Approach to MDR-TB microbiology in children

IMPAACT Annual Meeting

May 2017

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Desmond Tutu TB Centre

UNIVERSITEIT · STELLENBOSCH · UNIVERSITY
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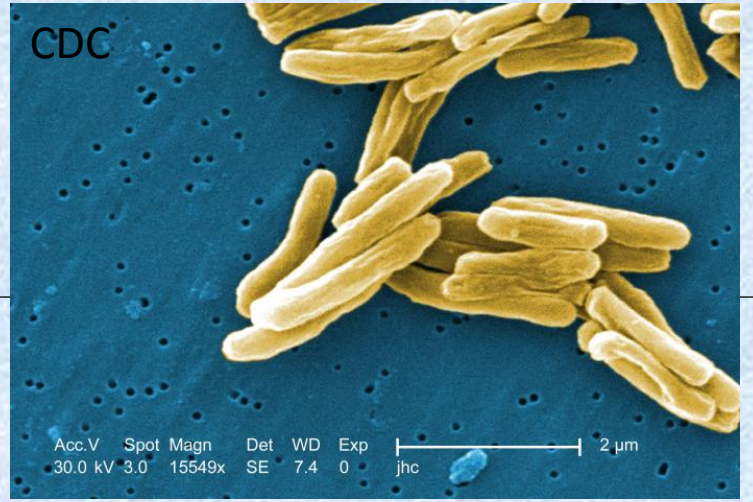
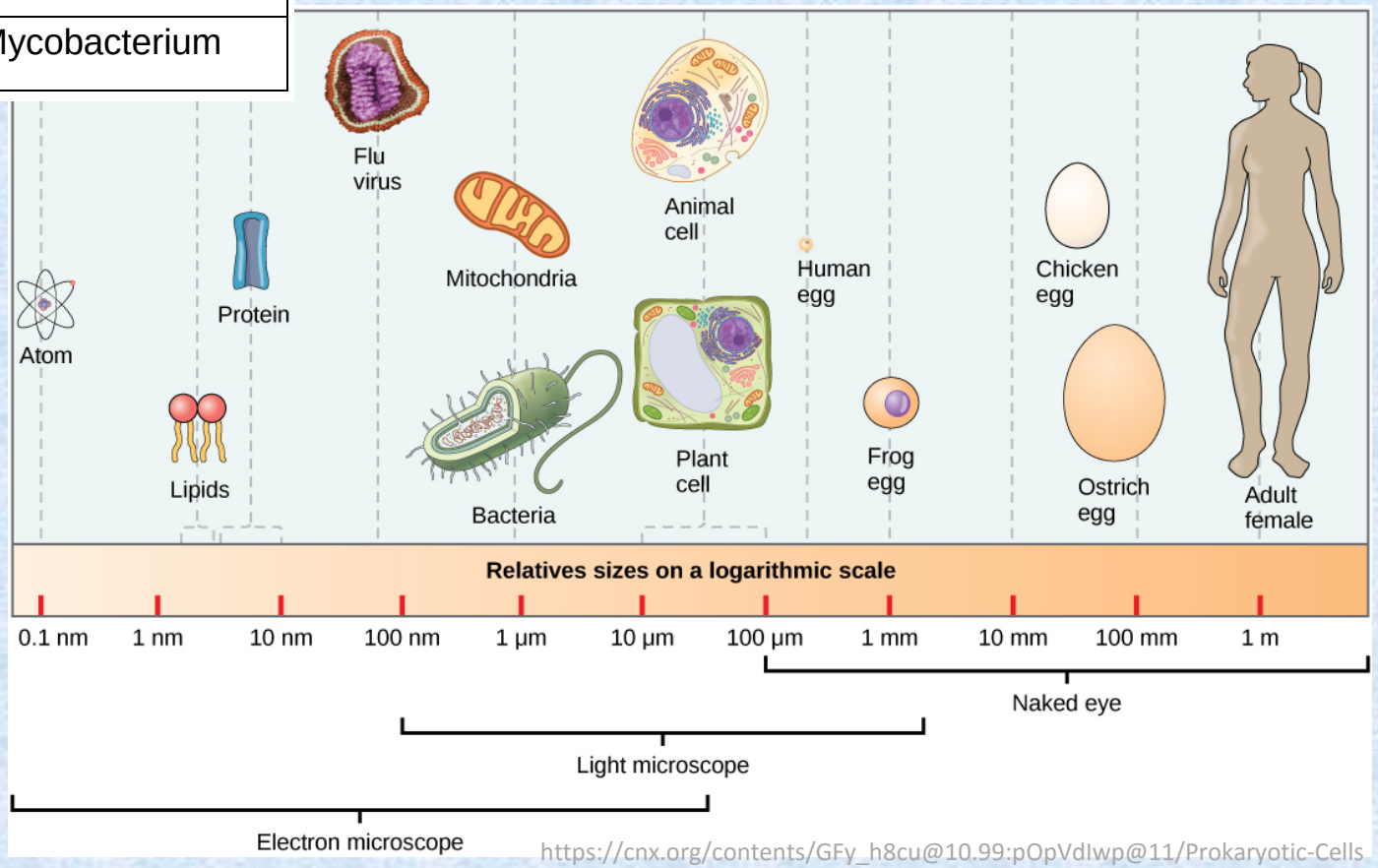
Objectives

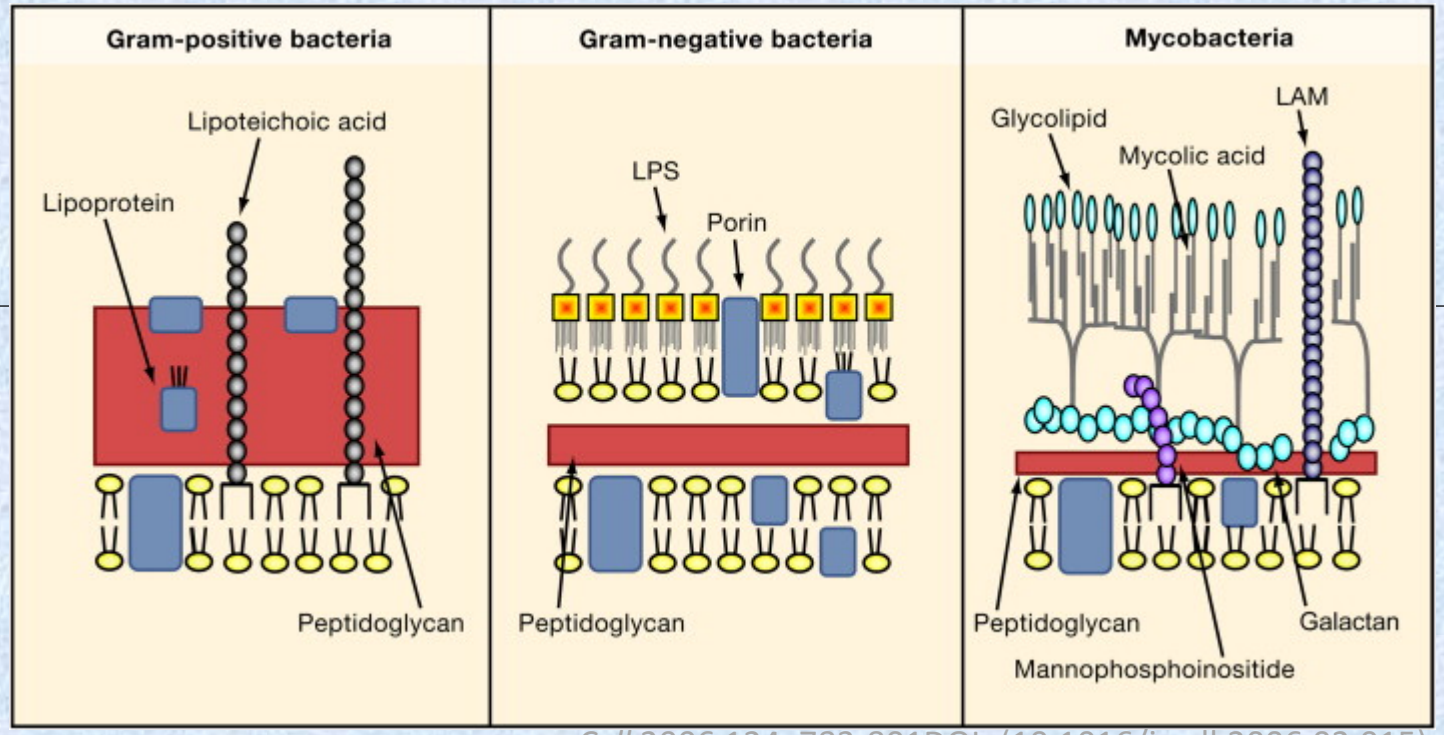
- Characteristics of mycobacteria
- Microbiology diagnosis of tuberculosis
- Research context
- Phases of lab testing
- Specimen Collection
- Smear, Culture, Nucleic Acid Amplification Tests (NAATs), Drug Susceptibility Testing (DST)
- Reporting
- Videos

Domain	Bacteria
Phylum or Division	Actinobacteria
Class	Actinobacteria
Subclass	Actinobacteridae
Order	Actinomycetales
Suborder	Corynebacterineae
Family	Mycobacteriaceae
Genus	Mycobacterium

