask to dissolve the dye. Add another 0.5 liter of water and mix again.

Label the bottle **"0.5% Potassium permanganate"**, record date of preparation and sign your initials. Write date when bottle is first opened. Stocks and solutions should not be used beyond 3 months. For study purposes only 0.5% Potassium permanganate will be used at all trial sites.

Next steps

Let the flasks with freshly prepared reagents stand (covered) until quality control procedures have been performed.

After these reagents have passed quality control, pour the solutions into clean bottles and label them. If bottles need to be reused, clean thoroughly, use acid alcohol and a bottlebrush to remove any residue. On the label of the bottle, clearly print the reagent name, concentration and preparation date.

1.4. Quality control

Refer to SOP for internal quality control of newly prepared batches of reagents for microscopy. *Quality controls have to be performed by microscopists*.

1.5. Handling and storage

Well-prepared reagents can be kept for at least six months to one year, even at higher temperatures. Store all reagents in clean and tightly closed bottles with a label showing the name of reagent and the date of preparation. Keep these bottles out of direct sunlight. If clear bottles are used, keep stocks of reagents in a closed cabinet.

Concentrated Sulfuric acid
Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use only in a well-ventilated area. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Do not ingest or inhale. Do not allow contact with water. Discard contaminated shoes.
Storage: Keep container closed when not in use. Store in a cool, dry, well-ventilated area and away from incompatible substances. This is a very corrosive substance.

Concentrated Hydrochloric acid
Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Use with adequate ventilation. Do not get on skin or in eyes. Do not ingest or inhale.

Storage: Keep away from heat and flame. Do not store in direct sunlight. Store in a cool, dry, well-ventilated area and away from incompatible substances.

Phenol
Phenol should be stored in a cool, dry, well-ventilated area in tightly sealed containers. Containers of phenol should be protected from physical damage and kept away from combustibles and strong oxidizers such as calcium hypochlorite, acids, and halogens.

Auramine O
Storage: Tightly closed, in a well-ventilated place.
Storage temperature: +5ºC to +30ºC
All prepared stains are stored in amber colored bottles.

Potassium permanganate
Storage: Keep tightly closed.
Storage temperature: no restrictions.
Store in cool, dry place.
Keep away from combustible materials, heat, sparks, and open flame.

2. Smear preparation

2.1. Equipment and materials for smear preparation

- 70% alcohol sand jar
- Bunsen burner or spirit lamp
- Diamond pencil or regular pencil, if frosted end slides are available
- Forceps
- Plastic bag for the waste disposal
- Bamboo/wooden sticks or loops
- Slide staining rack
- Slide box
- New and clean slides

2.2. Instructions for smear preparation

- Label the slide properly using the laboratory register serial number mentioned on the sputum container
- Proceed to smearing
  - For a direct sputum smear select a small portion of purulent or mucopurulent material with the stick/loop and transfer it to the slide
  - **If a loop is used, it has to be sterilized before use by heating until red-hot within the cone of the blue flame of the Bunsen burner. After using, immerse the loop in the 70% alcohol sand jar, move it up and down to wash off the remaining material and heat it again until red-hot!**
  - Prepare the smear in an oval shape in the center of the slide. Spread the material carefully over the area equal to about 2 cm long by 1 cm wide, do not touch the border and take care to avoid placing too much on the slide; the thickness of a smear should be such that a newspaper held under the slide can be read through the smear
  - The slides bearing the smear should be left to dry at room temperature
  - As soon as a slide has dried, hold it using forceps and fix it by passing the slide through a Bunsen or spirit flame three times in quick succession

3. Staining of slides

3.1. Equipment and materials for staining of slides

- Bunsen burner or spirit lamp
- Filter paper, small, adapted to the funnel size (Whatman No. 1-3)
- Forceps
- Funnels, small for filtering solutions in use
- Plastic bag for the waste disposal
- Slide staining rack
- Timer
- Staining solutions

3.2. Ziehl-Neelsen stain

- Do not stain more than 15 slides at once
- Place the slides on a staining rack over a sink. **There must** be a distance of at least 1 cm between every slide. Otherwise there is the possibility that acid-fast bacilli might float off one slide and become attached to the next
- Flood with filtered 1% Carbol-fuchsin staining solution. Heat gently until steaming and allow to steam for 5-10 min
- Wash gently with running water and drain
- Decolorize with acid decolorizing solution with two changes of reagent for 3 min until there is no more red color in washing
- Wash gently with running water and drain
- Counterstain with 0.1% Methylene blue for 30 sec
- Wash gently in running water and drain
- Air-dry on a slide rack

3.3. Auramine stain

- Do not stain more than 15 slides at once
- Place the slides on a staining rack over a sink. **There must** be a distance of at least 1 cm between every slide. Otherwise there is a possibility that acid-fast bacilli might float off one slide and become attached to the next
- Flood with 0.1% auramine solution. **Do not heat.** Leave for 20 minutes. Make sure that the smear area is continuously covered with auramine solution by adding more if needed.
- Wash gently with distilled or running water
- Pour 0.5% acid-alcohol solution over the slides and allow to act for 3 min
- Wash gently with distilled or running water until all macroscopically visible stain has been washed away.
- Flood smear with 0.5% potassium permanganate for 1 min. Time is critical because counterstaining for a longer time may quench the acid-fast bacilli fluorescence
- Wash off with distilled or running water
- Stand the slide on edge to drain, and air dry on the slide rack out of strong light

