FIND

Because diagnosis matters

Architecture of an evaluation study and protocol components

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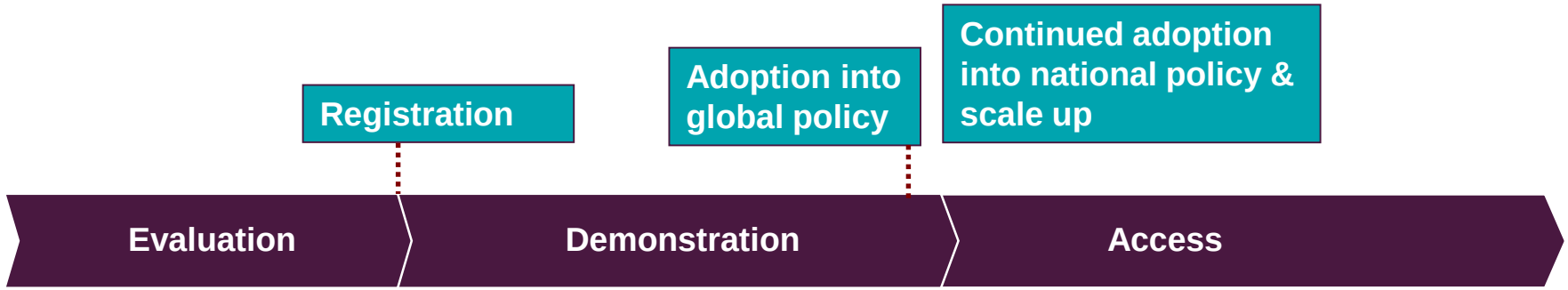
December 2014

India's trial focus spans probably across, with the strongest need for Access studies



Evaluation	Demonstration	Access
• Test performance • Ease of use • Surrogate patient important outcomes • Cost-comparisons	• Test performance in settings of intended use • Comparative effectiveness (patient important outcomes, case detection); • Cost of diagnostic process / treatment, incl. for patients • Operational requirements for implementation (infrastructure and human)	• (Comparative) validation for product selection to assess best fit • Algorithms, operational requirements • Test and resource utilization • Patient impact • Epidemiological impact • Economic impact • Health system impact

On the menu

- Evaluation studies: definition and value
- Study approach and protocol components
- Examples

Evaluation studies: definition

- Performed on a product that has completed development, and is design-locked and ready for scaled up manufacture.
- Intended to produce high quality data that can be used by regulatory authorities and should thus be collected in a manner that conforms to relevant external quality standards.
- The type of endpoints commonly studies include: accuracy (including patient follow up to determine clinical specificity), operational performance and surrogate markers for impact asessment.
- Carried out in referral settings or settings of intended use

Evaluating value of a diagnostic test

- The ideal diagnostic test:
  - Always gives the right answer:
    - -Positive result in everyone with the disease
    - -Negative result in everyone else
  - Be quick, safe, simple, painless, reliable & inexpensive

- But few, if any, tests are ideal.

- Thus there is a need for clinically useful substitutes

Is the test useful ?

	Evaluation	Demonstration
Reproducibility (precision)	*Usually done earlier*	-
Accuracy (compare test to «gold std»	X	-
Feasibility of implementation (robustness, costing)	X	X
Effects on clinical decisions and fit in clinical pathway	-	X
Effects on outcomes (and is it worth the costs)	*Surrogate marker data*	X

Determining Usefulness of a Diagnostic Test

Question	**Possible Designs**	**Statistics for Results**
How *reproducible* is the test?	Studies of: *- intra- and inter observer &* *- intra- and inter laboratory variability*	Proportion agreement, kappa, coefficient of variance, mean & distribution of differences

Determining Usefulness of a Diagnostic Test

Question	Possible Designs	Statistics for Results
How *accurate* is the test?	*Cross-sectional* designs in which *test result is compared with a "gold standard"*	Sensitivity, specificity, PV+, PV-, ROC curves, LRs

Determining Usefulness of a Diagnostic Test

Question	Possible Designs	Statistics for Results
What is the *feasibility of implementing* the test (costs, risks, user acceptability, robustness, training requirements, etc.) of the test?	*Assessment accompanying prospective* studies	Mean cost, proportions experiencing adverse effects, proportions (operators) willing to use the test, failure rate, mean training duration

Determining Usefulness of a Diagnostic Test

Question	Possible Designs	Statistics for Results
Does doing the test improve *clinical outcome*?	Assessment of *surrogate markers for clinical utility* accompanying prospective studies	Mean time to diagnosis, mean time to reporting compared to conventional results

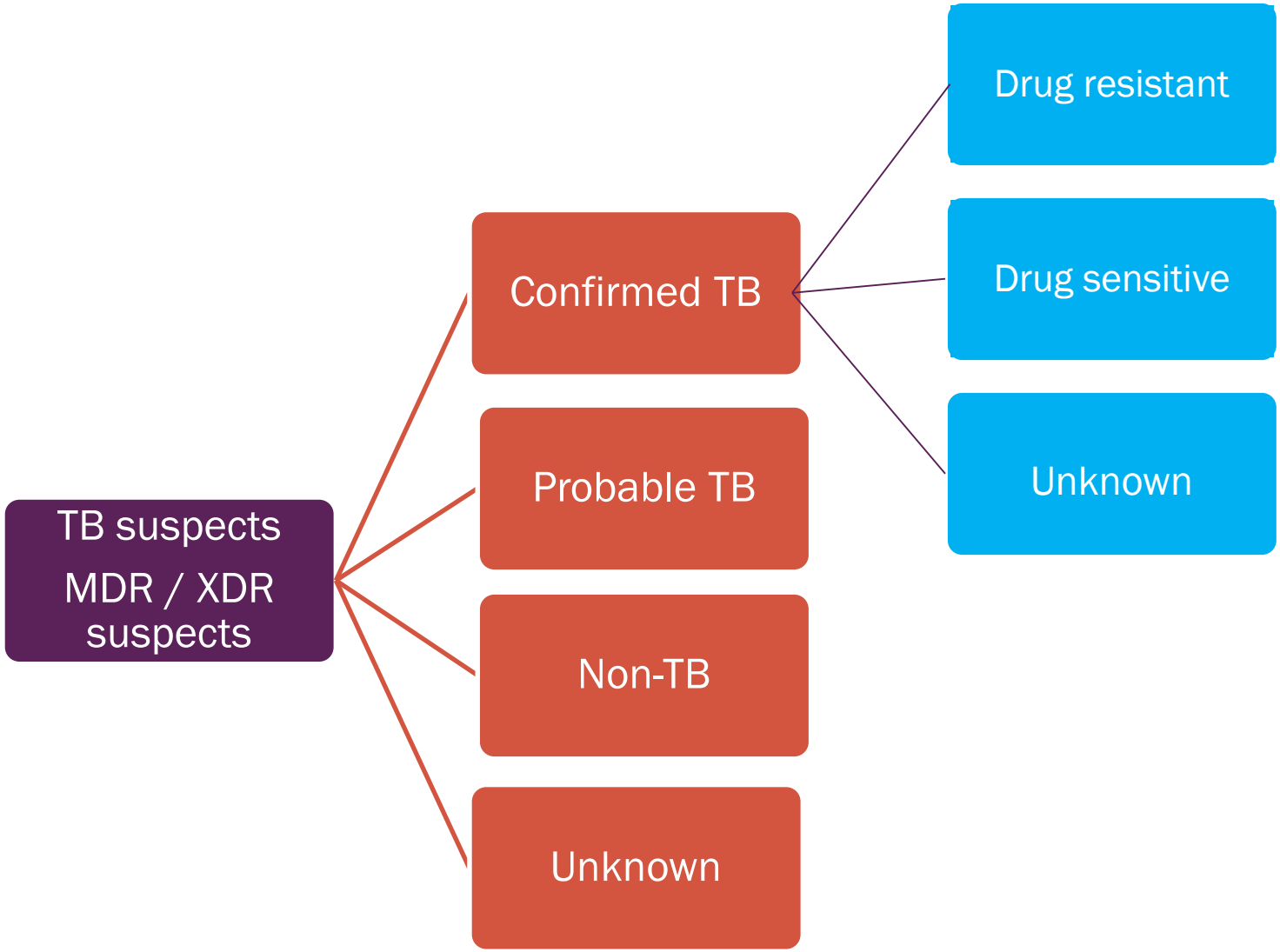
Validating tests against a gold standard

- A test is valid if:
  - It detects most people with disorder (high Sen)
  - It excludes most people without disorder (high Sp)
  - a positive test usually indicates that the disorder is present (high PV+)
- Another measure of the usefulness of a test is the LR: how much more likely a positive test is to be found in someone with, as opposed to without, the disorder

A test can separate *the very sick from the very healthy* does not mean that it will be useful in distinguish patients with mild cases of the disease from others with similar symptoms

Just accuracy? – Actually it is quite challenging

- Key groups for analysis in an accuracy study for a new test



Problem 1: Suboptimal sensitivity of microbiological gold standard, sputum variability

Table : Comparison of the overall sensitivity of a single LJ culture, a single MGIT culture and a single, direct Xpert MTB/Rif test using the results of 3 smears and 4 cultures per patient as a reference standard.