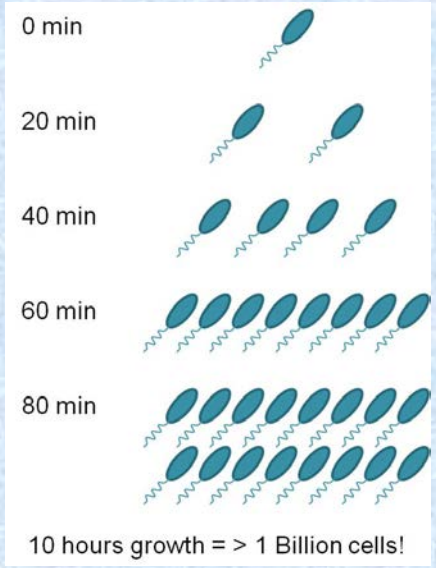


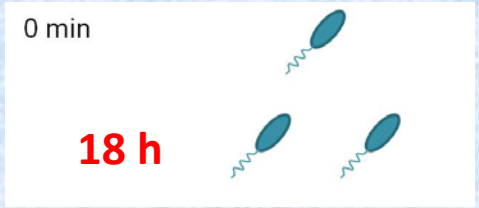


https://cnx.org/contents/GFy_h8cu@10.99:pOpVdIwp@11/Prokaryotic-Cells

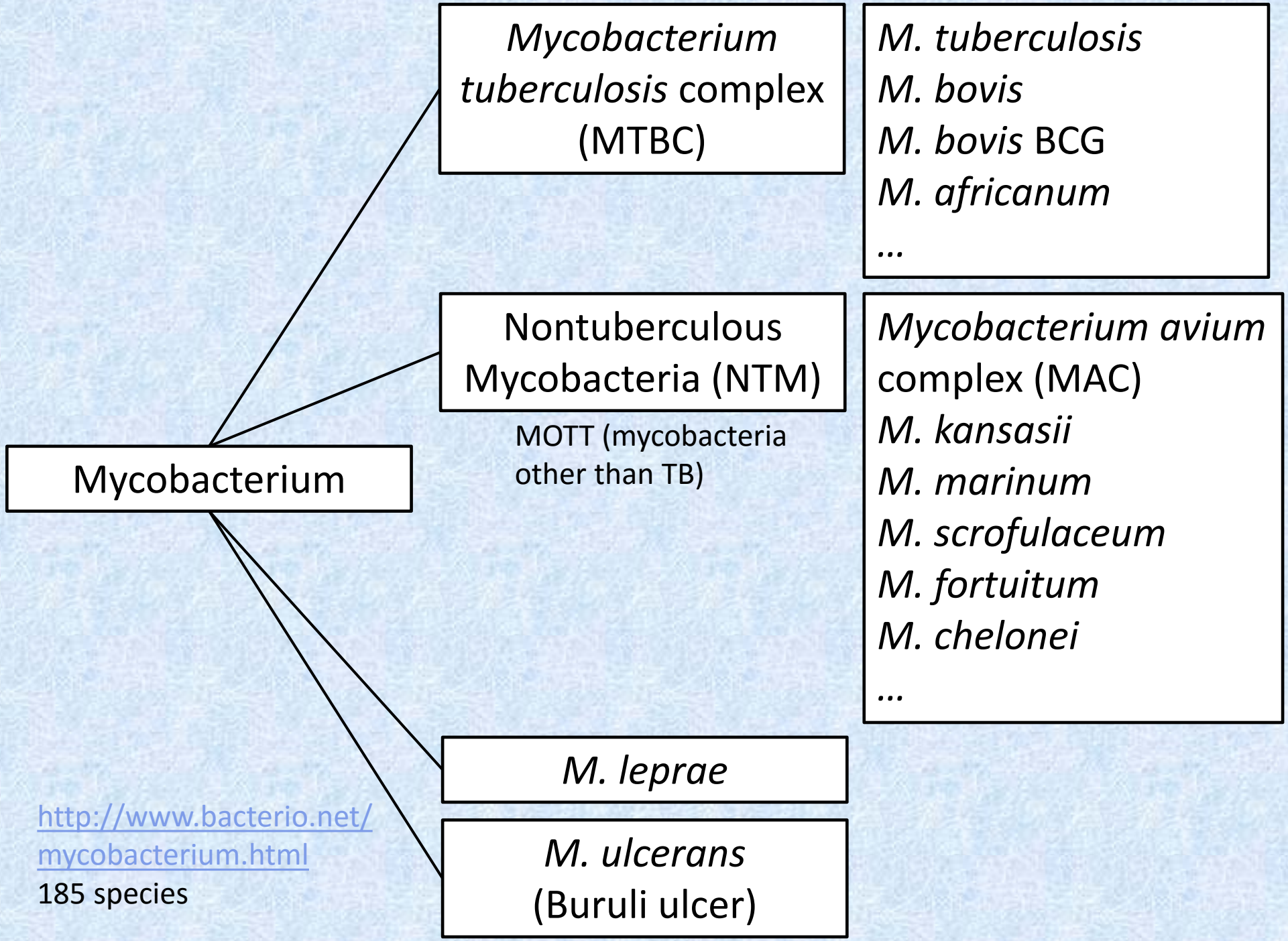


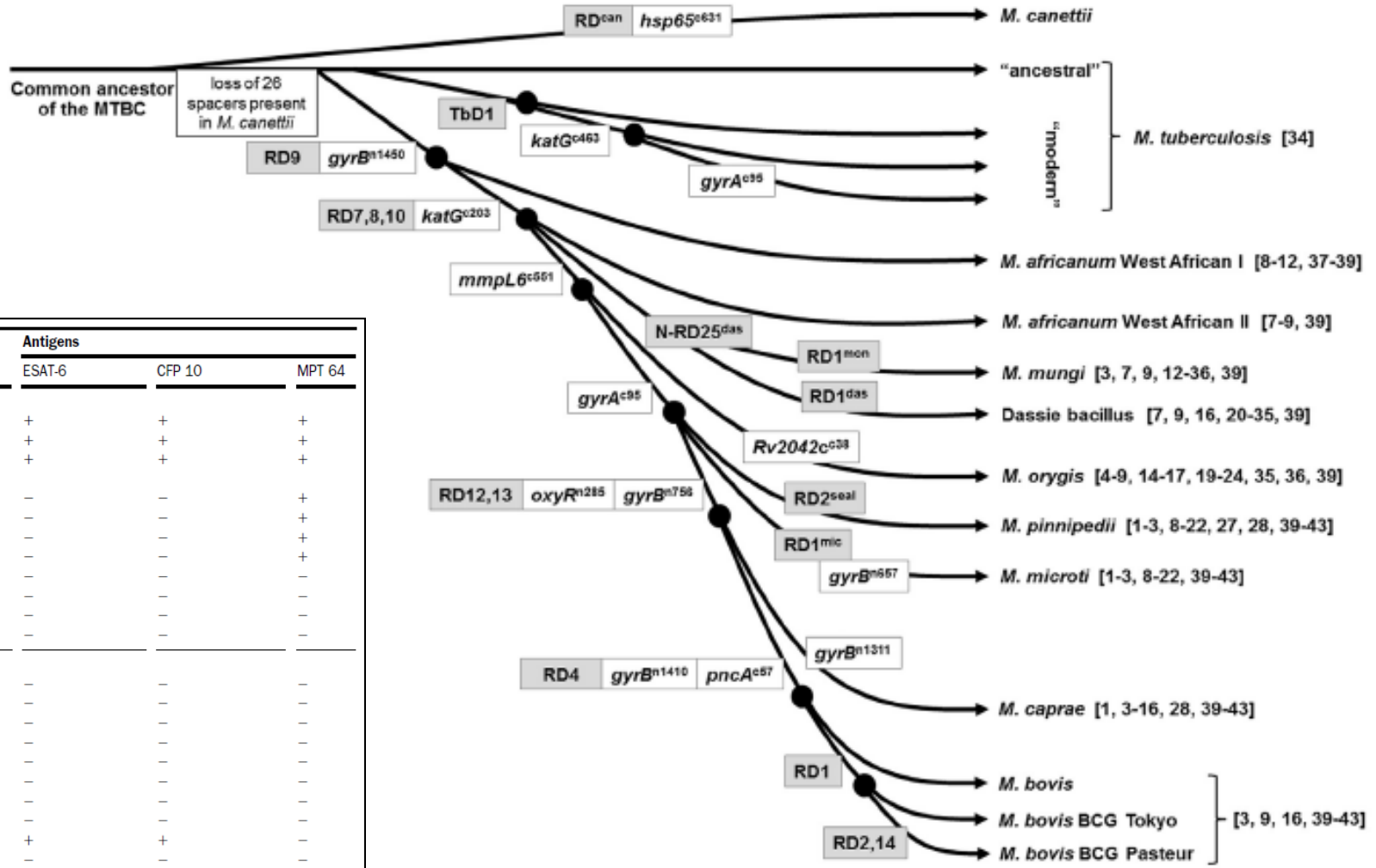
Cell 2006 124, 783-801DOI: (10.1016/j.cell.2006.02.015)





- Mycolic acids: special staining required
- Specific nutrients required
- Slow growth


Mycobacterium
Mycobacterium tuberculosis complex (MTBC)
M. tuberculosis
M. bovis
M. bovis BCG
M. africanum
…
Nontuberculous Mycobacteria (NTM)
MOTT (mycobacteria other than TB)
Mycobacterium avium complex (MAC)
M. kansasii
M. marinum
M. scrofulaceum
M. fortuitum
M. chelonei
…
M. leprae
M. ulcerans (Buruli ulcer)
http://www.bacterio.net/mycobacterium.html
185 species



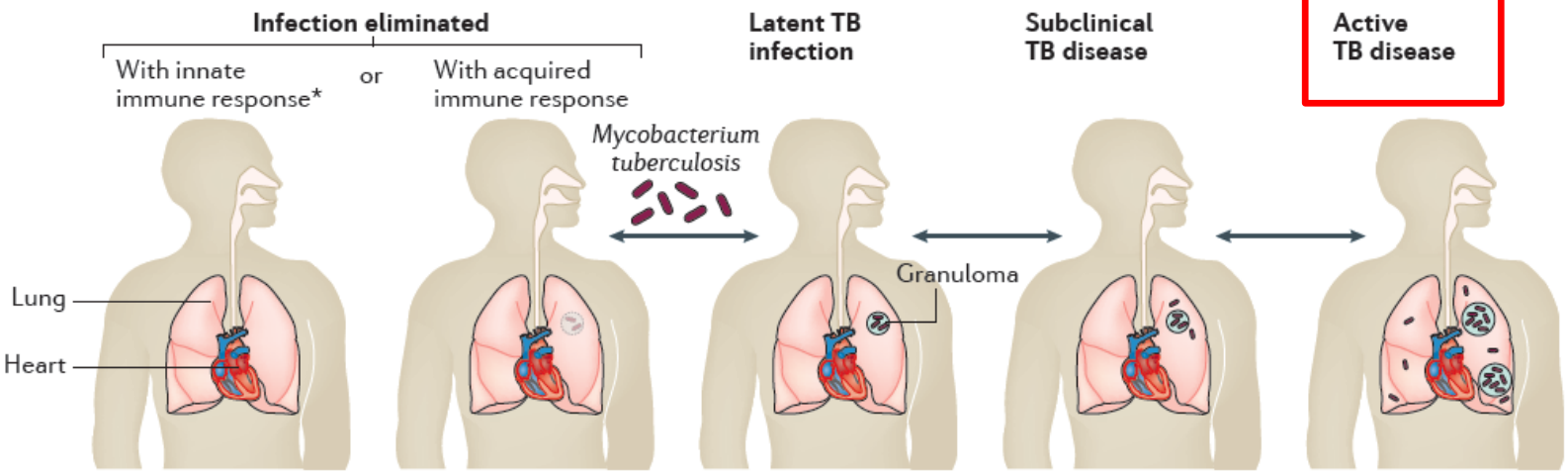
Strain tested	Antigens		
	ESAT-6	CFP 10	MPT 64
Tuberculosis complex			
M tuberculosis	+	+	+
M africanum	+	+	+
M bovis	+	+	+
BCG substrain			
gothenburg	–	–	+
moreau	–	–	+
tice	–	–	+
tokyo	–	–	+
danish	–	–	–
glaxo	–	–	–
montreal	–	–	–
pasteur	–	–	–
Environmental strains			
M abcessus	–	–	–
M avium	–	–	–
M branderi	–	–	–
M celatum	–	–	–
M chelonae	–	–	–
M fortuitum	–	–	–
M gordonii	–	–	–
M intracellulare	–	–	–
M kansasii	+	+	–
M malmoense	–	–	–
M marinum	+	+	–
M oenavense	–	–	–
M scrofulaceum	–	–	–
M smegmatis	–	–	–
M szulgai	+	+	–
M terrae	–	–	–
M vaccae	–	–	–
M xenopi	–	–	–

–=not present in species/strain, +=present in species/strain.

