

STUDY PROTOCOL

Demonstration Project iLED



Effectiveness of
LED-based Fluorescence
Microscopy for Detection
of Tuberculosis

Technical and Financial Agency:

**Foundation for
Innovative New Diagnostics**

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Demonstration Project iLED
Study Protocol
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Confidentiality statement

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EXECUTIVE SUMMARY

Background

Replacing light microscopy with fluorescence microscopy would be one of the immediate options to improve TB case finding in high burden countries. The recent application of ultra-bright LEDs to enable inexpensive fluorescence microscopy is a potentially significant advance in TB diagnostics. Recently completed and ongoing studies from a number of groups appear to confirm the equivalence of LED-powered microscopes to their much more expensive, conventional fluorescence counterparts. In collaboration with national TB programs and international partner organizations, FIND, the Foundation for Innovative New Diagnostics, will explore the feasibility and impact of scaling up the use of LED fluorescence microscopes to improve TB control.

Project Purpose

The project aims at a) confirming the performance and b) demonstrating the effectiveness of LED-based fluorescence microscopy for detection of tuberculosis using the Primo Star iLED developed by Zeiss in partnership with FIND. The data will be reported to national TB and public health authorities such as the Strategic Technical Advisory Board of the WHO to inform policy decision.

Study Design

The Primo Star iLED will be implemented in at least 20 routine microscopy centers 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 and cost-effectiveness. Participating microscopy centers will be grouped in clusters. Each cluster will consist of one supervisory site and two to three microscopy centers. The supervisory site will be responsible for training, monitoring, rechecking of slides and data management.

Partners

- ❖ **India** (9 demonstration and 3 supervisory sites: Christian Medical College Vellore, JALMA Agra and New Delhi TB Centre)
- ❖ **Vietnam** (6 demonstration and 2 supervisory sites: National Reference Laboratory, Hanoi and Pham Ngoc Thach Hospital, Ho Chi Minh City; coordinated by KNCV)
- ❖ **Peru** (3 demonstration sites and 1 supervisory site: Instituto Nacional de Salud, Lima)
- ❖ **Ethiopia** (3 demonstration sites and 1 supervisory site: EHNRI National Reference Laboratory, Addis Ababa)
- ❖ **Russia** (2 demonstration sites and 1 supervisory site: Samara District Laboratory and Queen Mary College)
- ❖ **Lesotho** (2 demonstration sites and 1 supervisory site: National Reference Laboratory in Maseru)
- ❖ **Tanzania** (2 demonstration sites and 1 supervisory site: Muhimbili National Reference Laboratory)

- ❖ **Cambodia** (2 demonstration sites and 1 supervisory site: National TB Reference Laboratory, Phnom Penh)
- ❖ **Thailand** (2 demonstration sites and 1 supervisory site: National TB Reference Laboratory, Bangkok)
- ❖ **Other countries:** 20-40 microscopes will be provided to FIND partners including WHO/TDR, IUATLD, CDC, PEPFAR, USAID, MSF, PATH, and BMGF to expand the study to other countries and/or answer specific questions.

Timelines

ACTIONS	
Stakeholder and investigators meeting	End of Mar 08
IRB approvals	By Jul 08
ZN Baseline	Sep 08
iLED Training (5 day training)	Sep 08
Validation (ZN performed in parallel)	Oct 08
Implementation (LED performed as routine method)	Nov 08 – Jan 09
Continuation	Feb – Jul 09
Compilation of final data set	Aug 09
Final report	Aug 09

STUDY PROTOCOL

Background

A lack of human resources is among the greatest challenges in TB control in many disease endemic developing countries. Staff shortages combined with increasing numbers of requests for sputum microscopy are creating unmanageable workloads with negative effects on TB case finding. The poor sensitivity of light microscopy even under optimal conditions further aggravates this situation.

Simple yet rapid approaches to reduce the laboratory workload and to increase the sensitivity of direct smear microscopy need to be explored urgently.