Patient group	Single LJ*	Single MGIT*	Single, direct Xpert
Smear-positive, Culture-positive	93.0% (1031/1109)	97.7% (1106/1132)	98.2% (551/561)
Smear-negative, Culture-positive	69.4% (222/320)	84.5% (283/335)	72.5% (124/171)
All Culture-positive	87.7% (1253/1429)	94.7% (1389/1467)	92.2% (675/732)

Sensitivity (cfu/ml) of pulmonary TB tests in portfolio

LC

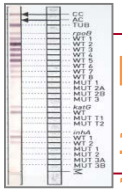
10-100/ml

Automated NAAT

100-150/ml

LJ

50-150/ml



Line-probe*

1000-10,000/ml

LED fluorescent microscope

10,000/ml

Rapid speciation dipstick

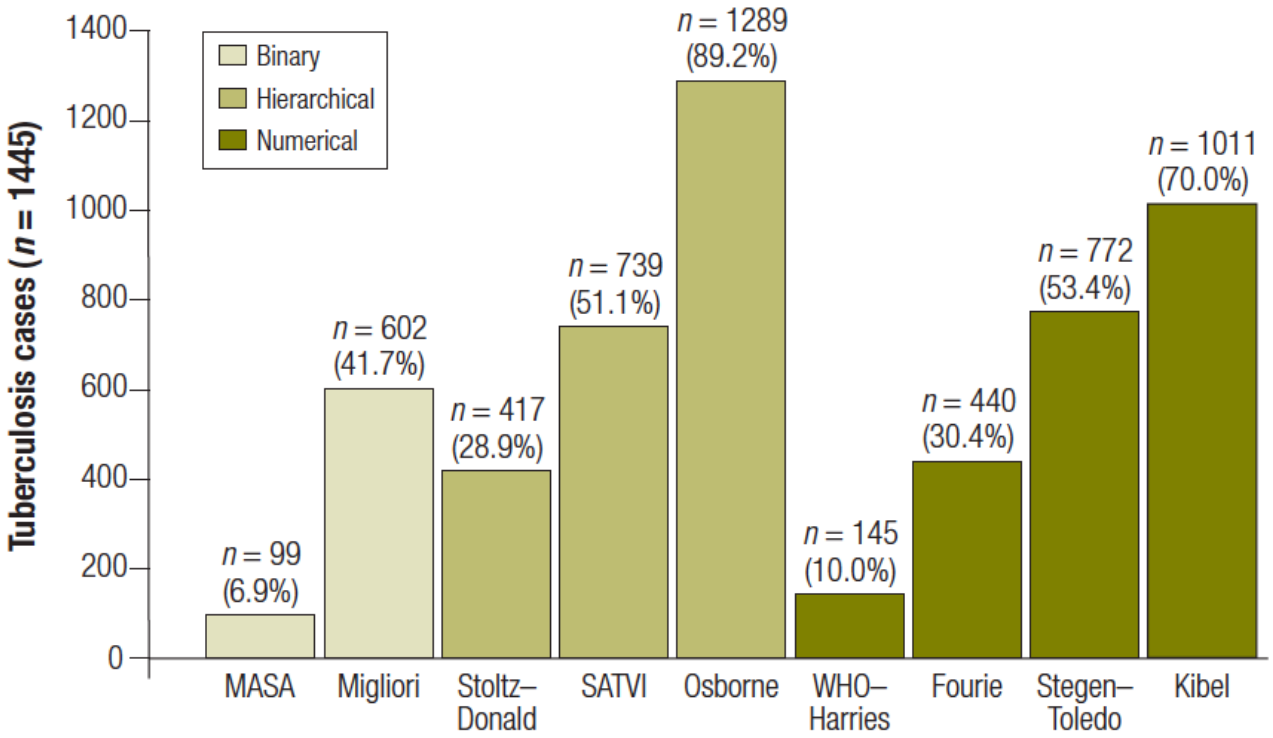
1,000,000/ml

0 1 2 3 4 5 6

Log cfu/ml

Problem 2: The definition of probable TB
Example: Interpretation of CXR is highly inconsistent

Fig. 1. **Frequency of cases classified as tuberculosis with various scoring systems, with hierarchical and numerical outcomes condensed to a binary "tuberculosis/not tuberculosis" output , South Africa, 2001–2006**

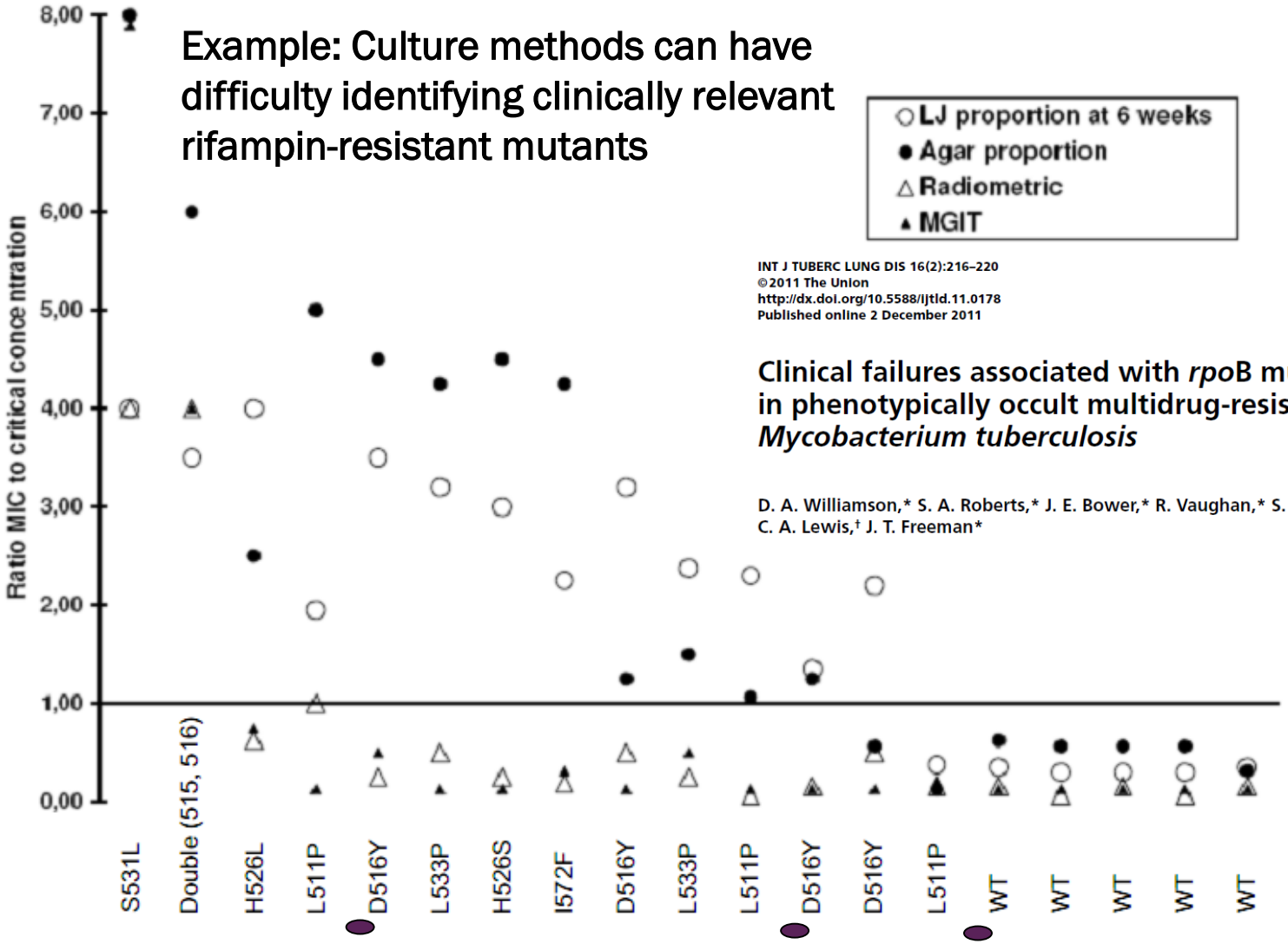


MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

Hatherill M et al. Bull World Health Organ 2010;88:312-320

Problem 3: Phenotypic vs genotypic

Example: Culture methods can have difficulty identifying clinically relevant rifampin-resistant mutants



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Published online 2 December 2011

Clinical failures associated with *rpo*B mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*

D. A. Williamson,* S. A. Roberts,* J. E. Bower,* R. Vaughan,* S. Newton,* O. Lowe,* C. A. Lewis,† J. T. Freeman*

[Adapted From] Wright et Al. *Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results.* J Clin Microbiol. 2009 Sep 16. [Epub ahead of print]

What to do in these situations?