4. Reading and interpretation of slides

4.1. Equipment and materials for reading of slides

- Immersion oil (for ZN only)
- Lens paper

- Stained slides
- Microscope: Light or Primo Star iLED
- Slide box

4.2. Ziehl-Neelsen stained slides

- Make sure that the smear is facing upwards when the slide is placed on the mechanical stage
- Focus the smear using low power objective 10X
- Put one drop of immersion oil on the stained smear, letting it fall freely onto the slide. Never allow the oil applicator to touch the slide
- Carefully rotate the 100X objective over the smear and focus it
- Systematically examine the smear under the 100X objective
- Scan smears by moving across the smear in a horizontal direction
- Stop and observe each field before moving onto the next field
- Read at least 100 fields before reporting a negative result. (Fewer than 100 fields may be read if the slide is found positive for AFB)
- Semiquantitative results will be documented according to the NTP guidelines. See below the most widely used grading scale by IUATLD/WHO

4.3. Auramine stained slides

- Make sure that the smear is facing upwards when the slide is placed on the mechanical stage
- Focus the smear using low power objective 20X
- **Do not use immersion oil for Auramine stained smears**
- Carefully rotate the 40X objective over the smear and focus it
- Systematically examine the smear under the 40X objective
- Scan smears by moving across the smear in a horizontal direction
- Stop and observe each field before moving onto the next field
- Read at least 40 fields before reporting a negative result. (Fewer than 40 fields may be read if the slide is found positive for AFB)
- Semiquantitative results will be according to the NTP guidelines. If no local guidelines are available it is recommended to follow either the below described grading scale of IUATLD or WHO

Table: Magnifications to be used

Microscope	Magnification
Bright field	1000X
iLED	400X (No dark room, no direct sunlight) for at least first 2 months. Then screening with 200X for high-volume sites.
Conventional fluorescence microscope	200X – 250X

IUATLD/WHO SCALE (1000x field=HPF) Result	MICROSCOPY SYSTEM USED		
	BRIGHTFIELD (1000x magnification; 1 length = 2cm = 100 HPF	CONVENTIONAL FLUORESCENCE (200-250x magnification; 1 length = 30 fields = 300 HPF	iLED FLUORESCENCE (400x magnification; 1 length = 40 fields = 200 HPF
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty (actual count)	1-9 AFB/1 length or 100 HPF	1-29 AFB/1 length	1-19 AFB/1 length
1+	10-99 AFB/1 length or 100 HPF (=1-9 AFB/10 fields)	30-299 AFB/1 length	20-199 AFB/1 length
2+	1-10 AFB/1 HPF on average	10-100 AFB/1 field on average	5-50 AFB/1 field on average
3+	≥10 AFB/1 HPF on average	≥100 AFB/1 field on average	>50 AFB/1 field on average

Table: Semi quantitative scales

5. Storage of slides

- All slides will be kept in slide boxes to allow re-reading of slides by supervisory site.
- The supervisory site will retrieve the stored slide boxes from each microscopy center for re-reading.

6. Data entry

- Results will be recorded in phase-specific forms provided by FIND.
- Data will be entered in the phase-specific study forms provided by FIND
- Electronic data capture will be done on-site and/or centrally at FIND Data management unit, India.
- A courier service will be arranged to retrieve all completed forms from the supervisory site to be shipped to FIND Data management unit, India.
- Please tick “Completed form sent to FIND” once it has been sent to FIND-India.
- The study supervisor might be contacted directly by FIND Data manager, Dr. M Muniyandi (m.muniyandi@finddiagnostics.org) to solve data queries.

Study phase	Frequency of retrieving slides & forms for data entry
ZN baseline	Once every second week
Validation	Daily
Implementation	Once every second week
Continuation	Once per month

7. Quality control

- The supervisory site will re-read all or - for high volume sites only - a percentage of all slides (see Instructions for Re-checking document).
- Discordant slides (i.e. ZN negative at study site and ZN positive at supervisory site) will be sent to the Supra National Reference Center in Germany for resolution. Double check slide ID and correct recording of results prior to shipping discordant slides.
- The results of the Supra National Laboratory overrule those of Demonstration and Supervisory sites
- Re-checking results will be provided to study sites and local authorities where required (to provide information for local QC).
- Quality control and evaluation of smear quality

7.1. Internal QC (to be carried out by Supervisory sites)

7.1.1. Internal QC of freshly made staining solutions

- Prepare batches of control slides from suitable sputum specimens. These specimens are negatives that have been thoroughly examined, and a low positive (1+, 10-99 AFB /100 fields) homogenized by standing overnight at room temperature. Prepare at least 20 smears of each, as identical in size and thickness as possible, giving each series the same QC identification number. Check 2-3 slides from each batch after good staining, and note the average number of AFB for the 1+ in the QC logbook.
- Check every newly prepared staining solution with unstained control specimens, using at least one positive slide with an approximate known number of AFB and one negative slide.
- Stain the positive smear(s) in one cycle as above; repeat the cycle for the negative(s) at least once to make sure contaminants present in decolorizer or counterstaining solution will be visible.
- Examine the controls as above, and note the results in the QC logbook, under the batch number (and/or preparation date) of the new solutions.
- Unacceptable control results for ZN can include:
  - Positive control AFB are not stained bright pink or red, or are too few in number
  - Negative control remains bright pink or red after decolorization
  - Background is too intense or contains too many artifacts
- Unacceptable control results for FM can include:
  - Positive control AFB are not stained bright yellow or are too few in number
  - Negative control remains bright yellow after decolorization
  - Background is too dark or contains too many fluorescent artifacts
- If one or more of these outcomes occurs, check if something went wrong with the solution preparation. If this seems unlikely, repeat the procedure with two more slides from each control, paying attention to correct staining technique. If these controls have the expected results, they can be accepted. However, if the repeat controls also show unacceptable results, discard the staining solutions and prepare new ones.