An immediate option for improving this situation in high burden countries would be by replacing light microscopy with fluorescence microscopy.

A systematic review by WHO/TDR and FIND has shown that¹:

- a) Fluorescence microscopy is, on average, 10% more sensitive than conventional light microscopy. The increased sensitivity is greatest in low grade positives.
- b) The specificity is comparable.
- c) Reading a fluorochrome stained smear takes only 25% of the time taken to read a ZN stained smear.

To date, the major constraints to the broader implementation of fluorescence microscopy are the high price of fluorescence microscopes and their lack of robustness and sustainability. 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 hours². Furthermore, the acceptability of darkrooms has generally been low in tropical settings due to local beliefs.

The recent application of ultra-bright LED (light emitting diodes) technology to enable inexpensive fluorescence microscopy is a potentially significant advance in TB diagnostics. Zeiss, in a joint development agreement with FIND, has developed a fluorescence microscope promising excellent performance at a most competitive price. Other LED-based approaches, such as the FRAEN After device, designed to attach to a bright field microscope, are or will become available shortly.

1. 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

2. 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

The demonstration project described here focuses on the evaluation of Primo Star iLED, although evaluation of other LED-based solutions will be fostered and the data will be included in the STAG report. One of the major innovations of Primo Star iLED compared to others is the use of ultrabright LED as a reflected light source. This improves the instrument's power consumption (lifespan \approx 10 000 hours) with a system that permits easy regulation of the light intensity. The new microscope will have high-quality optics and be as robust as the popular Primo Star microscope (e.g. complete antifungal coating). It will allow effortless switching from bright light to fluorescence light and can be battery operated. These innovations, in combination with the affordable price, may 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 Primo Star iLED 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, which was conducted in 4 reference laboratory settings, are summarized in the following tables:

Table 1: Assessment of the need for a darkroom at all sites showed that Primo Star iLED does not require a darkroom

	Peru	Thailand	Gambia	Germany
With darkroom	+++	+++	+++	+++
Comment:	"Contrast & brightness better than available standard FM"			
Without dark room; no direct sunlight	+++	+++	+++	++
Comment:	"Still better than many standard FM"			

Table 2: The time saved using the Primo Star iLED prototype was 45-75% compared to light microscopy and was at least equivalent to standard fluorescence microscopy

	Peru	Thailand	Gambia	Germany
Stand LM; ZN				
Mean time to result	120 s	77 s	229 s	130 s
Mean for pos	96 s	45 s	53 s	70 s
Mean for neg	169 s	102 s	334 s	161 s
Stand FM; Au/KMnO4				
Mean time to result	67 s	46 s	121 s	55 s
Mean for pos	34 s	31 s	77 s	33 s
Mean for neg	81 s	67 s	195 s	79 s
LED FM; Au/KMnO4				
Mean time to result	63 s	48 s	93 s	45 s
Mean for pos	43 s	28 s	47 s	33 s
Mean for neg	73 s	63 s	148 s	57 s

Table 3: Sensitivity and specificity were assessed in a slide set of 140 slides per study site. The table summarizes results for all 4 sites. LJ cultures were considered the gold standard. Performance of the Primo Star iLED prototype was at least equivalent to the performance of standard fluorescence microscopy

Microscope	Method	Sensitivity (%)	Specificity (%)
Standard FM	direct	67.4	96.1
Standard FM	concentrated	66.6	99.2
LED FM	direct	71.6 (p=0.1025)	96.4
LED FM	concentrated	72.4 (p=0.0073)	98.8

Table 4: Lab technicians' appraisal of Primo Star iLED (0=very weak; +++ = excellent)