- No valid index test result or no valid culture
- Single pos culture <20 colonies in SC or >28 days in LC
- S+C-
- Discrepant conventional DST results
- NTM vs mixed culture
- Culture pos at FU only
- Clinical diagnosis of TB in S-C-
- Culture negative patients on TB treatment

Study approach and protocol components

Defining the clinical question: PICO or PIRT

Patient/Problem

- How would I describe a group of patients similar to mine?

Index test

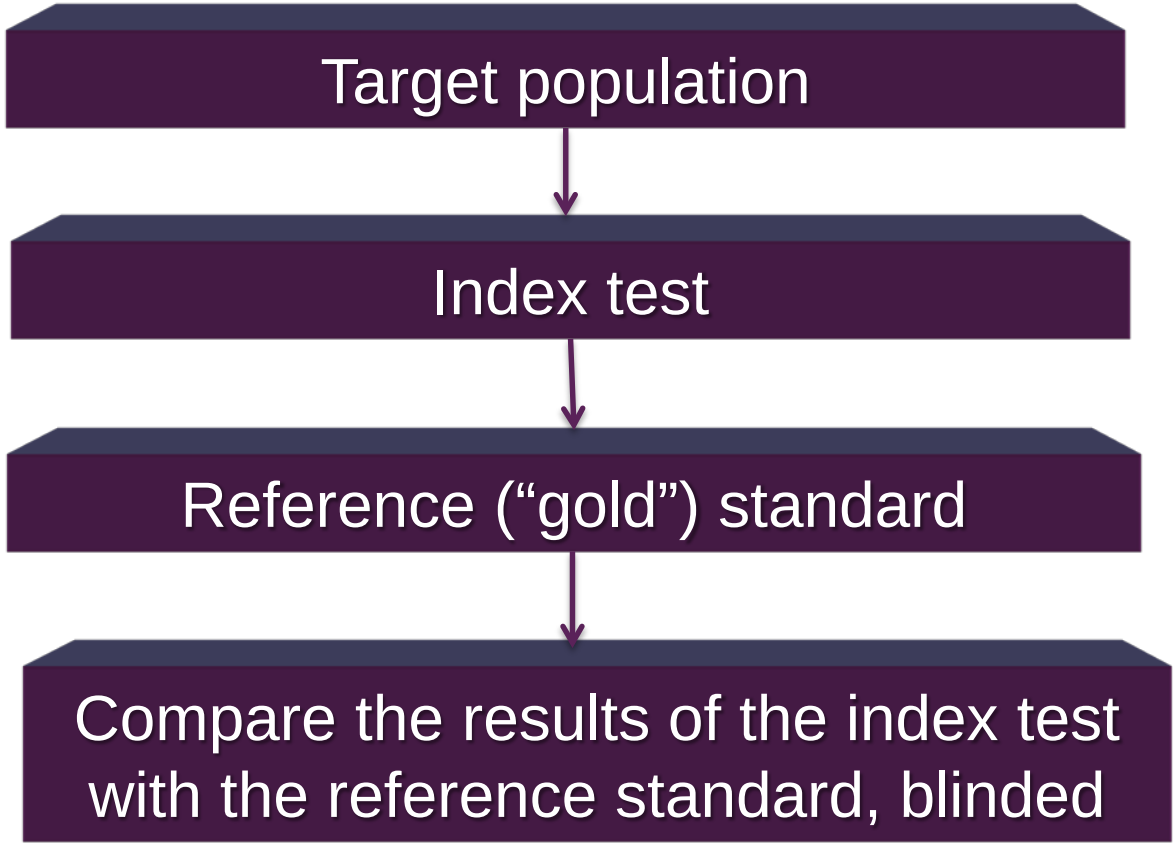
- Which test(s) am I considering?

Comparator… or …**R**eference Standard

- What is the best reference (gold) standard to diagnose the target condition?

Outcome….or….**T**arget condition

- Which condition do I want to rule in or rule out?


Target population
Index test
Reference (“gold”) standard
Compare the results of the index test with the reference standard, blinded

Example: *TB LAMP*

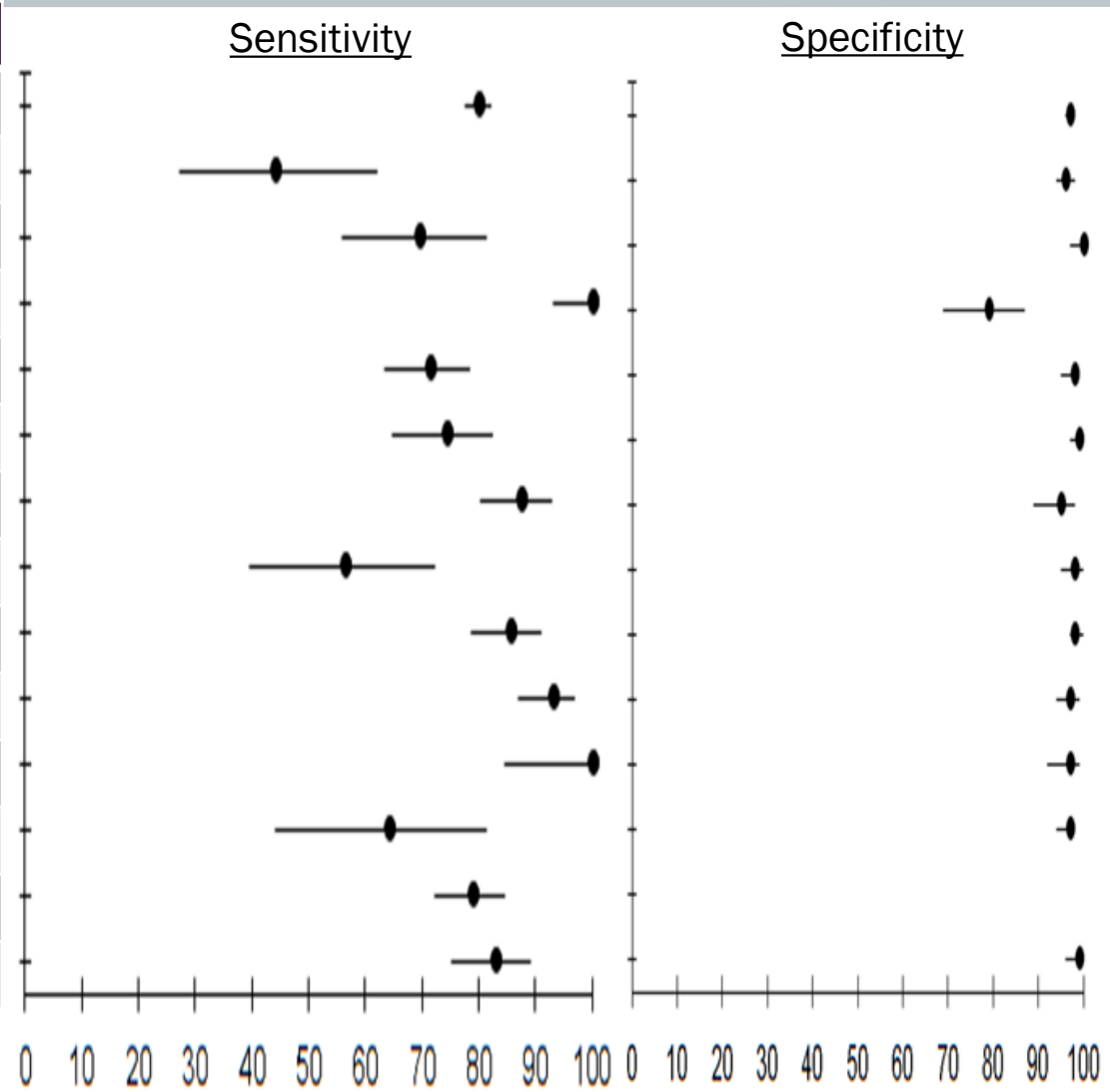
What is the diagnostic accuracy of LAMP compared to liquid culture for detection of pulmonary TB in adults, where LAMP is used as replacement test for ZN/Xpert or as add on to ZN?

- Diagnostic accuracy in terms of True positives (TB), True negatives (TN), false positives (FP), False negative (FN) results, stratified by HIV and smear status
- Cases of MDR not diagnosed with LAMP (compared to Xpert)
- Cost
- Reduced invalid rate

- **P**opulation targeted by the action/intervention: *Adults suspected of having pulmonary TB*;
- **I**ntervention being considered: *TB-LAMP assay*
- **C**omparator: Liquid Culture (gold standard); ZN microscopy, Xpert (comparator)
- **O**utcome: *Sensitivity/Specificity of TB-LAMP compared to Xpert and ZN sputum smear microscopy using culture as reference standard*.