7.1.2. Internal QC of staining solutions in use and of staining procedure

- Include positive and negative controls with each day's reading. Read control slides before patient smears; this will help with adjusting the focus and checking the proper functioning of the instrument.

- If unacceptable results described above are found, re-stain that day's routine smears together with new controls, paying attention to correct technique; if these new controls are also unacceptable, then prepared new staining solutions and repeat staining process.

7.1.3. Internal QC indicators

Monitoring of laboratory performance by monthly counts and plotting on a graph of :

- number of smears
- positivity rate
- positive cases detected

These indicators are an early warning of problems and indicate the need for corrective action. Internal monitoring, however, can contribute to staff motivation and self reliance.

7.2. External QC - In brief:

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

Table: Frequency of re-checking per study phase

As shown below, re-checking will be carried-out at two different levels. The supervisory site will be responsible for re-checking slides and only experienced staff will perform the rereading of slides. Discordant slides will be sent to the Supra National Reference Center for resolution.

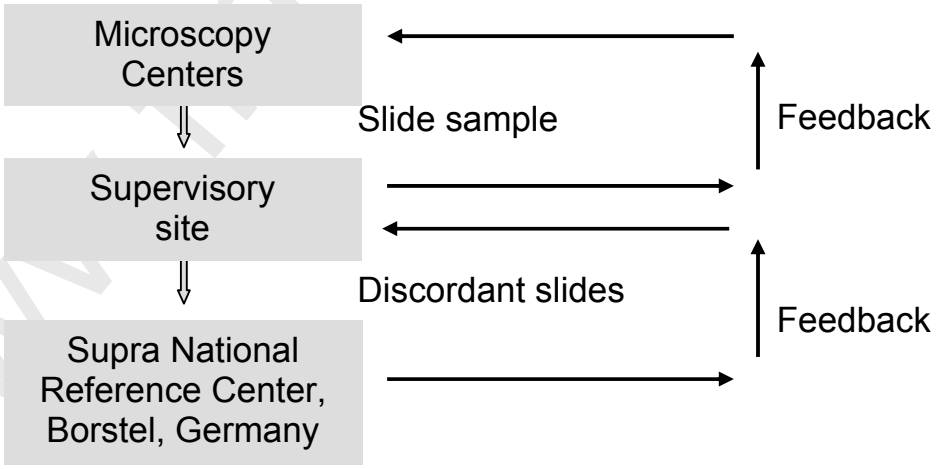


Figure: Organization of re-checking process

APPENDICES

Appendix I: Reagents and Solutions; waste management and other safety precautions

Concentrated Sulfuric acid

Appearance: colorless.

Danger! Harmful if inhaled. Corrosive and hygroscopic. Causes digestive and respiratory tract burns. Causes digestive and respiratory tract irritation. Causes severe eye and skin irritation and burns. Target Organs: None known.

First Aid Measures:
Eyes: Get medical aid immediately. Do NOT allow victim to rub or keep eyes closed. Extensive irrigation is required (at least 30 minutes).

Skin: Get medical aid immediately. Flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. SPEEDY ACTION IS CRITICAL!

Ingestion: Do NOT induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Inhalation: Get medical aid immediately. Remove from exposure to fresh air immediately. If breathing is difficult, give oxygen.

Notes to Physician: Treat symptomatically and supportively. See Fire Fighting Measures.

General Information: Wear appropriate protective clothing to prevent contact with skin and eyes. Wear a self-contained breathing apparatus (SCBA) to prevent contact with thermal decomposition products. Contact with water can cause violent liberation of heat and splattering of the material.

Extinguishing Media:
Do NOT use water directly on fire. Use water spray to cool fire-exposed containers. Use carbon dioxide or dry chemical.

Concentrated Hydrochloric acid

Appearance: Clear, colorless to faintly yellow.

Danger! Corrosive. Causes eye and skin burns. May cause severe respiratory and digestive tract irritation with possible burns.

First Aid Measures:
Eyes: Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Get medical aid immediately. Do NOT allow victim to rub or keep eyes closed.

Skin: Get medical aid. Rinse area with large amounts of water for at least 15 minutes. Remove contaminated clothing and shoes.

Ingestion: Do NOT induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Get medical aid immediately.

Inhalation: Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

Notes to Physician: Treat symptomatically and supportively.

Fire Fighting Measures: General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Not flammable, but reacts with most metals to form flammable hydrogen gas. Use water spray to keep fire-exposed containers cool.

Extinguishing Media:
Substance is nonflammable; use agent most appropriate to extinguish surrounding fire.

Phenol

Appearance: Clear, colorless crystals

Liquid phenol corrodes rubber, coatings, and some forms of plastic. Hot liquid phenol attacks aluminum, magnesium, lead, and zinc metals. If phenol contacts the skin, workers should immediately wash the affected areas with soap and water.

Clothing contaminated with phenol should be removed immediately, and provisions should be made for the safe removal of the chemical from the clothing. Persons laundering the clothes should be informed of the hazardous properties of phenol, particularly its potential for causing irritation and tissue corrosion.