Criteria	Scores (0 to +++)
LED principle	+++ No waiting time period unlike regular FM No darkroom needed No bulb replacement; no heat development <ul style="list-style-type: none"> • Option to use battery
<ul style="list-style-type: none"> ▪ Contrast ▪ Resolution ▪ Depths of focus ▪ Signal-to-noise ratio ▪ Homogeneity of illumination 	All +++ "We have always been rather satisfied with our fluorescence microscope until we used this one. It is less cumbersome to read with this microscope since the fluorescence of the AFBs is very bright and easy to recognize in the dark background. "
Switching between bright field & fluorescence	+++ But bright light LED needs filter to shift towards yellow
Suitability of alternative stains for LED	All +++ (Au/KMnO ₄ ; Au-Rhod/KMnO ₄ ; Au/MethBlue) Preferred stain: Au/KMnO ₄ (AFBs appeared brightest); due to the dark background, it was initially difficult to focus for readers with little experience
Product design	++ (including focus mechanism, comfort)

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

Study Design

This is a laboratory-based study to evaluate the effectiveness of a new LED-based fluorescence microscope (Primo Star iLED) for the detection of *M. tuberculosis* in sputum specimens submitted for mycobacterial evaluation.

The Primo Star iLED will be implemented in at least 20 routine microscopy centers 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 and to answer any outstanding questions.

Hypothesis:

We postulate that the Primo Star iLED system is a feasible, advantageous and cost-effective replacement for ZN (and, where existing, conventional fluorescence) microscopy in low- to moderate-income laboratory settings. Especially in busy microscopy centers, it will increase the case detection rate while substantially decreasing the daily workload.

Endpoints:

FIND believes that large-scale demonstration projects are required to provide the evidence that diagnostic tests performing well in affluent countries can successfully be implemented and have significant medical and public health impacts in programmatic settings. The purpose of this demonstration project is to assess the implementation of Primo Star iLED as replacement for ZN in routine TB diagnosis in low- and moderate-income settings. Specifically, we are interested in the following:

Primary Objectives (*Basic Protocol*)

1. To assess the feasibility of implementing Primo Star iLED for TB diagnosis at microscopy centers without prior experience with 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 and to results from the supervisory site
3. To determine the development of false positivity and negativity rates of LED fluorescence reading over time (with increasing experience)
4. To assess the impact of this implementation on daily workload and case detection rates for low, middle and high-volume settings
5. Determine lab technicians' appraisal of Primo Star iLED
6. To evaluate detailed costs associated with LED-based fluorescence microscopy in comparison with conventional methods

Secondary Objectives (*Extended protocol - FIND partners*)

1. To identify minimal training needs and develop training modules accordingly
2. To identify the optimal fluorescence staining method and ensure continuous and affordable supply
3. To assess effects of fading speed on external quality assurance by rechecking

4. To assess whether combining LED fluorescence microscopy with concentration methods such as bleach sedimentation further increases sensitivity

Design:

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 developing country settings. Participating microscopy centers will be grouped in clusters. Each cluster will consist of one supervisory site and two to three microscopy centers. The supervisory site will be responsible for training, monitoring, rechecking of slides and data management.

a) Investigators' meeting (duration: 1 day)

An investigators' meeting was held in March 2008 to refine the master protocol and get input from national TB programs and other FIND partners, adapt questions to country-specific situations, identify supervisory sites and participating microscopy centers and to develop detailed, country-specific study plans.

b) Establishing ZN baseline (duration: 1 month)

In order to be able to compare false positivity and negativity rates for LED fluorescence with the respective rates for ZN reading during the implementation phase, it will be necessary to establish a baseline under study conditions. Using data available from the routine quality assurance program would possibly result in a bias, since performance is likely to be better under study conditions simply due to the fact that staff get more attention than usual and are therefore more motivated. As a result, participating laboratory technicians will receive instructions for the study and study-related data management prior to this phase. Supervision will be as intense as during the implementation phase (supervisory visit once every 2nd week). All incoming sputum samples will be examined by ZN microscopy according to program norms and kept in slide boxes provided by FIND. All slides will be rechecked by the supervisory site and discrepant cases resolved by the Supra National Reference Center in Germany (see details on rechecking below).