Distribution of diagnostic antigens in mycobacterial species

Lancet 2000; **356:** 1099–104

> 95% DNA homology



TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

NATURE REVIEWS | DISEASE PRIMERS
VOLUME 2 | 2016

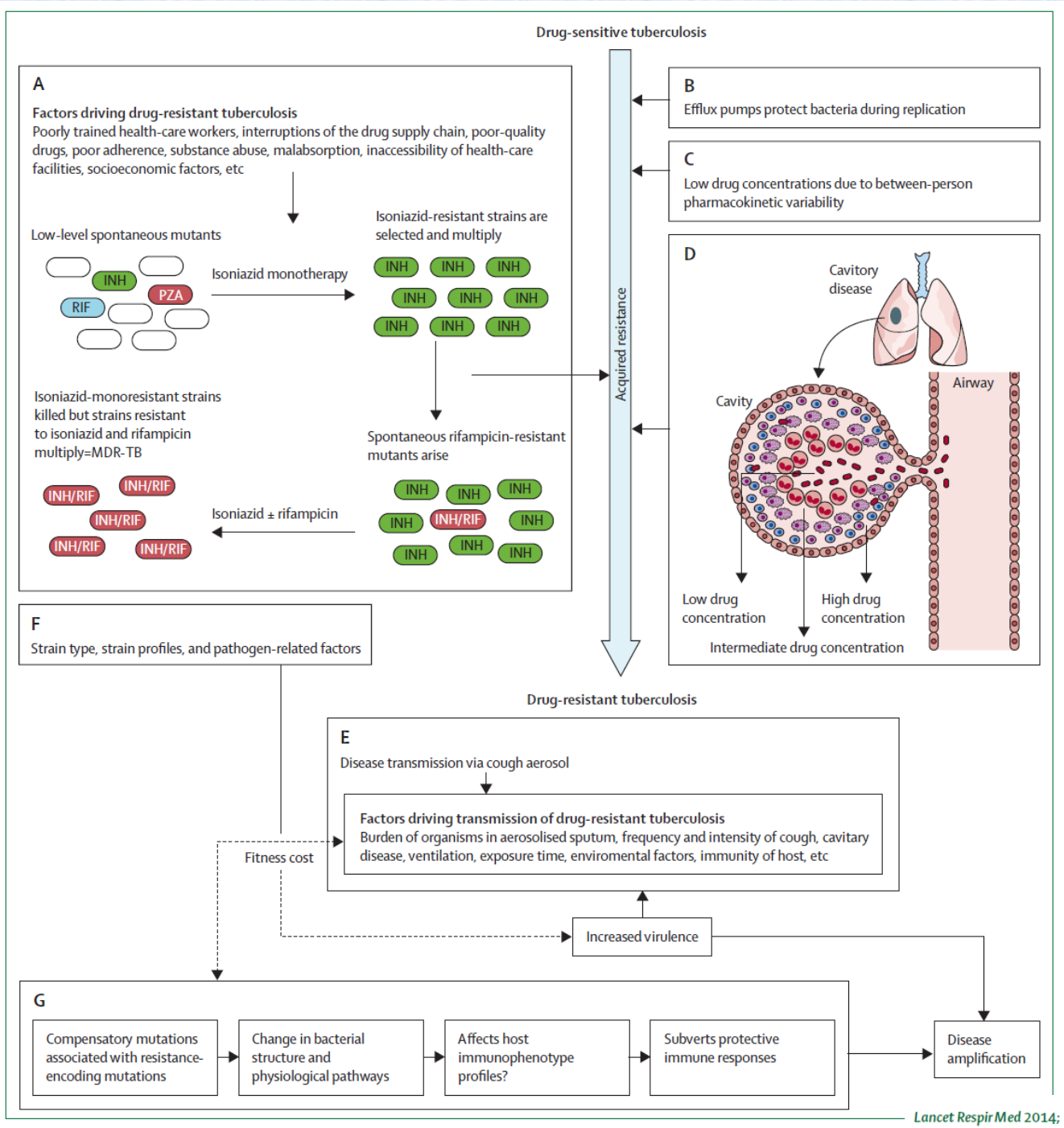


Figure 2: The pathogenesis of drug-resistant tuberculosis

Lancet Respir Med 2014; 2: 321–38

TB Laboratory Diagnosis Challenges
In both adults and children

TB diagnosis

- Respiratory specimens
  - Quality/variability of specimen
  - Infection control
- Culture still gold standard
  - Costly, specialized labs
  - Not perfect: contamination
  - Long
- No real point of care test
  - Xpert helps but other tests needed
- Variation in tests and procedures used, with many non-automated steps

Compared to HIV diagnosis

- Diagnosis on blood specimen
- Rapid point of care tests

Table 1 Timeline of the advances made in the diagnosis of tuberculosis

1880–1900	Robert Koch discovers that TB is caused by *M tuberculosis* Sputum smear microscopy using Ziehl–Neelsen staining *M tuberculosis* cultured on solid media (Lowenstein–Jensen slants) Tuberculin (purified protein derivative) isolated
1900–20	Rontgen discovers *x* rays: 1899 Tuberculin skin test developed: first used to diagnose *M bovis* in cows
1920–40	Use of attenuated *M bovis* BCG as TB vaccine: first given to a human (per os) in 1921 Sputum concentration using chemical flocculation Flourescent staining using auramine

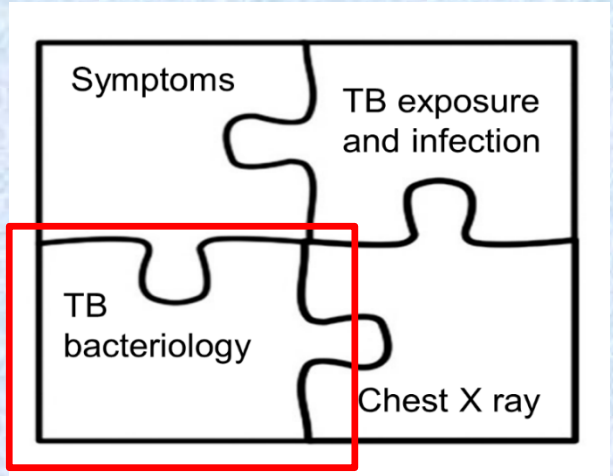
Arch Dis Child 2007;**92**:446–452.

TB Laboratory Diagnosis in children

- Additional challenges:
  - Paucibacillary nature of childhood TB
    - Culture confirmed in 30% cases
  - Specimen collection is invasive in young children
  - Variety of specimens used
  - Volumes collected often small
  - Difficult to evaluate new diagnostic tests
    - Tests evaluated in adult sputum → applicable to gastric aspirate and other specimens?

TB Laboratory Diagnosis in children

- Why confirm the diagnosis?
  - Clinical diagnosis not always easy



Courtesy of E. Walters

  - Guide treatment if MDR-TB
  - Research

Table 2. Revised Classification of Intrathoracic Tuberculosis Case Definitions for Diagnostic Evaluation Studies in Children

Case Definition	Refined Criteria[a]
Confirmed tuberculosis	Bacteriological confirmation obtained Requires *Mycobacterium tuberculosis* to be confirmed (culture or Xpert MTB/RIF assay) from at least 1 respiratory specimen

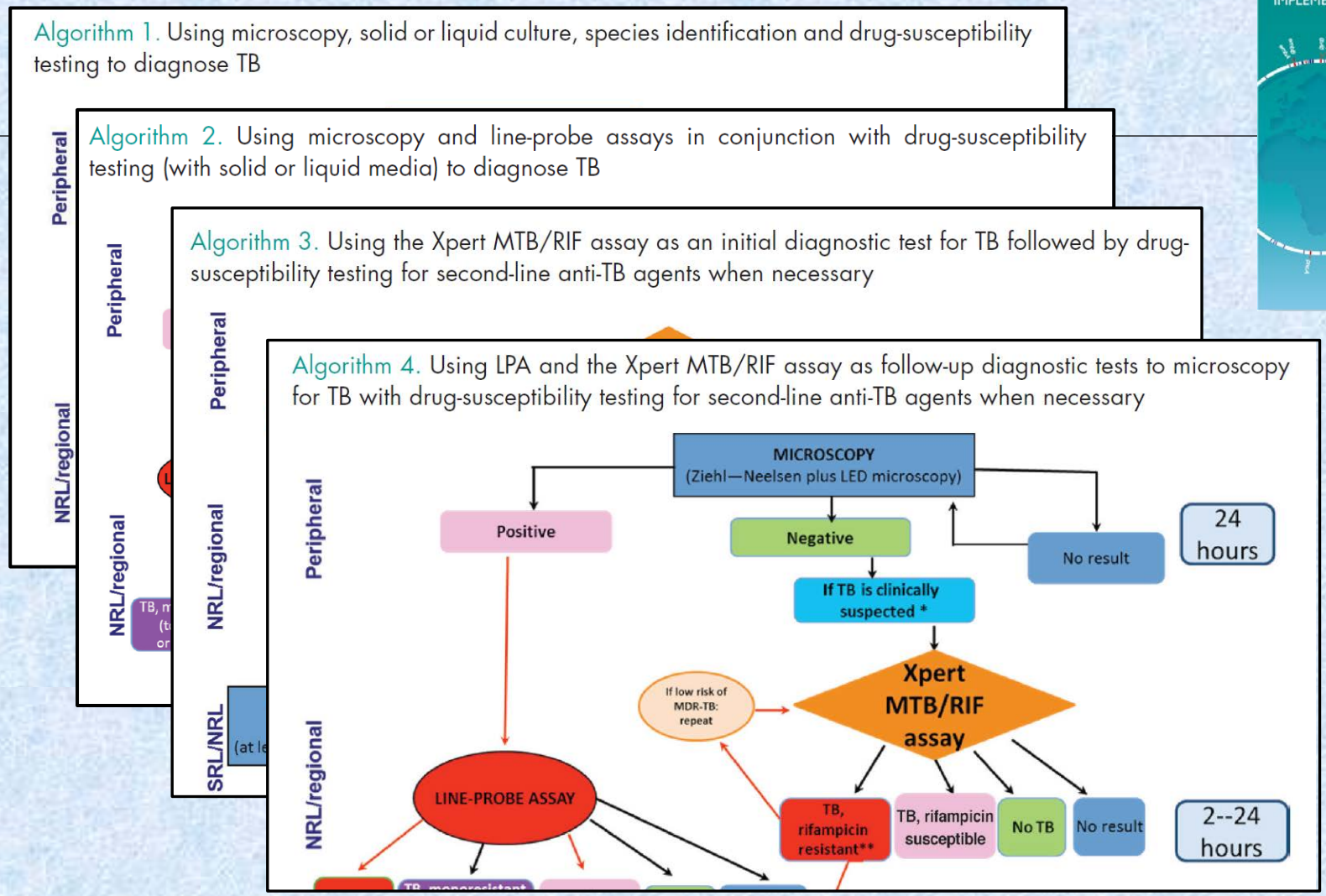
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following:
	• Symptoms/signs suggestive of tuberculosis (as defined)
	• Chest radiograph consistent with tuberculosis
	• Close tuberculosis exposure or immunologic evidence of *M. tuberculosis* infection
	• Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified)
	- With *M. tuberculosis* infection
	  • Immunological evidence of *M. tuberculosis* infection (TST and/or IGRA positive)
	- Without *M. tuberculosis* infection
	  • No immunological evidence of *M. tuberculosis* infection

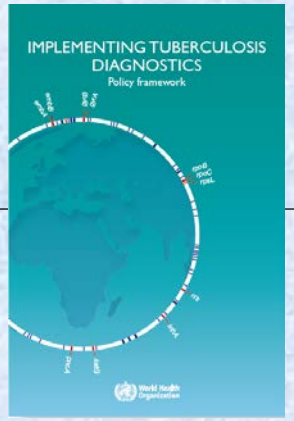
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND Criteria for "unconfirmed tuberculosis" NOT met
	- With *M. tuberculosis* infection
	  • Immunological evidence of *M. tuberculosis* infection (TST and/or IGRA positive)
	- Without *M. tuberculosis* infection
	  • No immunological evidence of *M. tuberculosis* infection

Variability

- Variability in procedures in TB labs worldwide
  - Smear: auramine, ZN, Kinyoun
  - Solid culture: LJ, 7H11
  - MGIT culture: automated and commercially available: however, identification of positive cultures varies
- If same procedure (non-automated), variability due to individuals performing them
  - e.g. smear reading, sputum processing
- Variability of diagnostic algorithms
  - Culture or culture based DST not always done

Variety of TB diagnosis algorithms





WHO. Implementing tuberculosis diagnostics. Policy framework.
http://www.who.int/tb/publications/implementing_TB_diagnostics/en/

Research Context

- Variability of tests/methods in TB labs worldwide
  - Not a problem for routine programs
  - Problem to compare results across sites in multi-centre studies
- ACTG-IMPAACT network labs
  - Approval process, EQA
  - Harmonisation efforts for TB labs based on Key Elements: Draft Sourcebook currently reviewed
- Not always possible to have all specimens tested in network labs
- Collecting extra specimen for research
  - Easier for adults to collect sputum, not so easy for invasive specimen in children

- P1108 and 2005
  - Children diagnosed as MDR-TB by routine services must be treated for a period before entering the study and have Bedaquiline or Delamanid added to OBR
  - Repeating specimen collection in young children (gastric aspirate or induced sputum) would be invasive, costly, time consuming and unlikely to yield positive results after many weeks of treatment.