A worker who handles phenol should thoroughly wash hands, forearms, and face with soap and water before eating, using tobacco products, using toilet facilities, applying cosmetics, or taking medication.

Workers should not eat, drink, use tobacco products, apply cosmetics, or take medication in areas where phenol or a solution containing phenol is handled, processed, or stored.

Spills and leaks:
In the event of a spill or leak involving phenol, persons not wearing protective equipment and clothing should be restricted from contaminated areas until cleanup has been completed. The following steps should be undertaken following a spill or leak:

1. Do not touch the spilled material; stop the leak if it is possible to do so without risk.
2. Notify safety personnel.
3. Remove all sources of heat and ignition.
4. Ventilate the area of the spill or leak.
5. Use non-sparking tools.
6. Water spray may be used to reduce vapors.
7. For small dry spills, use a clean shovel and place the material into a clean, dry container; cover and remove the container from the spill area.
8. For small liquid spills, take up with sand or other noncombustible absorbent material and place into closed containers for later disposal.
9. For large liquid spills, build dikes far ahead of the spill to contain the phenol for later reclamation or disposal.

Auramine O

Hazard identification: Harmful if swallowed. Irritating to eyes. Possible risks of irreversible effects. Toxic to aquatic organisms, may cause long tem adverse effects in the aquatic environment.

First aid measures:

After inhalation: Fresh air. Summon doctor, if necessary.

After skin contact: Wash off with plenty of water. Remove contaminated clothing.

After eye contact: Rinse out with plenty of water for at least 10 minutes with the eyelid held wide open. Immediately summon eye specialist

After swallowing: Immediately make victim drink plenty of water, induce vomiting, summon doctor.

Fire-fighting measures:

Suitable extinguishing media: Water, powder, foam.

Do not allow to enter sewerage system.

Personal protection:

Respiratory protection: Required when dusts are generated.

Eye protection: Required

Hand protection: Required

Protective clothing should be selected specifically for the working place, depending on concentration and quantity of the hazardous substances handled.

Disposal considerations:

Product: Chemical residues generally count as special waste.

Packaging: Handle contaminated packaging in the same way as the substance itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled

Potassium Permanganate

Oxidizing, toxic. Contact with combustible material may cause fire. Toxic if swallowed, causes burns.

Target organ(s): Central nervous system, blood

Keep away from combustible material.

In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Wear suitable protective clothing, gloves and eye/face

First-aid measures:

In case of contact, immediately flush eyes or skin with copious amounts of water for at least 15 minutes while removing contaminated clothing and shoes. Assure adequate flushing of the eyes by separating the eyelids with fingers.

If inhaled, move to fresh air. If individual is not breathing, give artificial respiration. If breathing is difficult, give oxygen.

If swallowed, wash out mouth with water provided person is conscious, and call a physician. Wash contaminated clothing before reuse. Discard contaminated shoes.

Fire fighting measures:

Extinguishing media: Water spray

Additional information:
Mixtures of organic nitro compounds ignite easily on heating, shock or contact with Sulfuric acid. Wash thoroughly after handling.

Waste management and personnel protection

Waste management

- At the end of each day, close contaminated material (used sputum containers, sticks, etc.) in a bag and incinerate as soon as possible. Keep the bag in a safe, closed bin or large bucket till then.
- In intermediate or central laboratories where autoclave is present, infectious waste should be autoclaved before incineration (using an autoclave bag).
- If a burning drum is used, collect contaminated material (containers with tightened caps, sticks, etc.) from the lab into a lined bucket containing phenol 5%. Burn bucket contents weekly. When cool, bury burning drum contents at least 1.5 meters deep.

Personnel protection

- Surgical masks do not protect against TB infection. TB bacilli pass through them and they may give a false sense of security. Effective respiratory masks, such as N95, FFP3, are expensive and unnecessary for microscopy activities.
- Gloves are not required for use in smear preparation since TB is acquired by airborne inhalation. Gloves may be used in settings where the incidence of HIV is high and lab personnel may be exposed to HIV when handling specimens. If used, gloves have to be changed at every interruption of activity and discarded as contaminated material. Each country must evaluate the risks and decide on the level of protection that is appropriate with the resources that may be available.
- Hand washing and careful techniques are appropriate practices and should be followed.
- Gowns should be worn in the laboratory, never outside, and be changed on a regular basis.
- For staining procedures, especially for decolorization with acids, protective glasses should be worn.

Appendix II: Investigation of Errors

False positive results	False negative results
Re-use of contaminated containers or positive slides	Poor quality of specimen
Contaminated stain prepared with water containing environmental mycobacteria	Taking not proper portion of specimen for smear preparation
Use of scratched slides	Excessive decolorization
Acid-fast bacilli floated off one slide and became attached to another during the staining procedure because of no distance between each slide	Use of poorly prepared staining solution
Inadequate decolorization	Less time staining with auramine
Lack of experience, confusion with artifacts (more if stains are not or poorly filtered; more with inexperienced readers, and takes more time to get experienced with this technique)	Overstaining with permanganate
Bad condition of the microscope (lamp) or poorly adjusted: taking some glitter for AFB	Overheating during fixing
Poor quality of staining solutions	Reading fewer than one length
	Slide too long exposed to daylight
	Too long interval between staining and reading, particularly if slides were poorly stained or not kept in the dark