c) Training and validation (duration: 1 month)

The supervisory site(s) in each country (supported by FIND and its partner institutions) will be responsible for training the laboratory staff from participating microscopy centers. This training shall take approx. 5 days, but can be shorter for staff with prior experience in fluorescence microscopy. An important outcome of this demonstration study shall be to help define minimal training needs and best training modules. FIND/Zeiss will provide training materials. The training will be followed by a validation phase of 2-4 weeks (2 weeks is sufficient for higher volume laboratories), during which an Auramine slide will be prepared for all incoming sputum samples and will be examined by Primo Star iLED using 20x magnification for screening and 40x for confirmation in case of suspicion of artifacts.

On a daily basis, all slides will be rechecked by the supervisory site using conventional fluorescence microscopy. Results will be provided to the microscopy center the following day, and treatment will be started on the basis of the conventional fluorescence results. At the end of this validation, a proficiency slide panel of 10 ZN and 10 Auramine slides will be provided to all participating sites.

Performance targets for validation phase and proficiency panel:

- *>95% accordance between results of the demonstration site 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*

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.

d) Implementation phase (duration: 3-4 months)

After a successful training and validation phase in each participating laboratory, all incoming sputum samples will undergo routine microscopic examination with the Primo Star iLED according to program norms, and patients will be managed based on Primo Star iLED results for a period of 3-4 months. All slides will be stored in slide boxes which will be provided by FIND/supervisory sites. Quality control (QC) and assurance (QA) will follow the standards of the national TB programs. Supervisors will visit once every second week to discuss and record any problems with staining solutions, the microscope or result interpretation. Rechecking (after re-staining of slides with Auramine) will take place at the respective supervisory laboratory: This will allow determining the false positivity / false negativity rate over time for each demonstration site. However, in order to be able to compare these rates to ZN, it will be necessary to establish a false positivity / false negativity baseline for ZN beforehand (see paragraph b) above). Rechecking will be performed by experienced staff only and all discrepant slides (slides for which the result of the microscopy center is positive but the one from the rechecking supervisory site negative, or the other way round) will be reexamined by the Supra National Reference Laboratory. Prevalence data during implementation will be compared with preceding quarters. Laboratory technicians' appraisal of the new method will be assessed both initially and after 3 months with the help of a questionnaire. Difficulties with reading at 20x magnification will also be assessed at the beginning and the end of the implementation phase. In addition, cost data for LED fluorescence, compared to ZN and standard fluorescence microscopy, will be collected for selected sites and countries.

e) Continuation and expansion phase (duration: 6 months)

In collaboration with other international partner organizations and national TB programs, the demonstration project will be expanded to other countries/sites. In general, rechecking of slides, supervision and monitoring will be reduced compared to the implementation

phase. Rechecking will be done according to national TB guidelines. Specific operational issues with respect to fluorescence microscopy will also be addressed during this phase. For example:

- Identify optimal fluorescence staining method (methylene blue or pelican ink are possible counterstains that may produce a lighter background and therefore allow easier focus; phenol free staining kits have become available on the market and may be considered as environment-friendly alternatives)
- Determine effect of fading speed on external quality assurance by rechecking
- Ascertain effect of combining LED fluorescence microscopy with methods such as bleach sedimentation to further increase sensitivity
- Determine performance specifically in high HIV prevalence settings and overburdened microscopy centers

Training, technical assistance

Prior to implementing and validating Primo Star iLED in the laboratory, staff from each demonstration site will attend one intensive training session (5 days) on fluorescence microscopy. Proficiency will be ensured during the validation phase by rechecking and participation in an EQA panel testing. Each site will have access to technical assistance throughout the demonstration period, and site visits will be arranged once every 2nd week by the study oversight team to ensure that that protocol instructions are followed.