TB LAMP vs. Culture

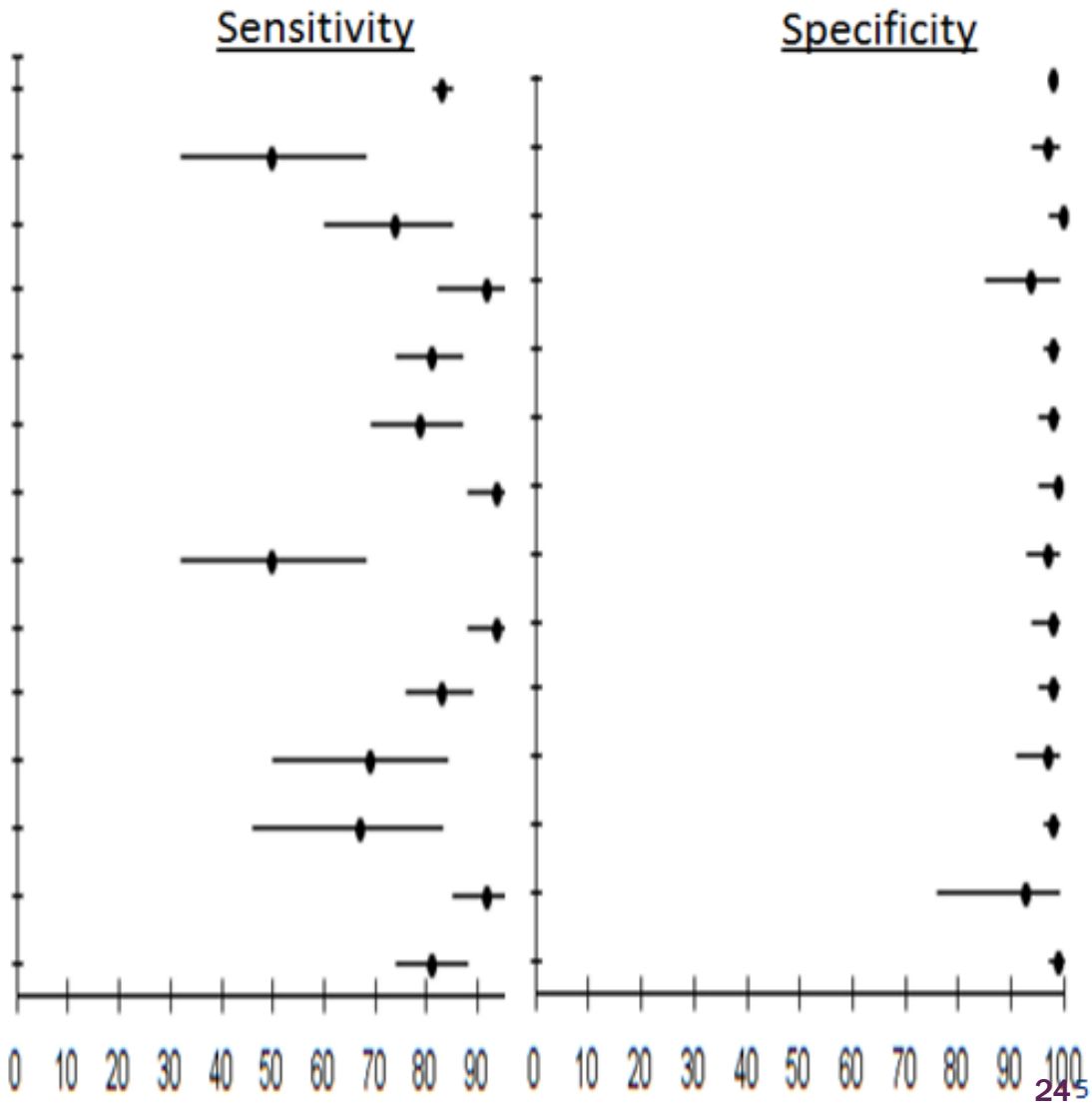
Site	Sensitivity (95% CI)	Specificity (95% CI)
Overall	**79.93 (77.58-82.23)**	**96.88 (96.13-97.51)**
Vietnam	44.12 (27.19-62.11)	96.40 (94.21-97.93)
Uganda	69.64 (55.90-81.22)	100.00 (96.61-100.00)
The Gambia	100.00 (93.15-100.00)	79.31 (69.29-87.25)
Tanzania	71.32 (63.40 -78.4)	97.58 (95.10-99.00)
South Africa	74.29 (64.83-82.32)	98.63 (96.52-99.62)
Mongolia	87.50 (80.22-92.83)	94.78 (88.99-98.06)
Malawi	56.41 (39.62-72.19)	98.39 (95.36-99.67)
Madagascar	85.61 (78.66-90.98)	100.00 (96.61-100.00)
Ivory Coast	92.97 (87.07-96.74)	97.18 (94.27-98.86)
India	100.00 (84.56-100.00)	96.95 (92.37-99.16)
Ethiopia (S-)	64.29 (44.07-81.36)	96.54 (94.27-98.10)
Cameroon	78.89 (72.19-84.61)	NA
Cambodia	82.93 (75.09-89.11)	98.91 (96.11-99.87)



- No DNA contamination or technical problems reported
- LAMP indeterminate rate <0.3% and <0.05% after repeat

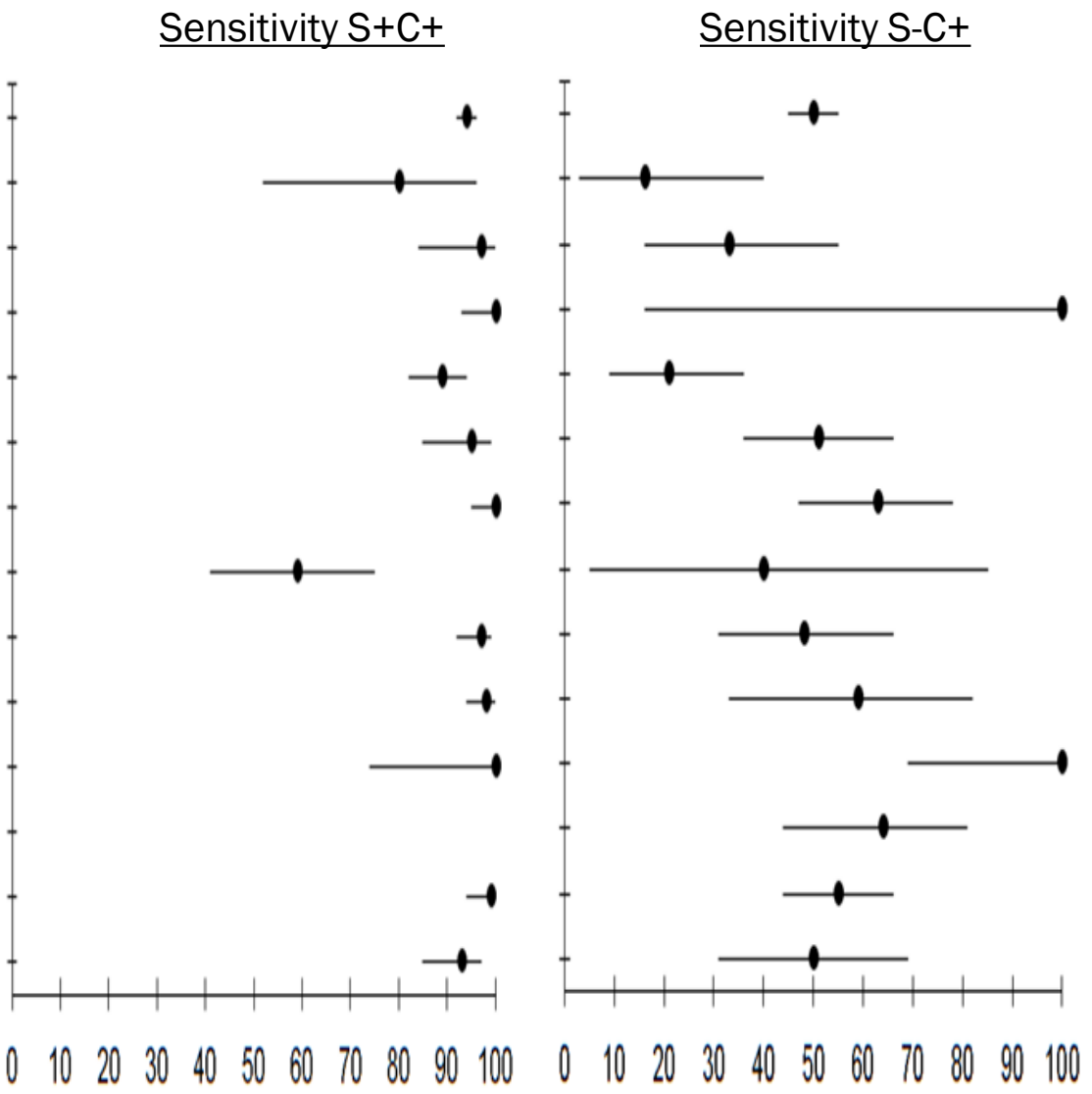
TB LAMP vs. Xpert

Site	Sensitivity (95% CI)	Specificity (95% CI)
Overall	**82.92 (80.56-85.11)**	**97.72 (97.05-98.27)**
Vietnam	50.00 (31.89-68.11)	96.54 (94.35-98.05)
Uganda	73.58 (59.67-84.74)	100.00 (96.70-100.00)
The Gambia	91.67 (81.61-97.24)	94.44 (84.61-98.84)
Tanzania	81.20 (73.52-87.45)	98.33 (96.14-99.45)
South Africa	79.31 (69.29-87.25)	97.78 (95.23-99.18)
Mongolia	93.91 (87.86-97.52)	99.15 (95.37-99.98)
Malawi	50.00 (32.43-67.57)	96.72 (93.00-98.79)
Madagascar	94.17 (88.35-97.62)	98.32 (94.06-99.80)
Ivory Coast	82.88 (75.77-88.6)	97.83 (95.00-99.29)
India	68.75 (49.99-83.88)	96.88 (91.14-99.35)
Ethiopia (S-)	66.67 (46.04-83.48)	97.92 (95.77-99.16)
Cameroon	91.54 (85.36-95.7)	92.59 (75.71-99.09)
Cambodia	81.75 (73.88-88.06)	99.44 (96.94-99.99)