Appendix III: Rechecking

INSTRUCTIONS FOR RECHECKING

1. General Information

- Re-checking is a process of rereading a sample of slides from a laboratory to assess whether that laboratory has an acceptable level of performance.
- Frequency:

Study phase	Nr of slides to be rechecked	Frequency of retrieving slides	Data courier to India & monitoring visit	Microscope for re-checking
ZN baseline	100%	Once every second week	Once every second week	Bright field (1000X)
Validation	100%	Daily	Once every second week	Conventional fluorescence (200 – 250X)
Implementation	As per LQAS	Once every second week	Once every second week	Primo Star iLED (400X)
Continuation	As per National Guidelines	Once per month	Once per month	Primo Star iLED (400X)

Table: Frequency of re-checking per study 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

Table: Lot quality assurance sampling (LQAS) for implementation phase

2. Rereading

- Re-staining of slides will only be necessary for Auramine slides for which the supervisory site considers the staining to be of low quality.
- Slides will be reread using the appropriate microscope and magnification for each study phase and the below described WHO/IUATLD scale.

IUATLD/WHO SCALE (1000x field=HPF) Result	MICROSCOPY SYSTEM USED		
	BRIGHTFIELD (1000x magnification; 1 length = 2cm = 100 HPF	CONVENTIONAL FLUORESCENCE (200-250x magnification; 1 length = 30 fields = 300 HPF	iLED FLUORESCENCE (400x magnification; 1 length = 40 fields = 200 HPF
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty	1-9 AFB/1 length or 100 HPF	1-29 AFB/1 length	1-19 AFB/1 length
1+	10-99 AFB/1 length or 100 HPF (=1-9 AFB/10 fields)	30-299 AFB/1 length	20-199 AFB/1 length
2+	1-10 AFB/1 HPF on average	10-100 AFB/1 field on average	5-50 AFB/1 field on average
3+	≥10 AFB/1 HPF on average	≥100 AFB/1 field on average	>50 AFB/1 field on average

Table: Semi quantitative scale

Result being re-checked	Result at Supervisory site				
	Negative	1-9 AFB	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
1-9 AFB	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Table: Classification of errors

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

3. Discordant results

- A list of all discordant slide IDs will be provided by FIND after central data entry.
- Discordant slides (i.e. ZN or Auramine negative at study site and ZN or Auramine positive at supervisory site) will be kept by the supervisory site in separate boxes.
- Boxes containing **discordant slides will be sent to the Supra National Reference Center in Germany for resolution at the end of implementation phase**. Double check slide ID and correct recording of results prior to shipment of discordant slides.
- The supervisory sites will contact FIND Logistics Officer, Nora Champouillon (nora.champouillon@finddiagnostics.org) for shipment instructions.
- The conclusions of the Supra National Reference Laboratory will overrule the results from the Demonstration and Supervisory sites.
- The microscopist responsible for resolving discrepant results will need to search long enough to find any AFB or to reliably exclude the presence of AFB, so at this point it will be helpful for both results to be known.
- Feedback on the results of discordant slides, along with the slides, will be returned to the first controllers and action will be taken to resolve any performance problems identified.
- The results from the re-checked slides will be communicated to study sites and local authorities when required (to provide information for local QC).

Completed form sent to FIND ☐

Re-checking Report
iLED Demonstration Project

Period _____

Microscopy center ID_____
Supervisory Site ID _____

Result of Microscopy Centre	**Result of Supervisory Site ***				
	Negative	1-9 AFB/ 100 fields	1+	2+	3+
Negative	Correct	LFN	**HFN**	**HFN**	**HFN**
1-9 AFB/ 100 fields	LFP	Correct	Correct	QE	QE
1+	**HFP**	Correct	Correct	Correct	QE
2+	**HFP**	QE	Correct	Correct	Correct
3+	**HFP**	QE	QE	Correct	Correct

* Enter the number of slides on each box

No. of False results		**Slide ID / Error**
False negative		
False positive		

Summary of errors identified				
Major Errors		Minor Errors		
HFP	HFN	LFP	LFN	QE
Total Major Errors:		Total Minor Errors:		

HFP= High False Positive; HFN= High False Negative; LFP= Low False Positive; LFN= Low False Negative;
QE= Quantification Error

Appendix IV: Proficiency testing

Proficiency Panel Testing
After training & validation & implementation phase

1. **General Information**

- Proficiency testing using a manufactured unstained EQA panel (10 ZN and 10 Auramine) will be conducted after training, validation and implementation phase.
- Panel testing is one method of External Quality Assessment. The aim is to evaluate proficiency of laboratory technicians on fluorescence microscopy using the Primo Star iLED.
- Slides will be provided by FIND.
- Reading time of slides will be determined by the supervisor.
- Duration: 1 day (may vary in case performance targets were not met).
- Study personnel from the supervisory site will closely monitor the proficiency panel testing at microscopy centers.

2. **Frequency of testing**

- At least 3 x: Following training and validation and implementation phase.
- If possible also: After three months from initiation of continuation phase.

3. **Staining of slides**

- A manufactured unstained EQA panel will be provided for each microscopy center.
- Staining of ZN slides will be done with the same staining solutions used for routine conditions. Staining of Auramine slides will be done using the staining solutions provided by the supervisory site once per month.
- Reagent preparation at supervisory site and staining of slides at microscopy centers will be done according to the instructions provided in this SOP.