Study sites

- ❖ **India** (9 demonstration and 3 supervisory sites): 3 microscopy centers in New Delhi supported by the New Delhi TB Centre; 3 microscopy centers in Vellore supported by Christian Medical College Vellore; 3 microscopy centers in/close to Agra supported by JALMA
- ❖ **Vietnam** (6 demonstration and 2 supervisory sites; supported by KNCV): 3 microscopy centers in Northern Vietnam and 3 in Southern Vietnam supported by Pham Ngoc Thach Hospital and the National Reference Laboratory in Hanoi in close collaboration with KNCV
- ❖ **Peru** (3 demonstration sites and 1 supervisory site): 3 microscopy centers supported by the Instituto Nacional de Salud in San Juan de Lurigancho district
- ❖ **Ethiopia** (3 demonstration sites and 1 supervisory site): 3 microscopy centers supported by the EHNRI (National Reference Laboratory in Addis)
- ❖ **Russia** (2 demonstration sites and 1 supervisory site): 2 microscopy centers in the department of Samara supported by Samara District Laboratory and Queen Mary College (Dr. Francis Drobniowski)
- ❖ **Lesotho** (2 demonstration sites and 1 supervisory site): 2 microscopy centers supported by the National Reference Laboratory in Maseru
- ❖ **Tanzania** (2 demonstration sites and 1 supervisory site): 2 microscopy centers supported by Muhimbili (National Reference Laboratory)
- ❖ **Cambodia** (2 demonstration sites and 1 supervisory site): 2 microscopy centers supported by National TB Reference Laboratory, Phenom Penh

- ❖ **Thailand** (2 demonstration sites and 1 supervisory site): 2 microscopy centers supported by National TB Reference Laboratory, Bangkok
- ❖ **Other countries:** In addition, 20 microscopes will be provided to FIND partners including WHO/TDR, IUATLD, CDC, PEPFAR, USAID, MSF, BMGF and PATH to expand the study to other countries and/or answer specific questions

Criteria for country selection

- Agreement at National Level (MOU with NTP and/or MOH)
- High-burden of TB
- Low or middle income
- At least 2 countries with high HIV prevalence
- Local presence of FIND or implementing partner
- Representative of global TB situation

Criteria for selection of microscopy centers

National TB Programs will select the microscopy centers that shall participate in the project as demonstration sites and will base their selection on the following criteria:

- Selected microscopy centers must be accessible by monitors from the respective supervisory site on a daily basis during validation phase and on a weekly or twice per month basis during the implementation phase
- Must have at least 10-15% smear positivity
- Must have at least 1 microscopist present who has undergone formal microscopy training
- For settings with 3 demonstration sites, it is desirable to select 1 high volume (>40 smears per day), 1 medium volume (20-40 smears per day) and 1 low volume microscopy center (approx. 10 smears per day)
- For settings with 2 demonstration sites, at least one of the sites should be a medium volume center
- At least 2 sites with only intermittent power supply will be included (1 located in India, and 1 in an African setting)
- At least 2 sites that have problems to cope with current workload

Population Source

Sputum samples from all known or suspected TB patients which are submitted for routine microscopic examination during the demonstration project period will be included in the study.

Ethical considerations

Patients will be treated on the basis of the Primo Star iLED result, which implies replacing the currently used routine ZN result.

Ethical approval will therefore be required for all participating sites/countries.

Risks: Numerous published studies have demonstrated that fluorescence microscopy is more sensitive than and equally specific as ZN microscopy. Fluorescence microscopy is a well established method for TB diagnosis, particularly in high-income countries. Thus, participation in this project poses no foreseeable risk to patients. No informed consent will be sought for this demonstration project and a waiver of informed consent will be requested from relevant Institutional Review Boards (IRBs). All patients will receive diagnosis and treatment according to program norms throughout the demonstration period.

Confidentiality: There are no risks with respect to confidentiality. No personal health information or demographic data will be collected for this laboratory study.