Sensitivity of LAMP compared to culture stratified by smear

Site	Sensitivity S+C+ (95% CI)	Sensitivity S-C+ (95% CI)
Overall	**94.24 (92.39-95.75)**	**49.74 (44.58-54.89)**
Vietnam	80.00 (51.91-95.67)	15.79 (3.38-39.58)
Uganda	96.88 (83.78-99.92)	33.33 (15.63-55.32)
The Gambia	100.00 (92.89-100.00)	100.00 (15.81-100.00)
Tanzania	89.19 (81.88-94.29)	20.51 (9.30-36.46)
South Africa	94.64 (85.13-98.88)	51.02 (36.34-65.58)
Mongolia	100.00 (95.44 -100.00)	63.41 (46.94-77.88)
Malawi	58.82 (40.70-75.35)	40.00 (5.27-85.34)
Madagascar	97.17 (91.95-99.41)	48.48 (30.80-66.46)
Ivory Coast	98.20 (93.64-99.78)	58.82 (32.92-81.56)
India	100.00 (73.54-100.00)	100.00 (69.15-100.00)
Ethiopia (S-)	NA*	64.29 (44.07-81.36)
Cameroon	98.69 (94.39-99.97)	55.42 (44.10-66.34)
Cambodia	92.63 (85.41-96.99)	50.00 (30.65-69.35)



Example: Urine lateral flow LAM assay for diagnosing active TB in people living with HIV

- **Participants** are adults and children (14 years and younger) with HIV infection who are thought to have active TB. We will perform separate analyses for adults and children, in and outpatients.

- **Index test** is the urine lipoarabinomannan (LF-LAM) assay; grade 1 and 2 refer to thresholds for test positivity. We will perform separate analyses for grade 1 and 2.

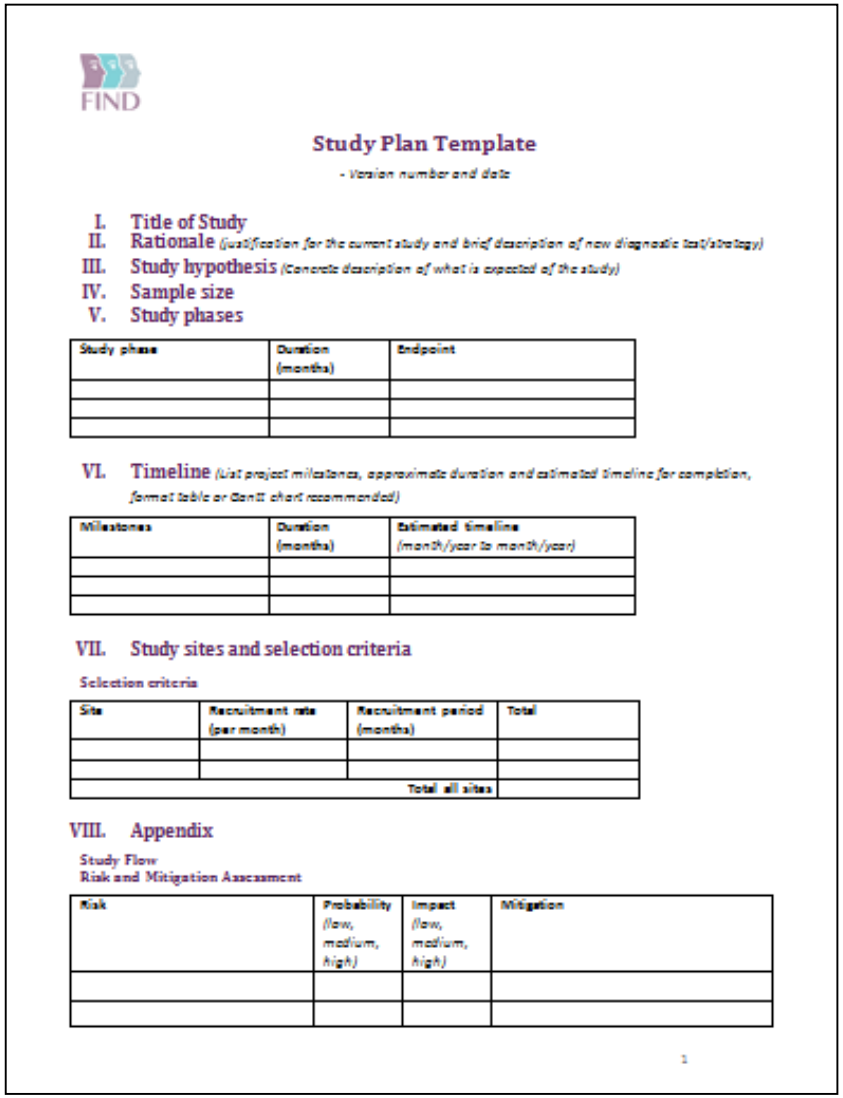
- **Target condition** is active TB, including pulmonary and extrapulmonary TB

- **Reference standards a**t least one of **1.Microbiological reference standard (Culture or NAAT) 2. Composite reference standard** at least one of culture, NAAT, smear, or clinical findings.

Evaluation study endpoints

Category	Details
Clinical performance	• Sensitivity/specificity/predictive values (stratified by site/smear/HIV)
Operational performance	• Assess robustness of reagents and equipment (temperature, dust, power irregularities, contamination rates) through indeterminate rates; intermittent testing of (blinded) controls; t/h log tags; customer support interventions • Determine minimal training needs / performance dependence on skills/motivation/workload/user fatigue through proficiency testing tool & performance (stratification by user / over time) • Assess user appraisal / requirements for implementation (such as waste management or storage) through user appraisal questionnaire and group discussions
Impact	• Surrogate markers such as time to detection of TB/DR & time to reporting compared to routine diagnostic algorithm

Developing a study plan



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Study Plan Template

- Version number and date

I. Title of Study
II. Rationale *(justification for the current study and brief description of new diagnostic test/strategy)*
III. Study hypothesis *(Concrete description of what is expected of the study)*
IV. Sample size
V. Study phases

Study phase	Duration (months)	Endpoint

VI. Timeline *(List project milestones, approximate duration and estimated timeline for completion, format table or Gantt chart recommended)*

Milestones	Duration (months)	Estimated timeline *(month/year to month/year)*

VII. Study sites and selection criteria

Selection criteria

Site	Recruitment rate (per month)	Recruitment period (months)	Total
		Total all sites	

VIII. Appendix

Study Flow
Risk and Mitigation Assessment

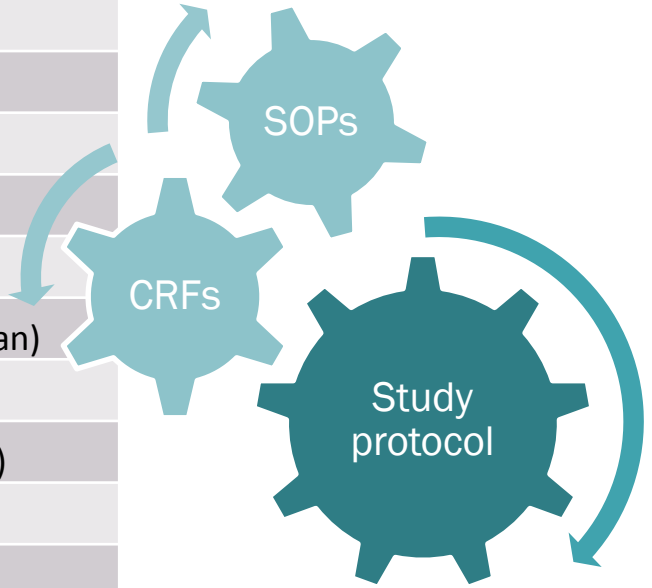
Risk	Probability *(low, medium, high)*	Impact *(low, medium, high)*	Mitigation

1

- To be prepared well in advance, basis for discussion with partners
- Short summary of most key study aspects
- Usually includes: title, rationale, study hypothesis, sample size, study phases, timelines, list of sites and study flow

What are the key components a study protocol?