- A5300/I2003
  - Evaluation of delamanid given to contacts of MDR-TB patients to prevent TB
  - MDR-TB Index cases already on treatment for weeks and may or may not be culture + when approached by the study team

Research context

- Considerations when MDR TB diagnosis made outside of network lab:
  - Isolates cannot be stored for further testing: DST for new drugs, MIC, WGS, etc.
  - Not all OBR drugs/mutations may have been tested
    - e.g. no INH R result with Xpert thus no inhA mutations to guide use of ethionamide and high dose INH
  - Not possible to definitively confirm the MDR diagnosis
    - Errors are rare but could occur
- Solution: obtaining baseline isolate

Research context

- Understanding TB Lab results from routine program
  - For inclusion or late exclusion criteria
  - To complete TB Lab CRF
- Challenging
  - Requires understanding of the different tests done in the TB laboratory
  - Results can be complex, especially for MDR-TB when drug susceptibility results done by different methods

Phases of laboratory testing

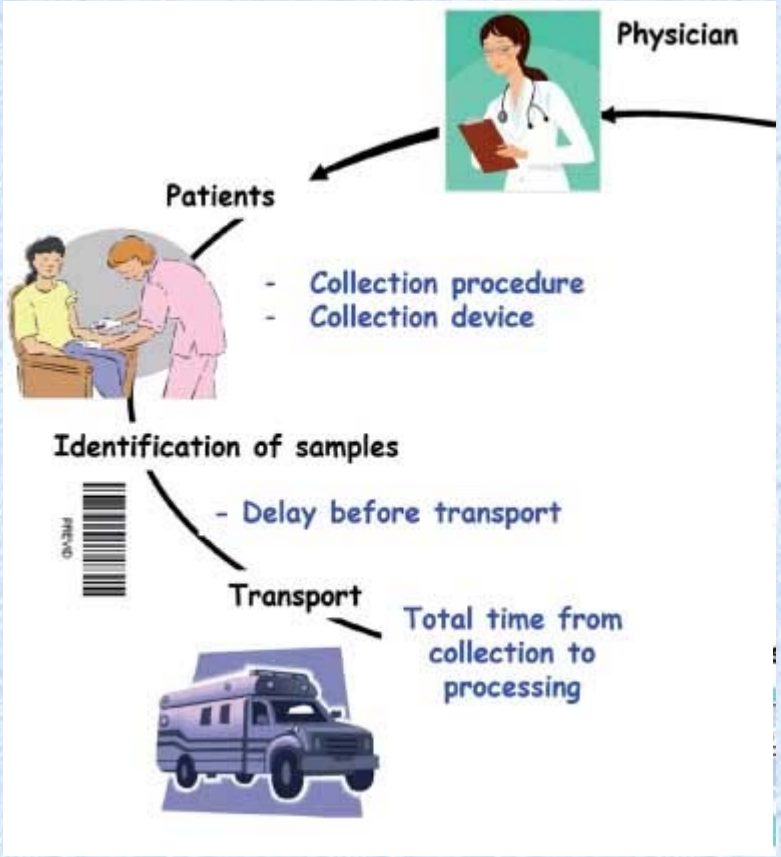
Pre-Analytical	Analytical	Post-Analytical

Phases of laboratory testing

Pre-Analytical

Pre-analytical phase

- Decision of
  - Test
  - Specimen type
- Specimen collection and transport
- Context
  - Routine diagnostic / National TB Program
  - Research



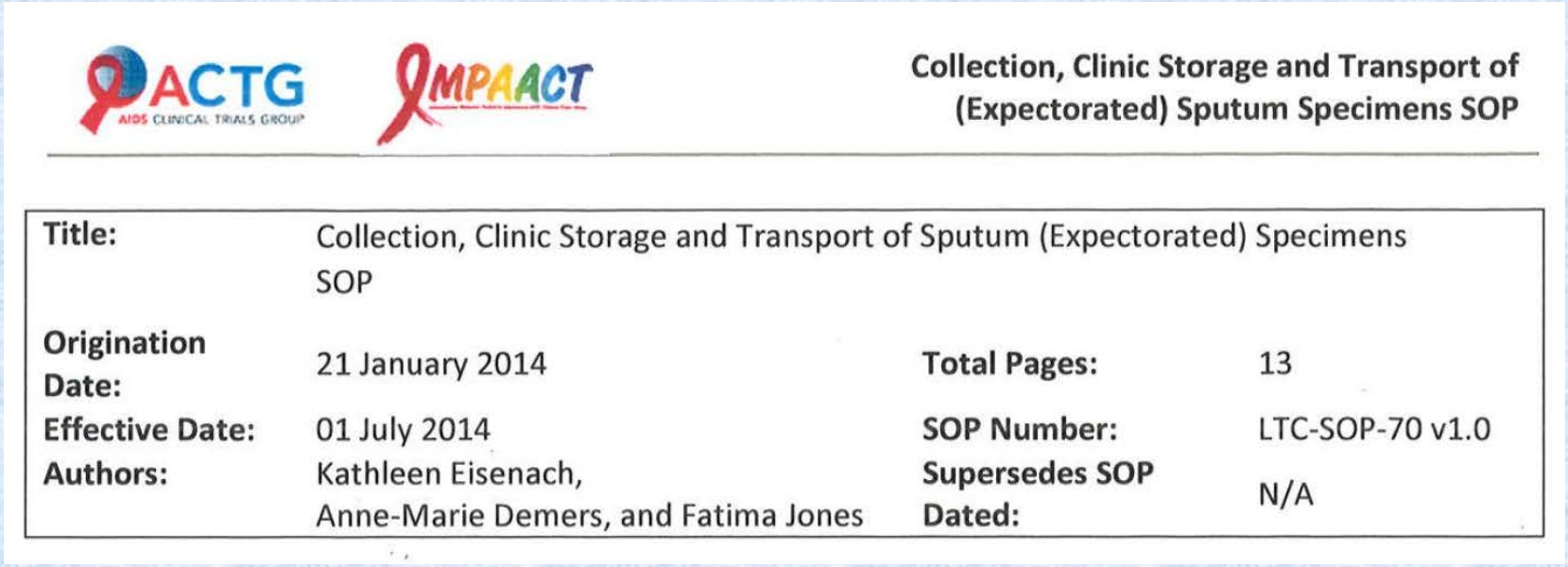
https://www.degruyter.com/view/j/cclm.2013.51.issue-5/

Specimens

- Respiratory vs non-respiratory
  - Sputum (expectorated, induced), Gastric aspirate/lavage, Naso-pharyngeal aspirate, Broncho-alveolar lavage (BAL), etc.
  - Fine needle aspirate (FNA) of lymphadenopathy, Cerebrospinal fluid (CSF), tissue, fluids, etc.
- Contaminated vs sterile
  - From normally sterile sites (e.g. CSF) vs from sites contaminated with normal flora (e.g. sputum)

Sputum (expectorated)

- Main specimen for diagnosis of TB disease in adults and older children
- ACTG IMPAACT SOP
  - Available on HANC website



ACTG AIDS CLINICAL TRIALS GROUP IMPAACT

Collection, Clinic Storage and Transport of (Expectorated) Sputum Specimens SOP

Title:	Collection, Clinic Storage and Transport of Sputum (Expectorated) Specimens SOP		
Origination Date:	21 January 2014	**Total Pages:**	13
Effective Date:	01 July 2014	**SOP Number:**	LTC-SOP-70 v1.0
Authors:	Kathleen Eisenach, Anne-Marie Demers, and Fatima Jones	**Supersedes SOP Dated:**	N/A

Sputum (expectorated)

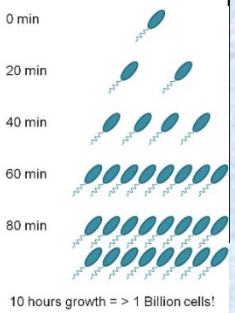
Key Elements

- Participants must rinse their mouth with boiled/sterile/bottled or distilled water prior to collection.
- Collect at least 3 to 5 mL of sputum. Larger volumes are preferred. A minimum of 1 mL is acceptable.

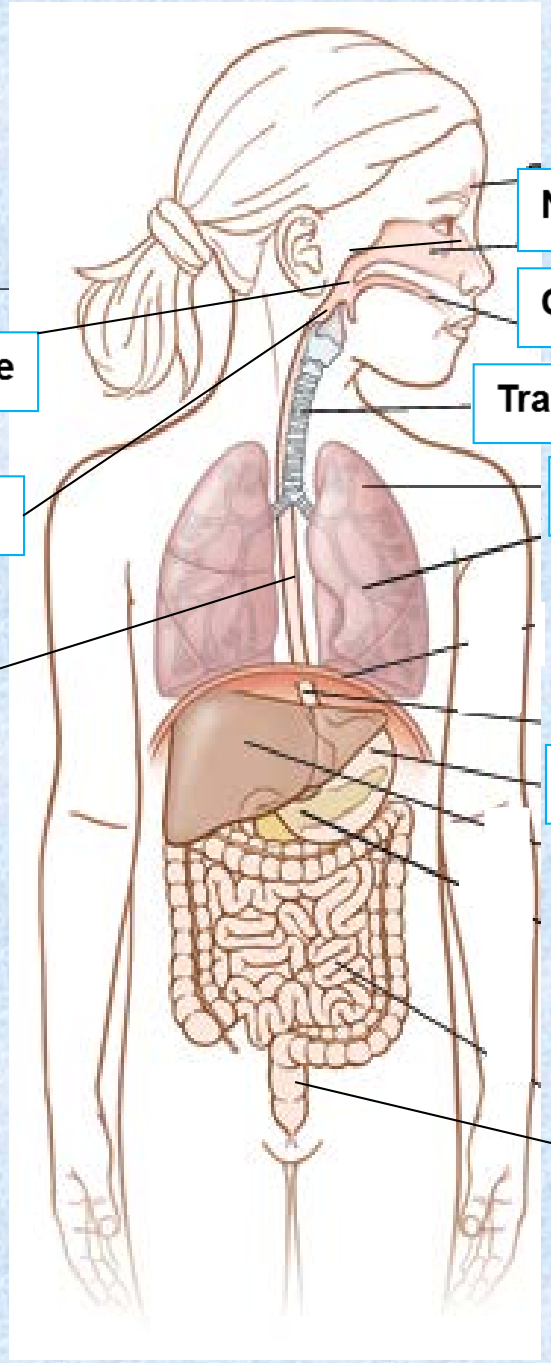
- Store specimens in a refrigerator or cool box (2-8°C) if not transported to the laboratory within 1 hour of collection
- Transport specimens to the laboratory in a cool box (2-8°C) as soon as possible after collection. Respiratory specimens must be delivered to the laboratory as soon as possible and/or within 24 hours of collection.