Data management and potential use of findings

All forms required for data entry will be provided by FIND. The electronic data entry tool for the study will be connected to FIND's central database through secured VPN, which offers the advantage that study monitors will have continuous access to the electronic data. Electronic data entry will be conducted by professional staff hired in India. This data entry staff will receive faxed source data from the supervisory sites once every 2nd week. The original forms must be stored on site for at least 3 years after study completion.

Toward the end of the project, basic protocol data will be pooled, analyzed, and the results disseminated by FIND in collaboration with all project sites. The data will be reported to local and national TB and public health authorities such as the WHO Strategic Technical Advisory Board to inform policy decision. Additionally, the findings of this project will be published in a peer-reviewed journal and may be used to encourage other TB programs to evaluate and implement LED-based fluorescence microscopy. In its charter, FIND states that it is responsible for ensuring such dissemination. Furthermore, FIND will compile and analyze the data from all the sites and prepare a first draft for review by the sites. A final publication will be prepared with input and authorship from all the collaborators. Site-specific data may be presented independently and in combination with other data, but only after the principal publication has been made available and/or accepted for publication. In the case where the combined multi-site data are not of publishable quality, FIND shall be the final arbiter on any joint publication. All scientific manuscripts and reports related to these studies should be sent to FIND for review prior to submission for publication.

Standardization and Quality Control (QC)

During implementation phase, staining solutions will be prepared by supervisory sites using reagents from Merck based on a SOP provided by FIND. Incoming QC and lot-to-lot QC will be performed according to this SOP. The solutions will be provided to participating microscopy centers once every 2nd week. During continuation phase, microscopy centers will be responsible for preparing staining solutions. Data will be captured on laboratory forms provided by FIND, which will then be sent to a central data entry unit in order to be captured electronically. Semiquantitative results for ZN will follow the scale used by the

local NTP and for fluorescence will follow the IUATLD/WHO scale. All slides will be stored on site according to an SOP. Rechecking will be standardized for all participating countries. In brief, highly experienced microscopists at the supervisory sites will read 300 fields per slide for negative smears, 100 fields for scanty smears until a semiquantitative result has been established for higher positive smears. Rechecking at supervisory sites will be done using the Primo Star iLED. All slides will be re-stained prior to reading. Slides with discrepant results will be rechecked by the Supra National Laboratory in Germany using LED microscopy and assessing the same number of fields. Their conclusions will overrule the results from the microscopy center and demonstration site.

Table 5: Semi-quantitative scale

IUATLD/WHO SCALE (1000x field=HPF) Result	MICROSCOPY SYSTEM USED		
	BRIGHTFIELD (1000x magnification; 1 length = 2cm = 100 HPF	FLUORESCENCE (200-250x magnification; 1 length = 30 fields = 300 HPF	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

Human resource needs

Microscopy centers: The workload at these sites will not increase compared to routine conditions. During the initial training and validation phase (4 weeks), the work may be felt to be more intense than under normal conditions due to the introduction of a new technology. During the implementation and continuation phase (9 months - LED fluorescence only), the workload is expected to be reduced compared to routine conditions. The staffing situation will be assessed for each microscopy center and support provided where needed.

Supervisory sites: Each supervisory site will monitor 2-3 microscopy centers and will have to recheck all slides that are read at these microscopy centers over a period of 4-5 months (validation and implementation phase). The workload will highly depend on the slide volume that is examined at the microscopy centers. A supervisor and 1-2 laboratory technicians will be needed on a part time basis. Additional staff may therefore have to be hired in some settings, whereas top up salaries for available staff may be sufficient in other settings.

Blinding

Blinding at the microscopy centers will not be required. The slides will be labeled according to routine procedures. During the implementation phase, rechecking at the supervisory site will be done without access to results from the microscopy centers.

Timelines

ACTIONS	2008										2009							
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Investigator Meeting																		
ZN Baseline																		
Training & Validation																		
Implementation																		
Continuation & Expansion																		
STAG/WHO submission																		