Study protocol components	
1	General information (statement of compliance, signature page, key roles)
2	Background information and scientific rationale
3	Study objectives and purpose
4	Study design (phases and endpoints)
5	Study population and enrolment (inclusion, exclusion criteria)
6	Study sites
7	Study procedures and study flow
8	Investigational products
9	Monitoring
10	Timelines
11	Statistical considerations (study hypothesis, sample size, analysis plan)
12	Ethical considerations (Informed Consent Form, confidentiality)
13	Data management (responsibilities, data capture, records retention)
14	References
15	Appendices



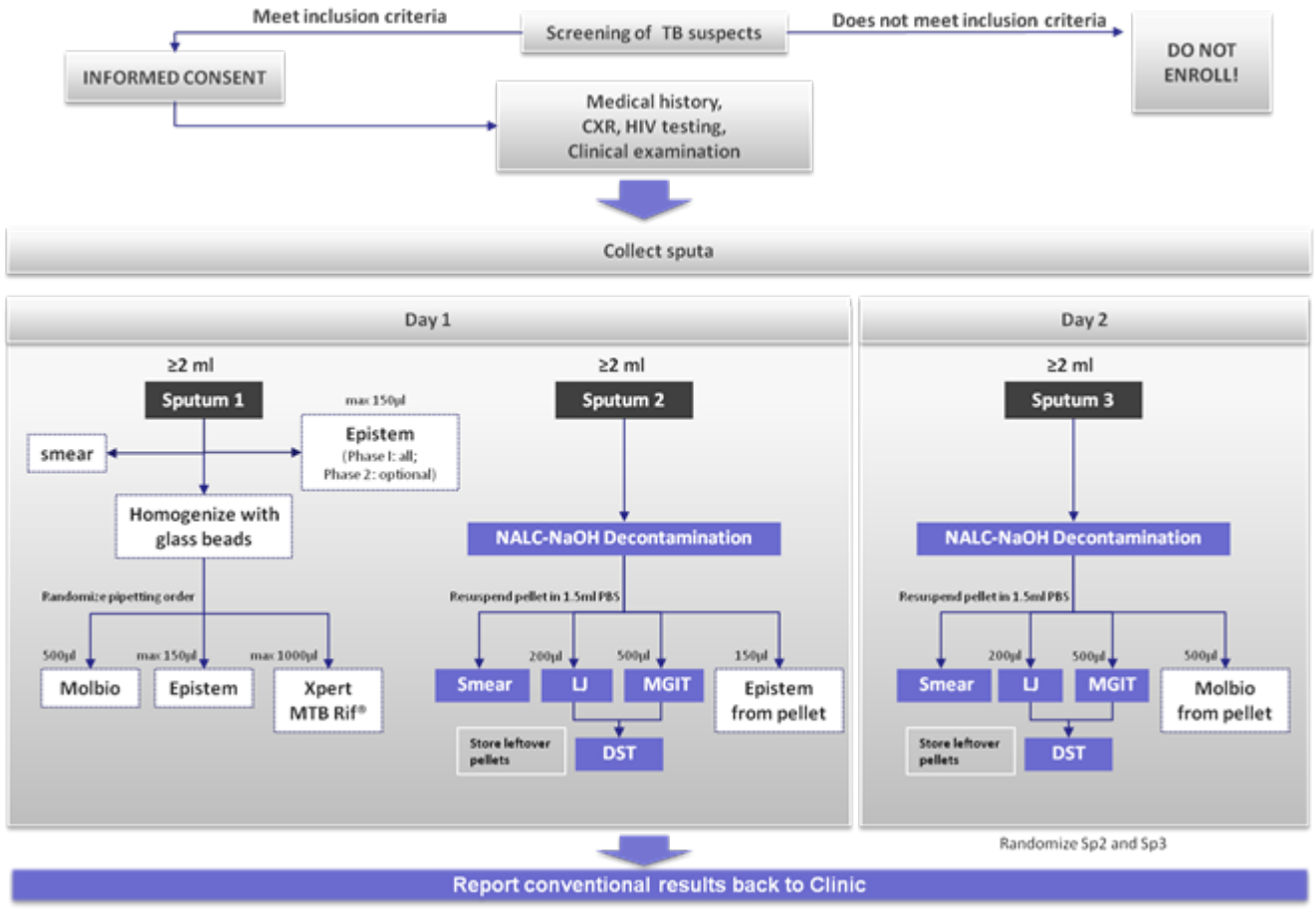
Example: *Accuracy of Epistem Genedrive MTB/RIF ID and Molbio Truenat MTB*

Table of Contents

Example: Accuracy of Epistem Genedrive MTB/RIF ID and Molbio Truenat MTB

Study flow: considerations

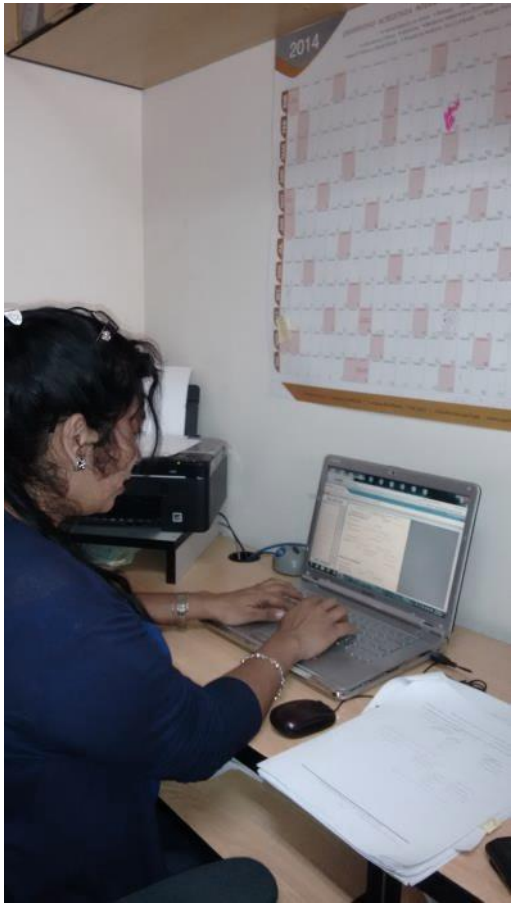
- Follow the flow: clinic, lab and reference lab (if different)
- Include timing
- Specify tests per sample
- Detailed, but keep it simple and short
- Index test done on the same sample than reference test and/or comparator



Hypothesis: Epistem Genedrive® MTB/RIF and Molbio Truenat™ MTB tests have diagnostic accuracy that is non-inferior to that of the GeneXpert® MTB/RIF assay

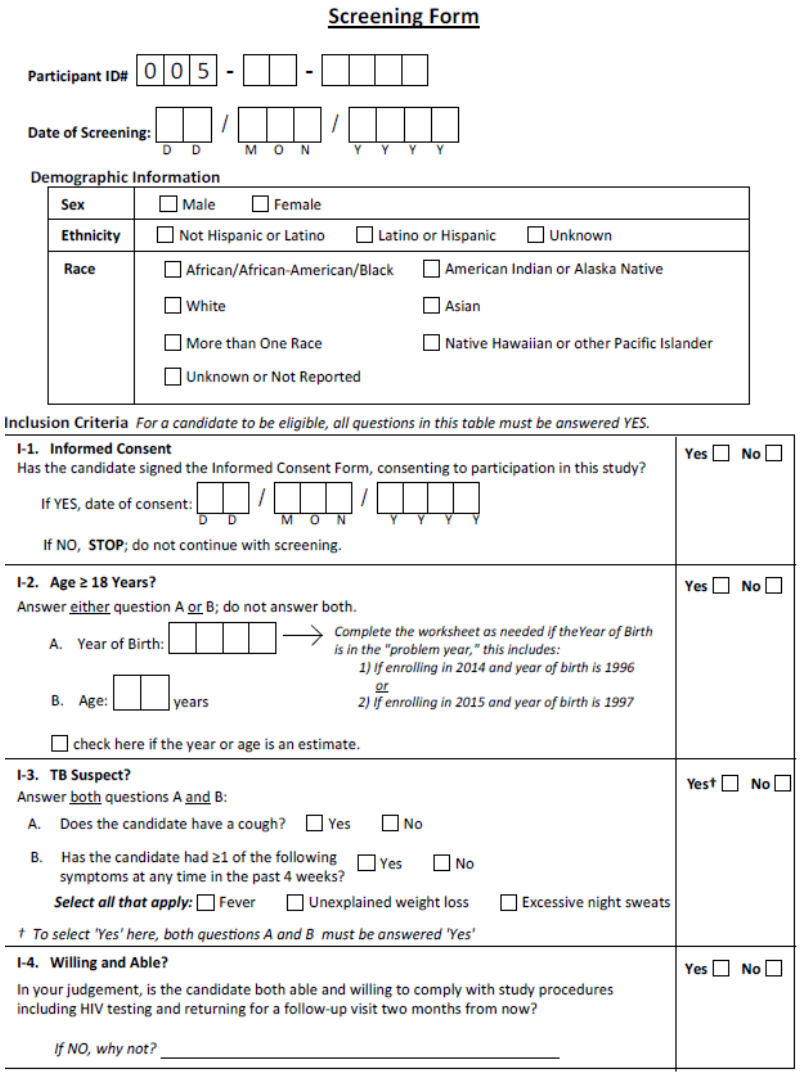
Data management considerations

- Appropriate training to ensure integrity, privacy on storage, transmision and protection of data
- Documents storage
  - Regulatory documents: e.g. IRB approvals, amendments (trial site & sponsor)
  - Source documents: document existence and integrity of trial data collected (trial site)
  - Signed, dated & completed CRFs: confirms observations recorded (trial site & sponsor)
- Electronic double data entry preferable, password protected databases, frequent backup
- Data validation/verification by study monitor