Critical Technical Points

- Procedures for the collection, transport and receipt of all mycobacteriology spec
- Infection control measures during specimen collection



Specimens

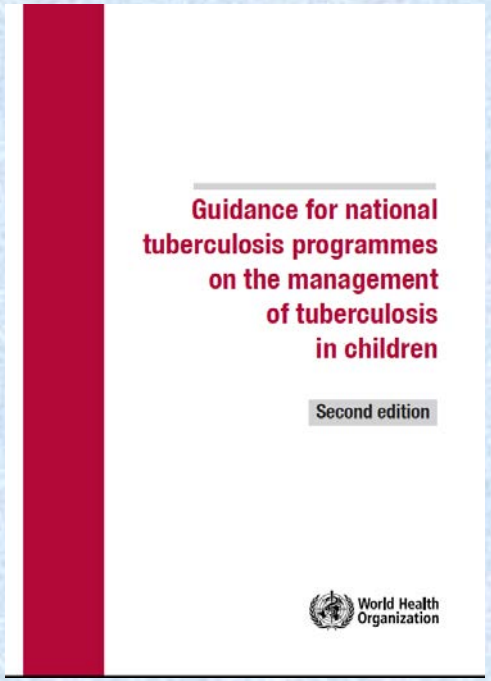


Potential specimens for bacteriological confirmation of PTB

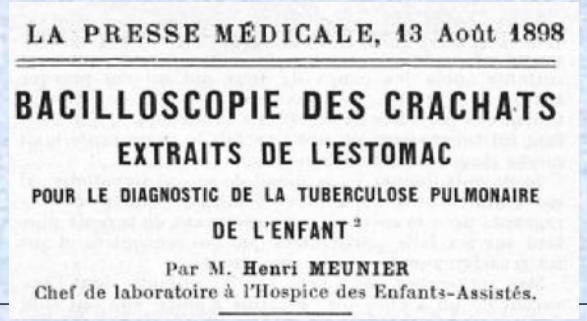
Slide courtesy of E. Walters

Induced Sputum

- Saline nebulisation inducing cough
- Can be done in adults, children and young infants
  - Naso-pharyngeal aspiration
- Nebulisation material
- Infection control measures
- No network SOP yet
  - WHO guidance



Gastric aspirate



LA PRESSE MÉDICALE, 13 Août 1898

BACILLOSCOPIE DES CRACHATS

EXTRAITS DE L'ESTOMAC

POUR LE DIAGNOSTIC DE LA TUBERCULOSE PULMONAIRE

DE L'ENFANT [2]

Par M. Henri MEUNIER

Chef de laboratoire à l'Hospice des Enfants-Assistés.

- Collection of secretions swallowed overnight
- Naso-gastric tube inserted to aspirate stomach content after fasting
  - Traditionally in hospital x 3 consecutive days
- Aspirate vs lavage
  - Aspiration only
  - Lavage using sterile water or saline. Dilution factor
- Inpatient vs outpatient
- Timing
  - Early morning

Gastric aspirate

- Neutralisation of gastric aspirates with bicarbonates is recommended by many organisations including WHO, American Society of Microbiology
  - Acid is detrimental to mycobacteria
  - JCM 2013 Parashar et al. questioning need to neutralise GA specimens
  - More research is needed
- Different neutralisation methods and formulations
  - Solution vs solid/powder form
  - Added by clinical team or by laboratory at reception
  - Unless processing done < 4 hours of collection
- No network SOP yet
- Various references including WHO and videos

Gastric aspirate

Key Elements

- Collect at least 5 to 10 mL of gastric aspirate. Larger volumes are preferred. A minimum of 1 mL is acceptable.
- Collect gastric aspirate after a minimum fasting period of at least 4 hours. Early morning collection is preferred.
- Gastric aspirate must be pH neutralized as soon as possible after aspiration unless the laboratory can neutralize or process the specimen within 4h of collection.

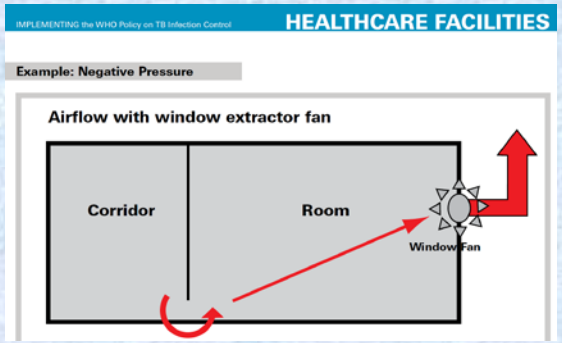
- Store specimens in a refrigerator or cool box (2-8°C) if not transported to the laboratory within 1 hour of collection
- Transport specimens to the laboratory in a cool box (2-8°C) as soon as possible after collection. Respiratory specimens must be delivered to the laboratory as soon as possible and/or within 24 hours of collection.

Critical Technical Points

- Procedures for the collection, transport and receipt of all mycobacteriology specimens
- Infection control measures during specimen collection
- Collect the gastric content by aspiration first as lavage introduces dilution. If adequate volumes are not obtained, lavage can be performed using sterile water or saline.

Infection control measures

- Personal protective equipment (PPE)
- Well ventilated area:
- Sufficient time for air changes in room in between procedures
- Guidance available



Material preparation

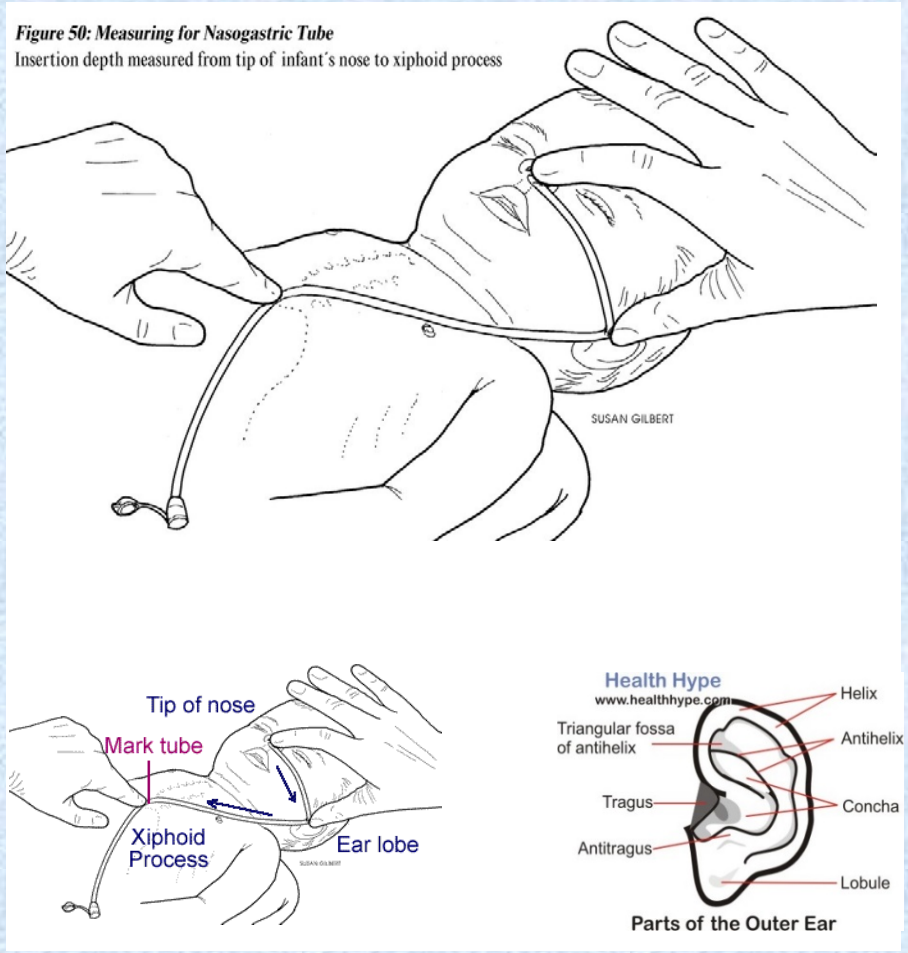
5 Procedures

5.1 Equipment required for gastric aspirate collection

Table 1.

1.	Disposable Gloves (non-sterile)
2.	Particulate respirator masks (N95 or equivalent)
3.	Disposable aprons
4.	Disposable linen saver
5.	Paper towel
6.	3 bed sheets or surgical drapes: one for the bed, one for wrappin

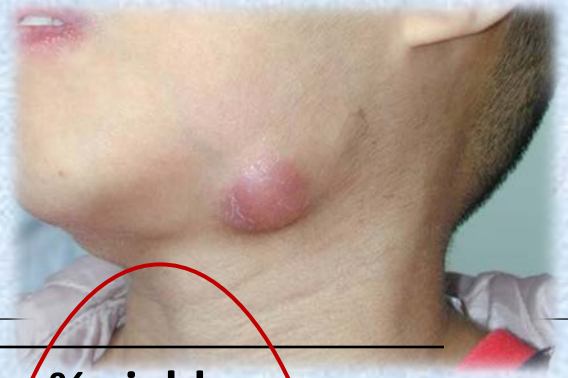
Technique preparation



Assistant to hold the child

Time and patience to obtain adequate volume

Fine needle aspiration



Study	Location	Population	% yield
Balaji 2009	India	Children	**34**% culture
Sharma 2010	India	Adults and children	**33**% (ZN, culture and PCR)
Wright 2010	South Africa	Children	**52**% culture
Cadmus 2011	Nigeria	Adults and children	**25**% culture
Van Wyk 2011	South Africa	Children	**69**% culture
Coetzee 2014	South Africa	Children	**58**% Xpert, cytology, culture

- **TB Lymphadenitis** = most common extrathoracic manifestation in children
- **FNA** = simple, feasible, minimally invasive, high yield

Slide courtesy of E. Walters

Fine needle aspiration

- No network SOP yet
- References including video

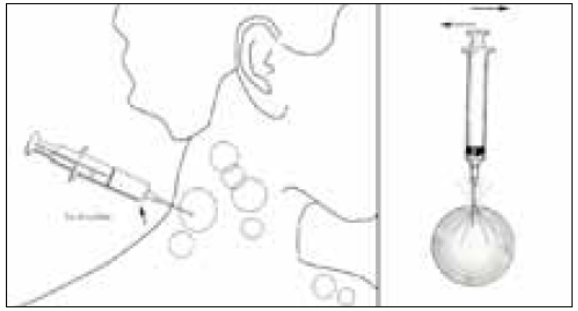


Fig. 1. Insert needle into node and aspirate with minimum of suction in a fan-like fashion, keeping needle in the lesion.

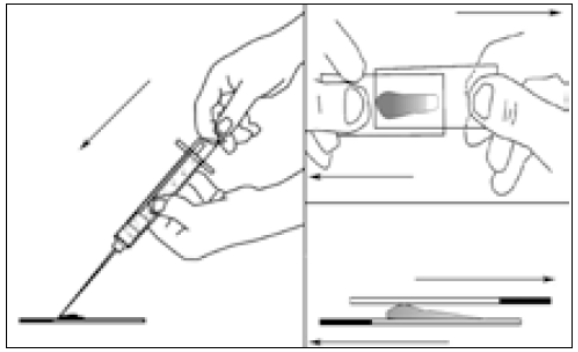


Fig. 2. Express material onto glass slide. Place second slide parallel to first, allow material to spread between slides and pull gently apart, keeping slides together at all times.