4. **Reading of slides**

- ZN slides will be read using the available brightfield microscope. Auramine slides will be read using the Primo Star iLED microscope.
- Reading time will be determined for every slide (start time when slide is placed under the microscope and the reading starts; stop time when a semiquantitative result has been established or for negative and scanty smears until 40 (Auramine) / 100 (ZN) fields have been read).
- Slide panels will only be examined by **one reader (with no consultation of entourage).**
- Semiquantitative results for ZN will follow the scale used by the local NTP. Semiquantitative results for Auramine slides will be documented using the IUATLD/WHO scale.

IUATLD/WHO SCALE (1000x field=HPF) Result	MICROSCOPY SYSTEM USED	
	BRIGHTFIELD (1000x magnification; 1 length = 2cm = 100 HPF	**iLED FLUORESCENCE** (400x magnification; 1 length = 40 fields = 200 HPF
Negative	Zero AFB/1 length	Zero AFB/1 length
Scanty	1-9 AFB/1 length or 100 HPF	1-19 AFB/1 length
1+	10-99 AFB/1 length or 100 HPF (=1-9 AFB/10 fields)	20-199 AFB/1 length
2+	1-10 AFB/1 HPF on average	5-50 AFB/1 field on average
3+	≥10 AFB/1 HPF on average	>50 AFB/1 field on average

Table: Semi quantitative scale

- Technicians should spend the same amount of time reading proficiency panel slides as they routinely spend on patient smears. A supervisor should monitor the time spent reading the panel smears since technicians can spend an excessive amount of time reading slides when they know they are being tested.
- Reading time will be determined.

5. Data entry

- Results and reading time will be recorded in a special form provided by FIND (see below).
- Completed forms will be sent to a central data entry unit to be captured electronically. Please tick “Completed form sent to FIND” once it has been sent to FIND-India.
- Study personnel will collect all boxes at the end of the Proficiency Panel Testing from microscopy centers and transport them to the supervisory site for re-reading. Please tick “Completed form sent to FIND” once it has been sent to FIND-India.

6. Performance targets for validation phase and proficiency panel

- 95% accordance between results of the microscopy center and supervisory site for fluorescence readings.
- Quality of Auramine stains acceptable in 100% of panel slides examined.
- < 2 false results in the proficiency testing panel.
- For evaluation of 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 meet performance targets will receive additional training and undergo proficiency testing until targets are met.

Completed form sent to FIND ☐

RESULT FORM: PROFICIENCY PANEL TESTING

(User: Microscopy Center)
iLED Demonstration Project

Supervisory Site ID # _____
Microscopy Center ID # _____

Slide ID	Date of slide staining	Date of slide reading	Results*		Lab Tech ID**	Reading time***
			Neg	Pos (scanty 1+2+3+)		
Z						
Z						
Z						
Z						
Z						
Z						
Z						
Z						
Z						
A						
A						
A						
A						
A						
A						
A						
A						
A						
A						

* If negative, tick "neg"; if positive register "scanty", "1+", "2+" or "3+" according to IUATLD/ WHO scale.
** Number assigned by the supervisory site.
*** Stop time for every slide: Start=when slide placed and focused under microscope; Stop=when reading completed

Supervisor _____ Date _____

Appendix V: Supervisory visits

Supervisory Visits

1. **General information**

   - Close supervision will be required during the study. One Supervisory site will be assigned to 2-3 microscopy centers.
   - Supervisory sites will be contacted monthly by the FIND Study Coordinator.

2. **Frequency of supervisory visits to Microscopy centers**

   - According to study phase. However, additional supervisory visits may be required for some Microscopy centers.

   Table: Frequency of supervisory visits

Study phase	Frequency of visits
ZN baseline	Monthly
Validation	Every second week
Implementation	Monthly
Continuation and expansion	Monthly

3. **Topics to be covered during supervisory visits**

   - Supervisory sites will coordinate and ensure the provision of staining solutions, slide boxes and study forms to microscopy centers on a periodic basis.
   - The On-site Evaluation Checklist (see below) will be completed and will be sent to FIND Study Coordinator. Please tick the box “Completed form sent to FIND” once it has been sent to FIND either by fax (+41 22710 0599) or via e-mail:

     catharina.boehme@finddiagnostics.org.

Completed form sent to FIND ☐

ON-SITE EVALUATION CHECKLIST: SUPERVISORY VISIT

(User: Supervisory Site)*

iLED Demonstration Project

I General Information

Supervisor:	
Supervisory Site (name/ID):	
Microscopy Center (name/ID):	
Date of Visit:	

II Laboratory infrastructure and equipment:

- Uninterrupted power supply	No problems ☐ Rare and short interruptions ☐ Regular or sometimes long interruptions ☐
- Running water supply	No problems ☐ Rare and brief interruptions ☐ Regular or sometimes long interruptions ☐
- Primo Star iLED microscope	No technical problems observed ☐ Technical problems observed ☐, Specify:

III Adequate stock (within expiry dates) and supply of:

Item	Adequate	Comments / Requirement
- Slides	Yes / No	
- Lens tissue	Yes / No	
- Smearing/staining equipment	Yes / No	
- 0.1% Auramine	Yes / No	
- 0.5% Acid alcohol	Yes / No	
- 0.5% Potassium permanganate	Yes / No	
- Slide boxes	Yes / No	
- Study forms	Yes / No	
- Other:		