Case Report Form

- The outcome of the study is only as good as the data you collect on the CRFs. Make sure your CRFs capture all intended variables.
- Make sure the biostatistician responsible for the analysis and the PI sign off/approve the CRFs BEFORE they are given to the DM for creation of the database



Screening Form

Participant ID# 0 0 5 - ☐☐ - ☐☐☐☐

Date of Screening: ☐☐ / ☐☐☐ / ☐☐☐☐ (D D / M O N / Y Y Y Y)

Demographic Information

Sex	☐ Male ☐ Female
Ethnicity	☐ Not Hispanic or Latino ☐ Latino or Hispanic ☐ Unknown
Race	☐ African/African-American/Black ☐ American Indian or Alaska Native ☐ White ☐ Asian ☐ More than One Race ☐ Native Hawaiian or other Pacific Islander ☐ Unknown or Not Reported

Inclusion Criteria *For a candidate to be eligible, all questions in this table must be answered YES.*

I-1. Informed Consent Has the candidate signed the Informed Consent Form, consenting to participation in this study? If YES, date of consent: ☐☐ / ☐☐☐ / ☐☐☐☐ (D D / M O N / Y Y Y Y) If NO, **STOP**; do not continue with screening.	**Yes** ☐ **No** ☐
I-2. Age ≥ 18 Years? Answer either question A or B; do not answer both. A. Year of Birth: ☐☐☐☐ → *Complete the worksheet as needed if the Year of Birth is in the "problem year," this includes: 1) If enrolling in 2014 and year of birth is 1996 or 2) If enrolling in 2015 and year of birth is 1997* B. Age: ☐☐ years ☐ check here if the year or age is an estimate.	**Yes** ☐ **No** ☐
I-3. TB Suspect? Answer both questions A and B: A. Does the candidate have a cough? ☐ Yes ☐ No B. Has the candidate had ≥1 of the following symptoms at any time in the past 4 weeks? ☐ Yes ☐ No ***Select all that apply:*** ☐ Fever ☐ Unexplained weight loss ☐ Excessive night sweats † *To select 'Yes' here, both questions A and B must be answered 'Yes'*	**Yes†** ☐ **No** ☐
I-4. Willing and Able? In your judgement, is the candidate both able and willing to comply with study procedures including HIV testing and returning for a follow-up visit two months from now? *If NO, why not?* _____	**Yes** ☐ **No** ☐

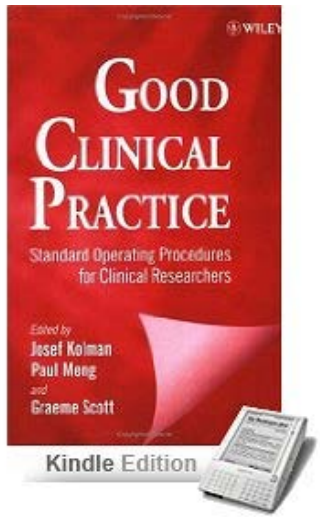
Considerations for creating effective CRFs

- The primary / secondary endpoints will drive data collected on CRFs
- Only collect what you really need
- Only ask each question once
- Make sure the way the question is worded is clear and will provide the answer you are looking for (test CRF for clarity and have a CRF key)
- Use validated instruments / methods for critical measures (especially important if submitting to the regulatory authorities for approval)
- CRFs/eCRFs set the framework for the database

Standard Operating Procedures (SOPs)

- SOPs required for all aspects of running clinical trials
- Needed centrally by the organization managing the overall conduct of the study and at the site level

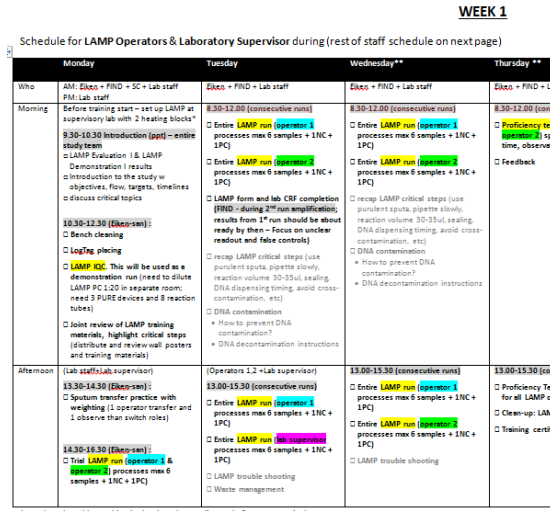
Ref: Good Clinical Practice: Standard Operating Procedures for Clinical Researchers; by Josef Kolman, Paul Meng, and Graeme Scott (Kindle Edition - Jun 15, 1998)



Typical site SOPs, tools and forms

- Site certification tool
- Costing tool
- Monitoring tool
- User appraisal /preferences tools
- Training curriculum / materials

- Roles and responsibilities
- Data analysis and management plan
- Monitoring plan
- Screening and informed consent
- Clinical examination
- Specimen collection
- Laboratory SOPs
  - Sample processing
  - Index test (processing, handling, inventory and troubleshooting)
  - Reference tests
  - Blinding procedure
  - RUO test and reagent inventory
- Document Archiving, Retention, Retrieval and Security
- Management of adverse events
- Participant withdrawal

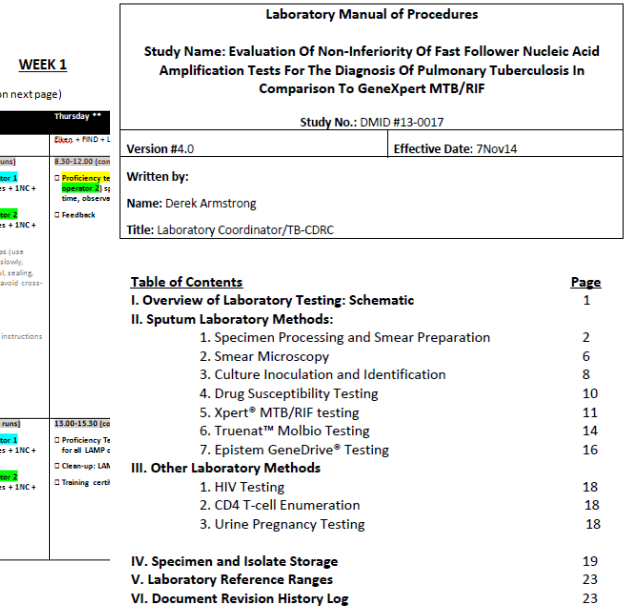


WEEK 1

Schedule for **LAMP Operators & Laboratory Supervisor** during (rest of staff schedule on next page)