Specimens

- Study specific information: type, number, timing of specimen etc.
- For the IMPAACT TB studies
  - Sputum
  - Gastric aspirate or Induced sputum
  - FNA
  - Other specimens can be collected

Phases of laboratory testing

Analytical


BIOHAZARD
AUTHORIZED
PERSONNEL
ONLY

Tests

- Diagnostic performance characteristics
- Prevalence
- Context: diagnostic vs research
  - Work-up done in National TB programs vs TB tests done to evaluate new drugs in multi-centre study

Tests

Clinical setting

Specialist referral hospital (high prevalence)

	Disease present	Disease absent
test +	50	10
test -	5	100

Sensitivity = 50/55 = 91%
Specificity = 100/110 = 91%

Prevalence = 55/165 = 33%

PPV = 50/60 = 83%
NPV = 100/105 = 95%

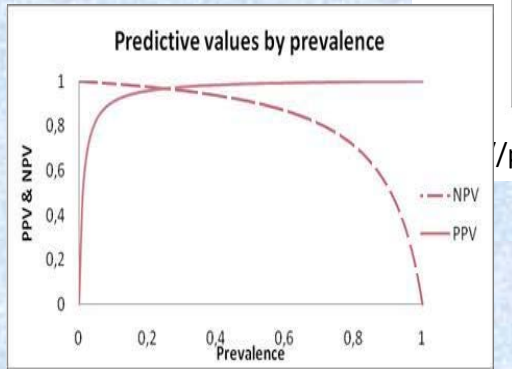
Primary care (low prevalence)

	Disease present	Disease absent
test +	50	100
test -	5	1000

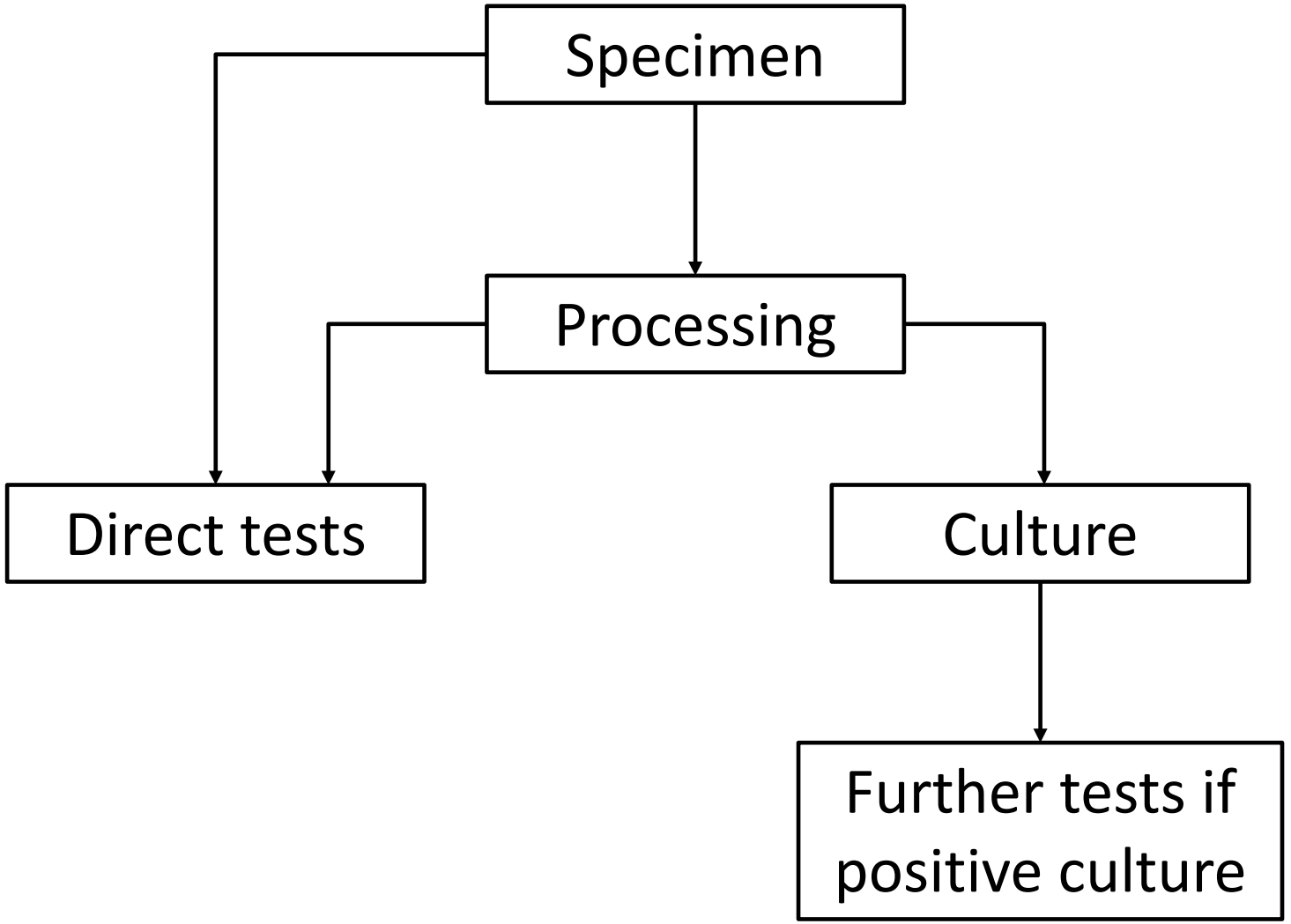
Sensitivity = 50/55 = 91%
Specificity = 1000/1100 = 91%

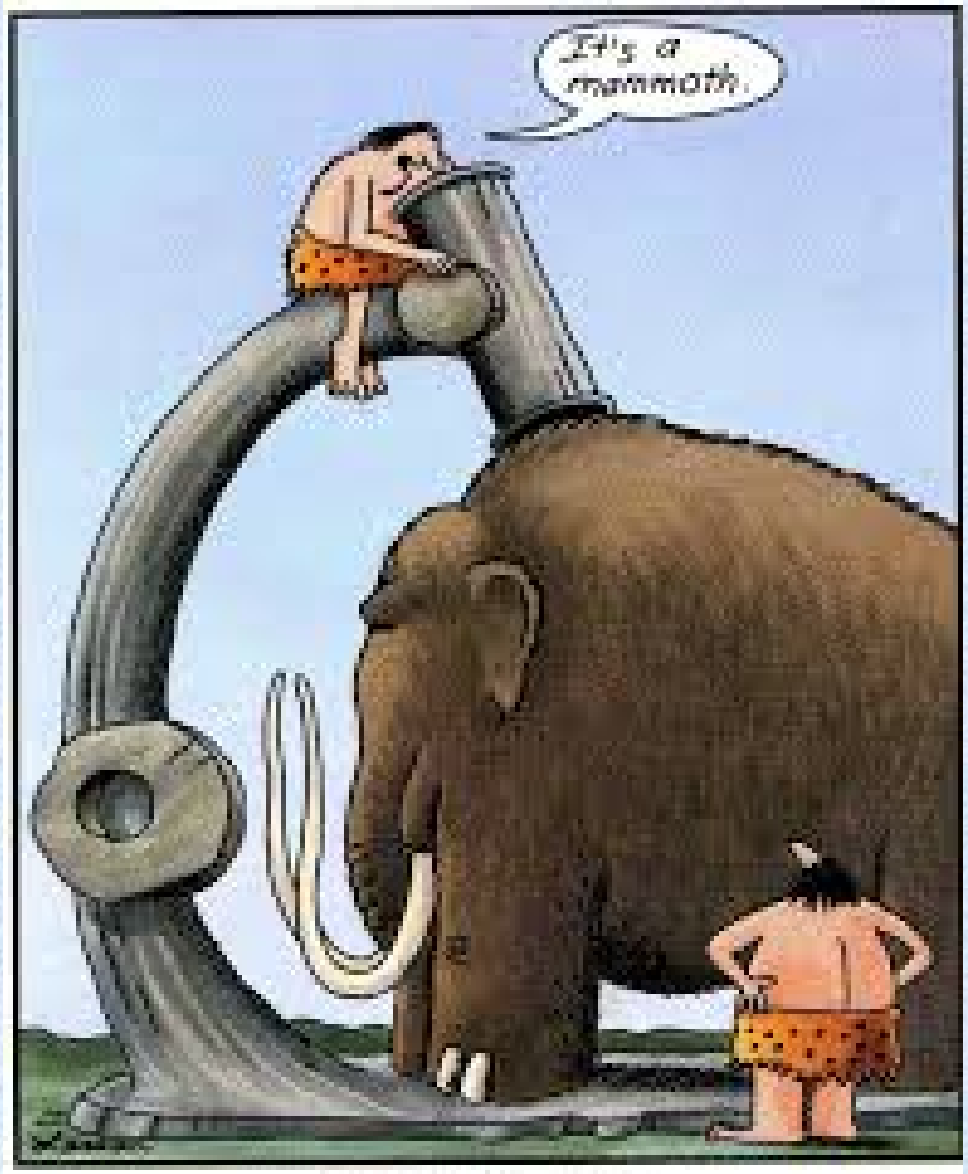
Prevalence = 55/1155 = 3%

PPV = 50/150 = 33%
NPV = 1000/1005 = 99.5%



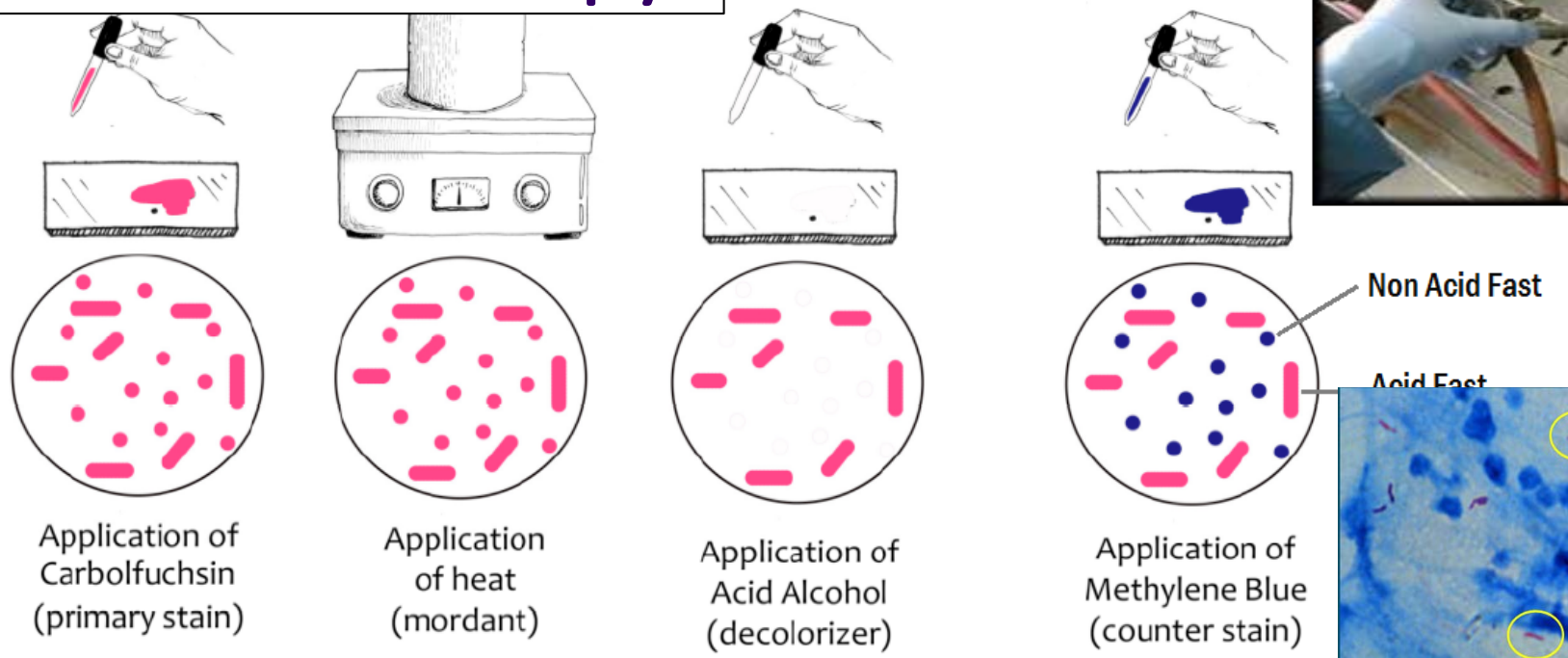
/phprimer.afmc.ca/Part2-MethodsStudyingHealth/Chapter6MethodsMeasuringHealth/Interpretingtestsonindividuals