**To be completed monthly during each study phase, except during Validation phase every 2nd week*

IV Study procedures and documentation

Item	Adequate / Acceptable	Problems identified / Requirement
Study-specific SOP followed		
- Smear preparation observation	Yes / No	
- Staining procedure observation	Yes / No	
- Slides properly stained** (thickness, field size)	Yes / No	
- Slides reading following grading chart	Yes / No	
- Slide boxes stored	Yes / No	
- All slides are available and stored as per lab register***	Yes / No	
- Storage of reagents: reagent bottles labeled with content, date of preparation, date of expiry	Yes / No	
- Storage of slides in boxes	Yes / No	
- Study forms have correctly completed	Yes / No	
- Completed study forms properly filed	Yes / No	
- Review of 5-10% of results forms for correctness compared to source data	N° of forms reviewed/ N° of forms completed: ___ / ___	

**Check 4 recent positive slides
***Check 20 IDs per visit

V Internal Quality Control

- Control smears are used for each new batch of staining solutions (register are available)	Yes / No	Comments:
- Control positive slides are used at least once a week	Yes / No	

VI Supervisory site tasks

- Supply study forms	Yes ☐ Not required ☐
- Supply slide boxes	Yes ☐ Not required ☐
- Supply staining solutions (Auramine at least once a month)	Yes ☐ Not required ☐
- Completed forms and slides retrieved for re-checking	Yes ☐

VII Overall remarks

Questionnaire – Appraisal of Primo Star iLED

Microscopy Center ID: (where applicable)

Supervisory Site:

Country:

Date of completion: (DD/ MM / YY)

Phase of completion: ☐Validation ☐Implementation ☐Continuation

Position: (Microbiologist, laboratory technologist, microscopist)

Instructions:

This questionnaire should be completed by at least 2 staff members per supervisory site and 1 from each microscopy center at a) the beginning of validation phase, b) the beginning of implementation phase, and c) the beginning of continuation phase.

Please check for each question the box of your selected evaluation category.

Please provide further details in text fields where applicable.

If you complete this form electronically, check fields by double-clicking on the selected box and by selecting “checked”. For text fields, double-click on the field and enter default text.

Please send completed forms to FIND Study Coordinator, either by fax (+41 22710 0599) or via e-mail: catharina.boehme@finddiagnostics.org

Part I: Installation and first use

Question #1:
Was the installation/first use of Primo Star iLED by a microscopist:

☐ Self-explanatory, can be done without reading the user manual
☐ Easy, but a user manual with instructions is required
☐ Rather difficult; some problems were faced during installation/first use
☐ Very difficult; cannot be expected of a microscopist

Describe difficulties that have occurred or may occur during installation:

Question #2:
Was the installation/first use of the battery pack by a microscopist:

☐ Self-explanatory, can be done without reading the user manual
☐ Easy, but a package insert is required
☐ Rather difficult; some problems were faced during installation/first use
☐ Very difficult; cannot be expected of a microscopist

Describe difficulties that have occurred or may occur during installation/first use:

Question #3:
How satisfied were you with the Primo Star iLED user manual:

☐ Easy to read and understand; covers all questions I had during installation/use
☐ Most sections easy to read and understand, with some weaknesses in sections:
Missing topics:
☐ Rather cumbersome to read (information required is not found easily; not enough pictures that allow understanding at first glance), weaknesses especially in the following sections:
Missing topics:

Comments:

Part II: Training

Question #1a:
For a microscopist trained in ZN microscopy, how intensive should the training for Primo Star iLED be? days

Question #1b:
For someone without prior training in smear microscopy, how intensive should the training for Primo Star iLED be? days

Comments:

Question #2: (*only to be completed by supervisory sites in phase II after training*)
How satisfied were you with the Primo Star iLED training manual:

☐ Can be used by NTPs for implementation of LED microscopy without major changes
☐ Can be used by NTPs for implementation of LED microscopy but requires some major changes

☐ Requires complete revision

Suggestions for changes:

Part III: Optics and Handling

Question #1:
How satisfied are you with contrast, color intensity and signal-to-noise (background) ratio of Primo Star iLED?

☐ Very satisfied (better than for the available light microscope [enter brand and model of microscope] and where applicable fluorescence microscope)
☐ Satisfied (comparable to available light microscope and where applicable fluorescence microscope)
☐ Not satisfied (not as good compared to those of the available light microscope and where applicable fluorescence microscope)

Comments:

Question #2:
How satisfied are you with the color impression for ZN stain of the Primo Star iLED (white LED) in comparison to a standard light microscope (halogen bulb)?

☐ AFBs can be better distinguished
☐ Same
☐ AFBs can be less well distinguished

Comments:

Question #3:
How satisfied are you with the resolution and depth of focus of Primo Star iLED?

☐ Very satisfied (better than for the available light microscope and where applicable fluorescence microscope)
☐ Satisfied (comparable to available light microscope and where applicable fluorescence microscope)
☐ Not satisfied (not as good compared to those of the available light microscope and where applicable fluorescence microscope)

Question #4:
Was there a difference between the homogeneity of fluorescence illumination in the field of view compared to your standard microscope?

☐ Field of view of Primo Star iLED is more homogenously illuminated
☐ Same
☐ Field of view of Primo Star iLED is less homogenously illuminated

Question #5:
How satisfied are you with the overall handling features of the microscope (on/off switch, intensity regulation of bright light and fluorescence light, variable viewing height, focus mechanism (coarse and fine focus))?

☐ Very satisfied (better than for the available light microscope and where applicable fluorescence microscope)

☐ Satisfied (comparable to available light microscope and where applicable fluorescence microscope)
☐ Not satisfied (less good compared to those of the available light microscope and where applicable fluorescence microscope)

Suggestions for improvements/comments:

Question #6:
Is the procedure for switching between bright field and fluorescence light convenient and do you easily understand the symbols used for white light and fluorescence light?