	Monday	Tuesday	Wednesday**	Thursday **
Who	AM: ~~Eikon~~ + FIND + SC + Lab staff PM: Lab staff	~~Eikon~~ + FIND + Lab staff	~~Eikon~~ + FIND + Lab staff	~~Eikon~~ + FIND + L
Morning	Before training start – set up LAMP at supervisory lab with 2 heating blocks* 9.30-10.30 Introduction (ppt) – entire study team □ LAMP Evaluation I & LAMP Demonstration I results □ Introduction to the study w objectives, flow, targets, timelines □ discuss critical topics 10.30-12.30 (Eikon-sen) : □ Bench cleaning □ LogTag placing □ LAMP IQC. This will be used as a demonstration run (need to dilute LAMP PC 1:20 in separate room; need 3 PURE devices and 8 reaction tubes) □ Joint review of LAMP training materials, highlight critical steps (distribute and review wall posters and training materials)	8.30-12.00 (consecutive runs) □ Entire LAMP run (operator 1 processes max 6 samples + 1NC + 1PC) □ Entire LAMP run (operator 2 processes max 6 samples + 1NC + 1PC) □ LAMP form and lab CRF completion (FIND - during 2nd run amplification; results from 1st run should be about ready by then – Focus on unclear readout and false controls) □ recap LAMP critical steps (use purulent sputa, pipette slowly, reaction volume 30-35ul, sealing, DNA dispensing timing, avoid cross-contamination, etc) □ DNA contamination • How to prevent DNA contamination? • DNA decontamination instructions	8.30-12.00 (consecutive runs) □ Entire LAMP run (operator 1 processes max 6 samples + 1NC + 1PC) □ Entire LAMP run (operator 2 processes max 6 samples + 1NC + 1PC) □ recap LAMP critical steps (use purulent sputa, pipette slowly, reaction volume 30-35ul, sealing, DNA dispensing timing, avoid cross-contamination, etc) □ DNA contamination • How to prevent DNA contamination? • DNA decontamination instructions	8.30-12.00 (con □ Proficiency te operator 2) s time, observe □ Feedback
Afternoon	(Lab staff+Lab supervisor) 13.30-14.30 (Eikon-sen) : □ Sputum transfer practice with weighting (1 operator transfer and 1 observe than switch roles) 14.30-16.30 (Eikon-sen) : □ Trial LAMP run (operator 1 & operator 2) processes max 6 samples + 1NC + 1PC)	(Operators 1,2 +Lab supervisor) 13.00-15.30 (consecutive runs) □ Entire LAMP run (operator 1 processes max 6 samples + 1NC + 1PC) □ Entire LAMP run (lab supervisor processes max 6 samples + 1NC + 1PC) □ LAMP trouble shooting □ Waste management	13.00-15.30 (consecutive runs) □ Entire LAMP run (operator 1 processes max 6 samples + 1NC + 1PC) □ Entire LAMP run (operator 2 processes max 6 samples + 1NC + 1PC) □ LAMP trouble shooting	13.00-15.30 (co □ Proficiency Te for all LAMP □ Clean-up: LA □ Training certi

* Needs to be addressed by the local study coordinator before FIND arrival.
Items in gray are points for discussion during the LAMP incubation time.



Laboratory Manual of Procedures

Study Name: Evaluation Of Non-Inferiority Of Fast Follower Nucleic Acid Amplification Tests For The Diagnosis Of Pulmonary Tuberculosis In Comparison To GeneXpert MTB/RIF

Study No.: DMID #13-0017

Version #4.0 | **Effective Date:** 7Nov14

Written by:

Name: Derek Armstrong

Title: Laboratory Coordinator/TB-CDRC

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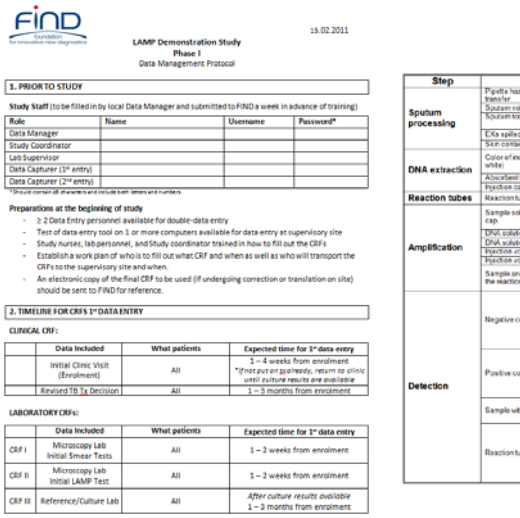


FIND — 19.02.2011

LAMP Demonstration Study
Phase I
Data Management Protocol

1. PRIOR TO STUDY

Study Staff (to be filled in by local Data Manager and submitted to FIND a week in advance of training)

Role	Name	Username	Password*
Data Manager			
Study Coordinator			
Lab Supervisor			
Data Capturer (1st entry)			
Data Capturer (2nd entry)			

*Should contain all characters and include both letters and numbers

Preparations at the beginning of study

- ≥ 2 Data Entry personnel available for double-data entry
- Test of data entry tool on 1 or more computers available for data entry at supervisory site
- Study nurses, lab personnel, and Study coordinator trained in how to fill out the CRFs
- Establish a work plan of who is to fill out what CRF and when as well as who will transport the CRFs to the supervisory site and when.
- An electronic copy of the final CRF to be used (if undergoing correction or translation on site) should be sent to FIND for reference.

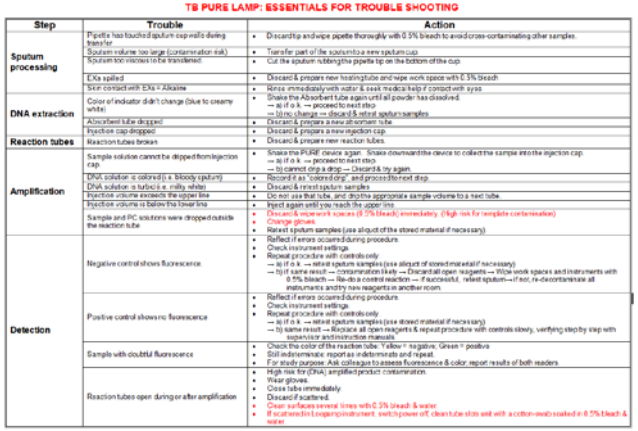
2. TIMELINE FOR CRFS 1st DATA ENTRY

CLINICAL CRF:

	Data Included	What patients	Expected time for 1st data entry
	Initial Clinic Visit (Enrolment)	All	1 – 4 weeks from enrolment *if not put on gatekeep, return to clinic until culture results are available
	Revised TB Tx Decision	All	1 – 5 months from enrolment

LABORATORY CRFs:

	Data Included	What patients	Expected time for 1st data entry
CRF I	Microscopy Lab Initial Smear Tests	All	1 – 2 weeks from enrolment
CRF II	Microscopy Lab Initial LAMP Test	All	1 – 2 weeks from enrolment
CRF III	Reference/Culture Lab	All	After culture results available 1 – 3 months from enrolment



TB PURE LAMP: ESSENTIALS FOR TROUBLE SHOOTING

Step	Trouble	Action
Sputum processing	Pipette has touched sputum cup walls during transfer	• Discard tip and wipe pipette thoroughly with 0.5% bleach to avoid cross-contaminating other samples.
	Sputum volume too large (contamination risk)	• Transfer part of the sputum to a new sputum cup
	Sputum too viscous to be transferred	• Cut the sputum rubbing the pipette tip on the bottom of the cup
DNA extraction	EXs spilled	• Discard & prepare new heating tube and wipe work space with 0.5% bleach
	Skin contact with EXs + Alkaline	• Rinse immediately with water & seek medical help if contact with eyes
	Color of indicator didn't change (blue to creamy white)	• Shake the Absorbert tube again until all powder has dissolved. → is it o.k. → proceed to next step → is no change → discard & retest sputum samples
	Absorbent tube dropped	• Discard & prepare a new absorbent tube
	Injection cap dropped	• Discard & prepare a new injection cap
Reaction tubes	Reaction tubes broken	• Discard & prepare new reaction tubes
Amplification	Sample solution cannot be dripped from injection cap	• Shake the PURE device again. Shake downward the device to collect the sample into the injection cap. → is it o.k. → proceed to next step → is cannot drop a drop. → Discard & try again.
	DNA solution is colored (i.e. bloody sputum)	• Record it as "colored drop", and proceed to next step.
	DNA solution is turbid (i.e. milky white)	• Discard & retest sputum samples
	Injection volume exceeds the upper line	• Do not use that tube, and drip the appropriate sample volume to a next tube.
	Injection volume is below the lower line	• Inject again until you reach the upper line.
	Sample and PC solutions were dropped outside the reaction tube	• Discard & wipe work spaces (0.5% bleach) immediately. (High risk for complete contamination) • Change gloves • Retest sputum samples (use aliquot of the stored material if necessary).
Detection	Negative control shows fluorescence	• Reflect if errors occurred during procedure • Check instrument settings • Repeat procedure with controls only → is it o.k. → retest sputum samples (use aliquot of stored material if necessary) → is it same result → contamination likely → Discard all open reagents → Wipe work spaces and instruments with 0.5% bleach → Re-do a control reaction → if successful, retest sputum→ if not, re-decontaminate all instruments and try new reagents in another room.
	Positive control shows no fluorescence	• Reflect if errors occurred during procedure • Check instrument settings • Repeat procedure with controls only → is it o.k. → retest sputum samples (use stored material if necessary) → is same result → Replace all open reagents & repeat procedure with controls slowly, verifying step by step with supervisor and instruction manuals
	Sample with doubtful fluorescence	• Check the color of the reaction tube: Yellow = negative; Green = positive • Still indeterminate: report as indeterminate and repeat. • For study purpose: Ask colleague to assess fluorescence & color; report results of both readers.
	Reaction tubes open during or after amplification	• High risk for (DNA) amplified product contamination. • Wear gloves. • Close tube immediately. • Discard if scattered. • Clean surfaces several times with 0.5% bleach & water. • If scattered in Loopamp instrument: switch power off, clean tube slots well with a cotton-swab soaked in 0.5% bleach & water.