Specimen
Processing
Direct tests
Culture
Further tests if positive culture



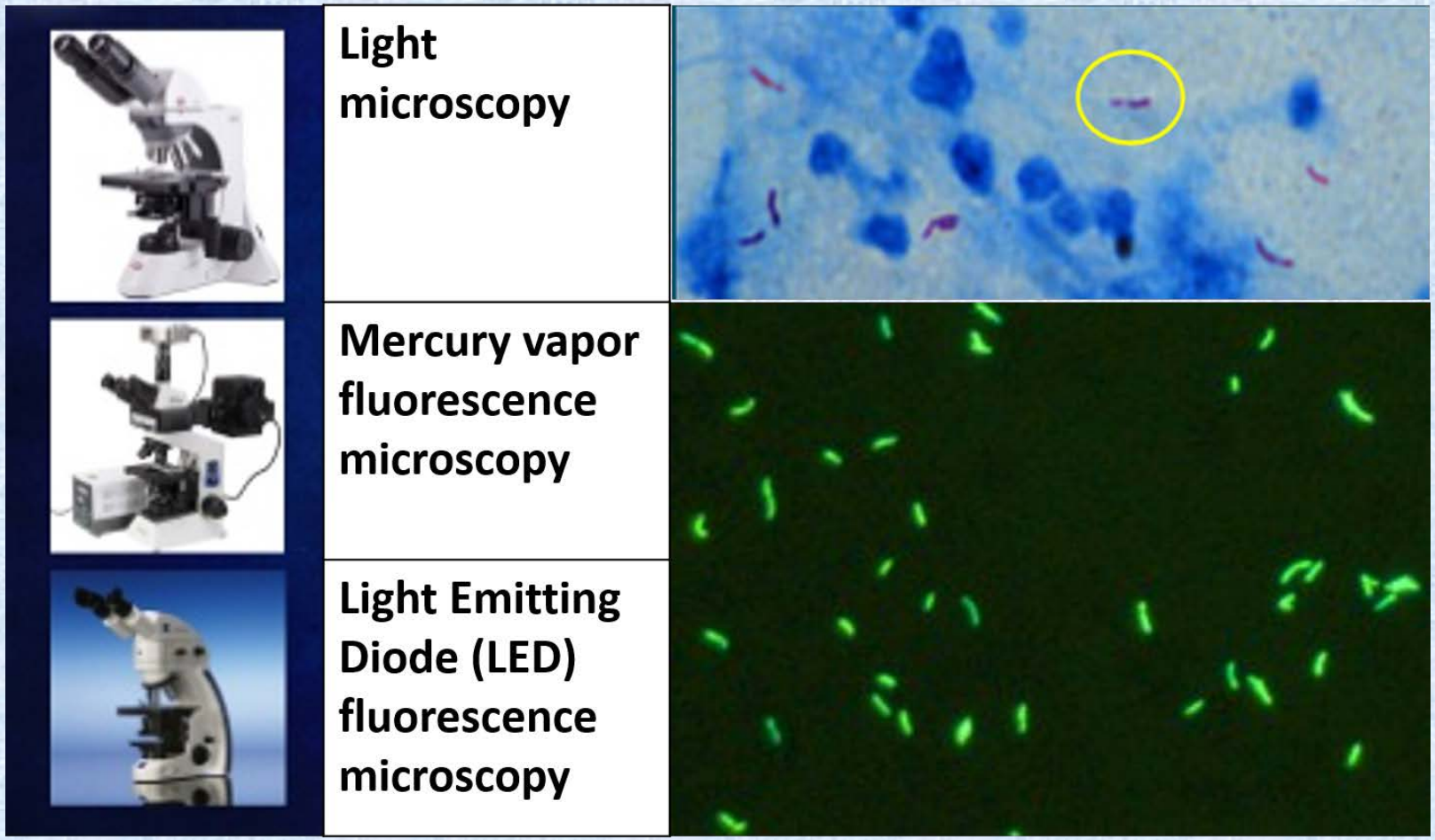
Early microscope

Smear microscopy



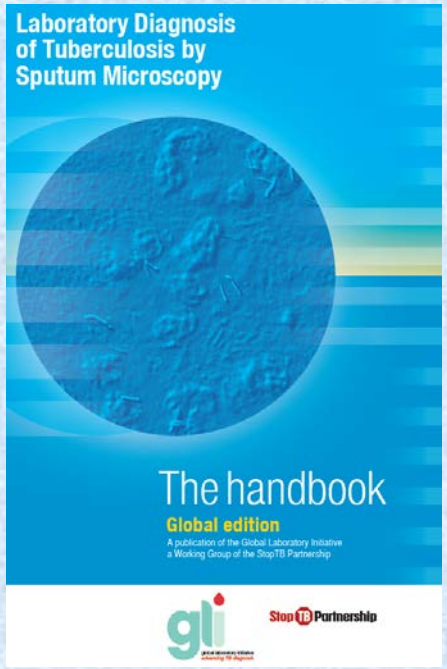
- Mycobacteria do not stain well with Gram stain
- Carbol fuschin stain: heat softens waxy lipid wall → penetration of stain (mixture of dye and phenol)
- Cooling → acid alcohol "decolouriser" poured over it
  - acid removes carbol-fuschin from normal cells
  - cold waxy coat of "acid fast" bacteria repel the decolouriser and remain stained: "acid fast" means acid cannot decolourise them

Test/Procedure	Smear microscopy
Type/Example	Carbol-fuchsin: Ziehl-Neelsen (ZN), Kinyoun Fluorochrome: Auramine, Auramine-Rhodamine
Done	• Directly on specimen: direct smear • On specimen after processing: concentrated smear • On culture if positive: ZN
Sensitivity	• High bacterial load 5,000-10,000 bacilli /mL is required for detection (culture detects 10 to 100 bacilli/ mL) • Compared to culture: Se 20-80% • ↑ 18% with processing (concentrated)* • ↑ 10% with fluorescent vs conventional microscopy*
Specificity	• All mycobacteria are acid fast • Does not provide species identification • Local prevalence of MTB and NTM determine the predictive values of a positive smear for MTB • Cording suggestive of MTBC but also seen in some NTMs
Turnaround time	• 24h
Reporting	• WHO-IUATLD, ATS *Steingart Expert Rev. Anti Infect. Ther. 2007



WHO Policy on Light Emitting Diode (LED) (2011):

- FM is 10% more sensitive and operational advantages
- Phased approach to change from brightfield to LED-based FM
- LED vs conventional FM: no dark room, less expensive lamps



Reporting How to report

Brightfield Microscopy | Method **A**

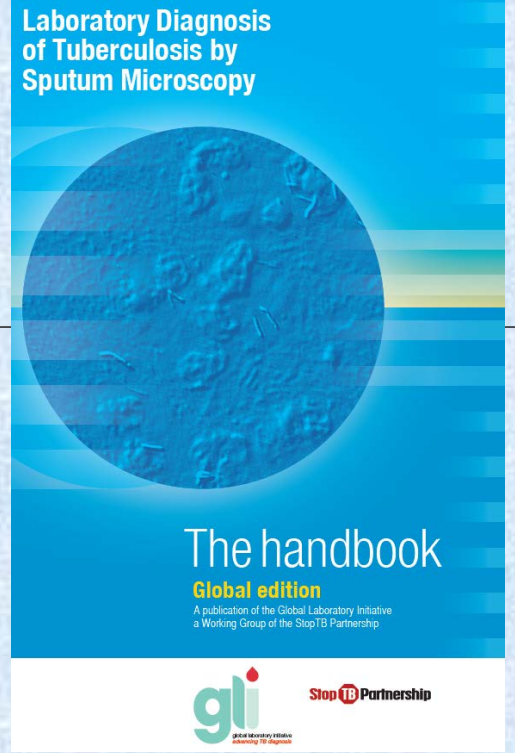
The number of AFB indicates how infectious the patient is. It is important to record exactly what you see.

1

What you see	What to report
No AFB in 100 fields	No AFB observed
1 – 9 AFB in 100 fields	Record exact number of bacilli
10 – 99 AFB in 100 fields	1+
1 – 10 AFB per field, check 50 fields	2+
More than 10 AFB per field, check 20 fields	3+

TABLE 1. IUATLD QUANTITATIVE GRADING CONVENTIONS

ZN-Smear Microscopy		
Grade		
IUATLD	ATS	AFB*
Negative	Negative†	0 AFB/100 fields
Scanty	1+	1–9 AFB/100 fields
1+	2+	1–9 AFB/10 fields
2+	3+	1–10 AFB/field
3+	4+	>10 AFB/field



Reporting How to report

Fluorescence Microscopy | Method B

What you see (200x)	What you see (400x)	What to report
No AFB in one length	No AFB in one length	No AFB observed
1-4 AFB in one length	1-2 AFB in one length	Confirmation required*
5-49 AFB in one length	3-24 AFB in one length	Scanty
3-24 AFB in one field	1-6 AFB in one field	1+
25-250 AFB in one field	7-60 AFB in one field	2+
>250 AFB in one field	>60 AFB in one field	3+