☐ Very convenient
☐ Convenient
☐ Not convenient
Comments:

Sub-question: Do you consider the toggle switch to be robust enough?
☐ Yes
☐ No

Question #7:
Is focusing with the fluorescence unit (due to black background):

☐ Very difficult
☐ Difficult, but only a matter of training
☐ Easy, I quickly got used to it

Question #8:
Do you use the option of opening the slider on the white light source to focus with the fluorescence unit (dark background gets structured which makes focusing easier)

☐ Yes, I always use this to facilitate focusing
☐ Sometimes
☐ Never

Question #9:
Are the blue LEDs on both sides of the microscope that indicate the intensity level of the bright field illumination convenient or rather disturbing/dazzling?

☐ Convenient
☐ Disturbing/Dazzling

Question #10:
Are the 4pcs objectives with magnifications: 10x, 20x 40x and 100x the best choice for the applications Auramine O fluorescence and Ziehl Neelsen bright field detection of pulmonary tuberculosis?

☐ Yes
☐ No; I would prefer to have a magnification
Comments:

Question #11:
Which magnification do you prefer for fluorescence detection of AFBs: 20 times or 40 times?
☐ 20x
☐ 40x

Question #12:
In your opinion, can Primo Star iLED be used without a darkroom?

☐ No darkroom is needed
☐ Darkroom is needed

Question #13:
Do you use the dazzling protection for the eyepieces?

☐ Yes, they are useful
☐ No, I do not need them (no dazzling)
☐ No, I would need them, but they are not comfortable/convenient

Question #14:
Did you have any technical problems with your microscope until now (repair, replacement)?

☐ Yes, describe:
☐ No

Part IV: Application questions

Question #1:
In your daily work, do you plan to switch between bright field and fluorescence contrast using just the Primo Star iLED microscope or would you rather use the iLED for fluorescence detection only and a second microscope for bright field detection (Ziehl-Neelsen)?

☐ I would use the Primo Star iLED for both fluorescence and bright field and would switch between the two modes at least once per day
☐ I would use the Primo Star iLED for fluorescence only and will use a second microscope for bright light microscopy
☐ I do not think a bright field microscope will be needed in the future anymore for TB detection, i.e. I will only use it for fluorescence

Question #2:
For which applications would you use the Primo Star iLED?

☐ for TB detection only
☐ for Malaria or HAT detection only
☐ for various applications (such as TB, Malaria, Blood Cell Counts, urine analysis, Trypanosomiasis)

Question #3:
Do you see a significant gain in speed when reading slides with Primo Star iLED (30 fields) compared to ZN (100 fields)?

☐ Yes
☐ Yes, for negative and low positive slides only
☐ No

Question #4:
If you had to decide whether to change a majority of microscopy centers in your country from light microscopy to LED based fluorescence microscopy, would you recommend to the Head of the National Health Program to switch to LED?

☐ Yes. Reasons:

☐ In principle, yes. But I would prefer using another microscope and not the Primo Star iLED. Reasons:

☐ Only for low volume microscopy centers. Reasons:

☐ Only for high volume microscopy centers. Reasons:

☐ Only in specific settings. Define setting: Reasons:

☐ No. Reasons:

☐ No. But I would switch centers that currently have and use a conventional fluorescence microscope to LED fluorescence. Reasons:

Thank you very much for helping us with your feedback!

REFERENCES

Steingart K, Henry M, Ng V, Hopewell P, Ramsay A, Cunningham J, Urbanczik R, Perkins M, Abdel Aziz M, Pai M. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6: 570-81

Anthony RM, Kolk AHJ, Kuijper A, Klatser PR. Light emitting diodes for auramine O fluorescence microscopic screening of Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2006; 10 (9): 1060-62

Hänscheid T. The future looks bright: low-cost fluorescent microscopes for detection of *Mycobacterium tuberculosis* and *Coccidiae*. Trans R Soc Trop Med Hyg 2008, doi: 10.1016/j.trstmh.2008.02.02

International Union Against Tuberculosis and Lung Disease. The Public Health Service National Tuberculosis Reference Laboratory and the National Laboratory Network. Paris; 1998.

Collins C, Kennedy D. Laboratory acquired infections: history, incidence, causes and prevention. 4th ed. Oxford: Butterworth-Heinemann; 1999.

Lumb R, Bastian I. Laboratory diagnosis of tuberculosis by sputum microscopy. Adelaide: Institute of Medical and Veterinary Science; 2005.

World Health Organization. Laboratory services in tuberculosis. Part II: Microscopy. Geneva; 1998.

Association of Public Health Laboratories. External Quality Assessment for AFB smear microscopy. Washington; 2002.

Kent PT, Kubica GP. Public Health Mycobacteriology: a guide for the level III laboratory. Atlanta, Ga: U.S. Department of Health and Human Services, Centers for Disease Control; 1985.

Smithwick R.W. Laboratory Manual for acid-fast microscopy. US Department of Health, Education and Welfare, CDCs, 1979

RNTCP, Central TB Division, Ministry of Health and Family Welfare, Government of India. Manual for Sputum Smear Fluorescence Microscopy; 2007.

Association of Public Health Laboratories and Centers for Disease Control and Prevention. External Quality Assessment for AFB Smear Microscopy; 2002

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