** Confirmation required by another technician or prepare another smear, stain and read*

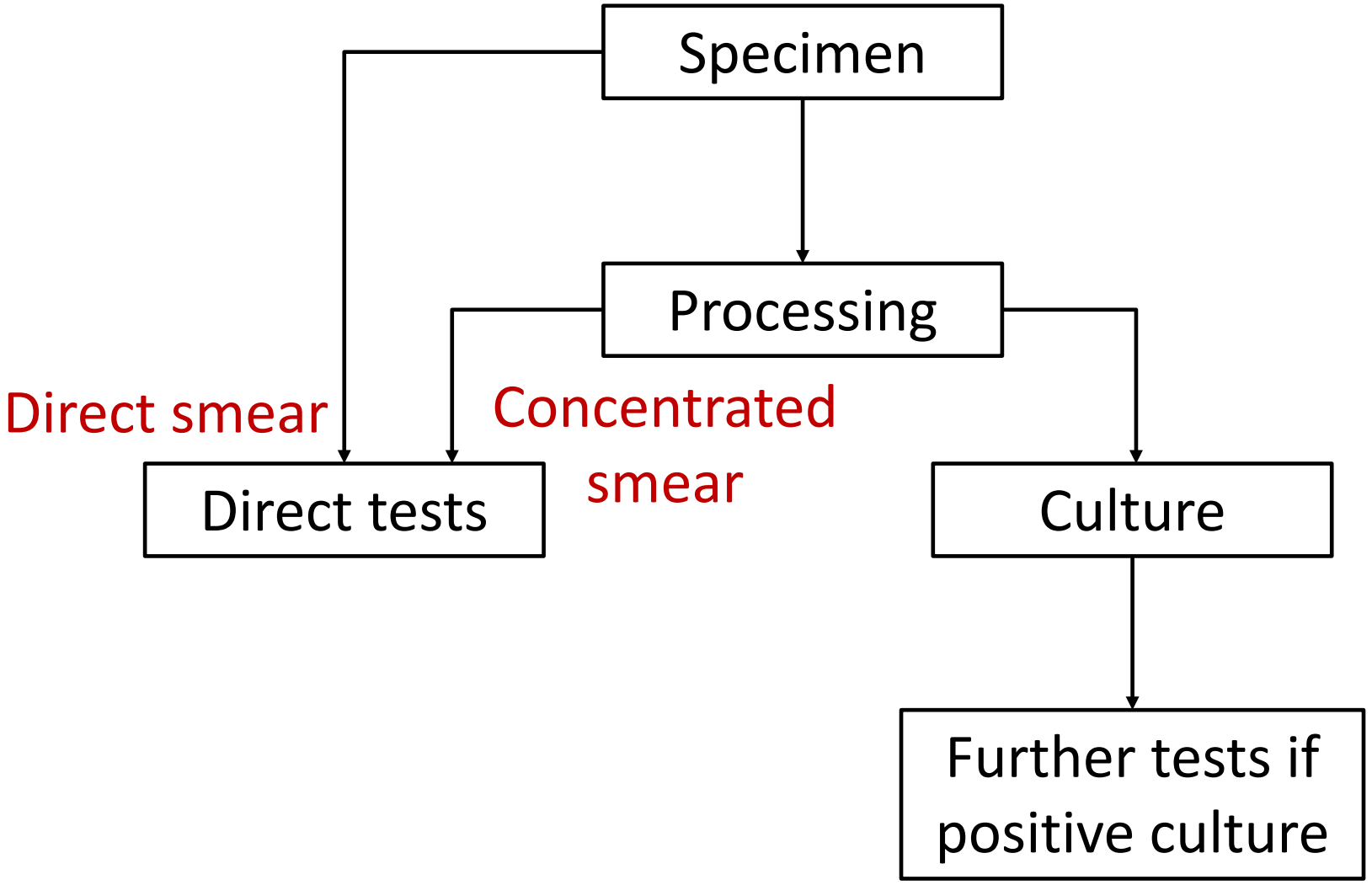
Reporting

Due to an historical inaccuracy, the FM reporting scale for positive smears has been revised because the actual field observed is larger than previously calculated.

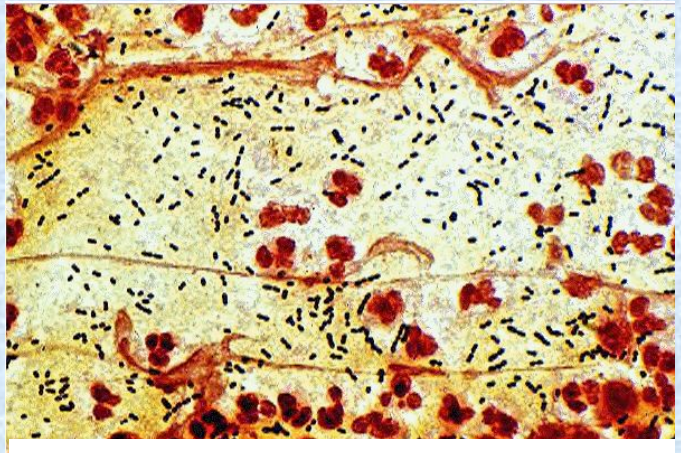
Low scanty positives, 1-4 AFB in one length at 200x magnification, or 1-2 in one length at 400x magnification should be confirmed by:

- **viewing additional fields**
- **having another technician check the AFB morphology or**
- **collecting another sputum sample**

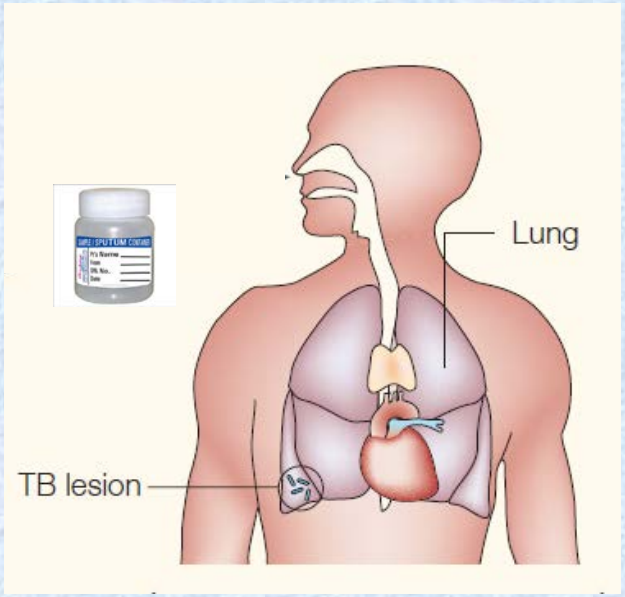
Confirmation of FM low-positive smears by re-staining with ZN should not be done.

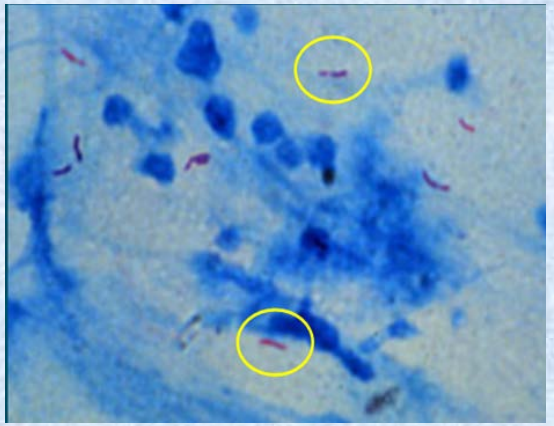

Specimen
Processing
Direct smear
Concentrated smear
Direct tests
Culture
Further tests if positive culture
Ziehl Neelsen on positive culture

Processing

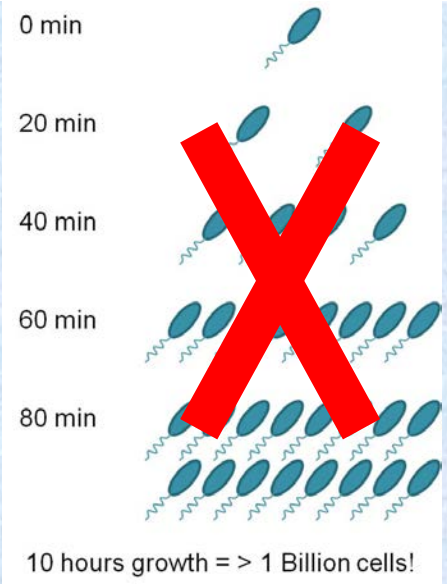


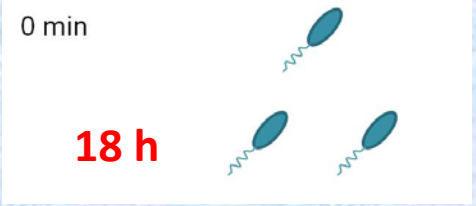
Normal respiratory flora



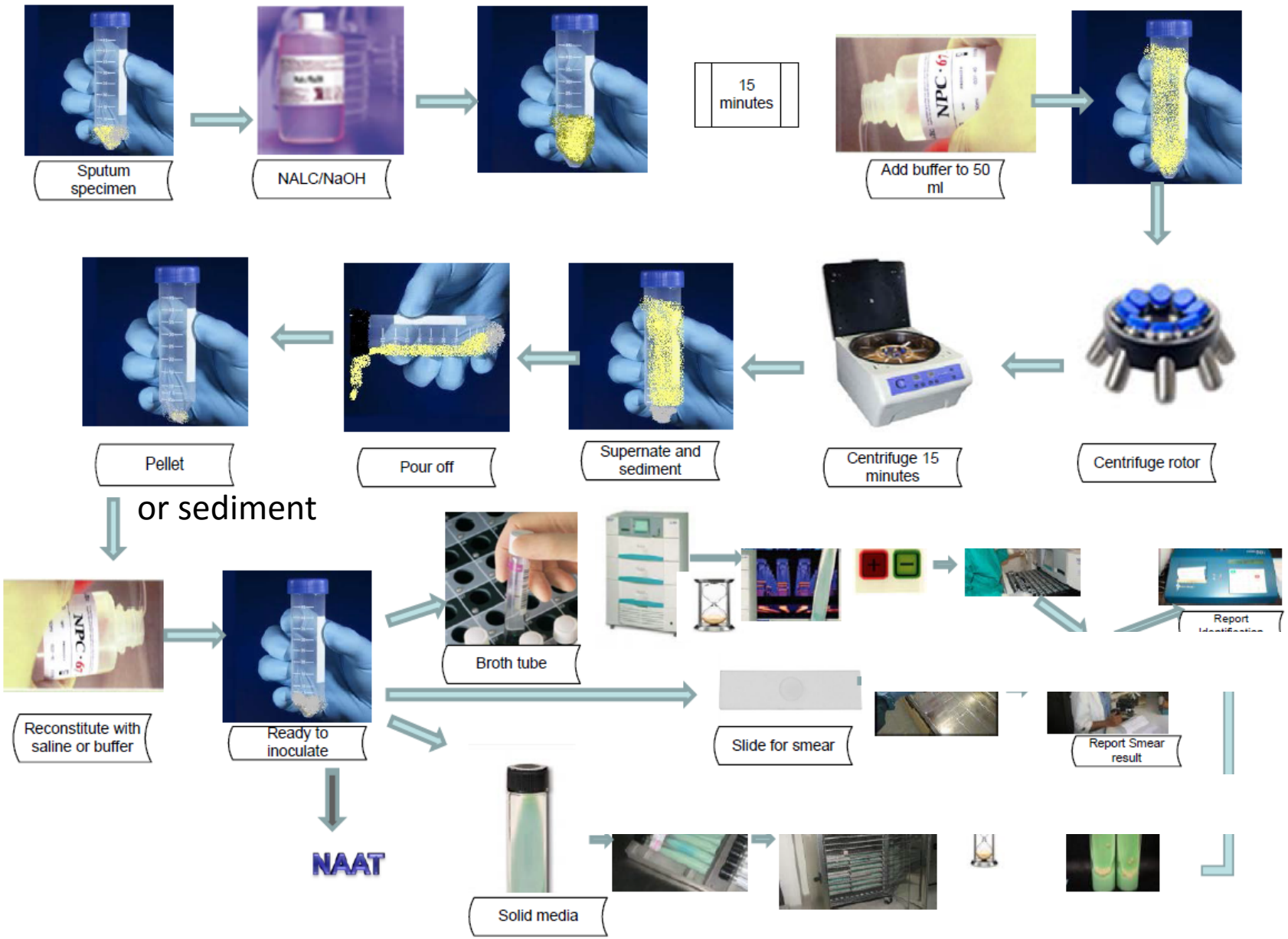


M.tuberculosis

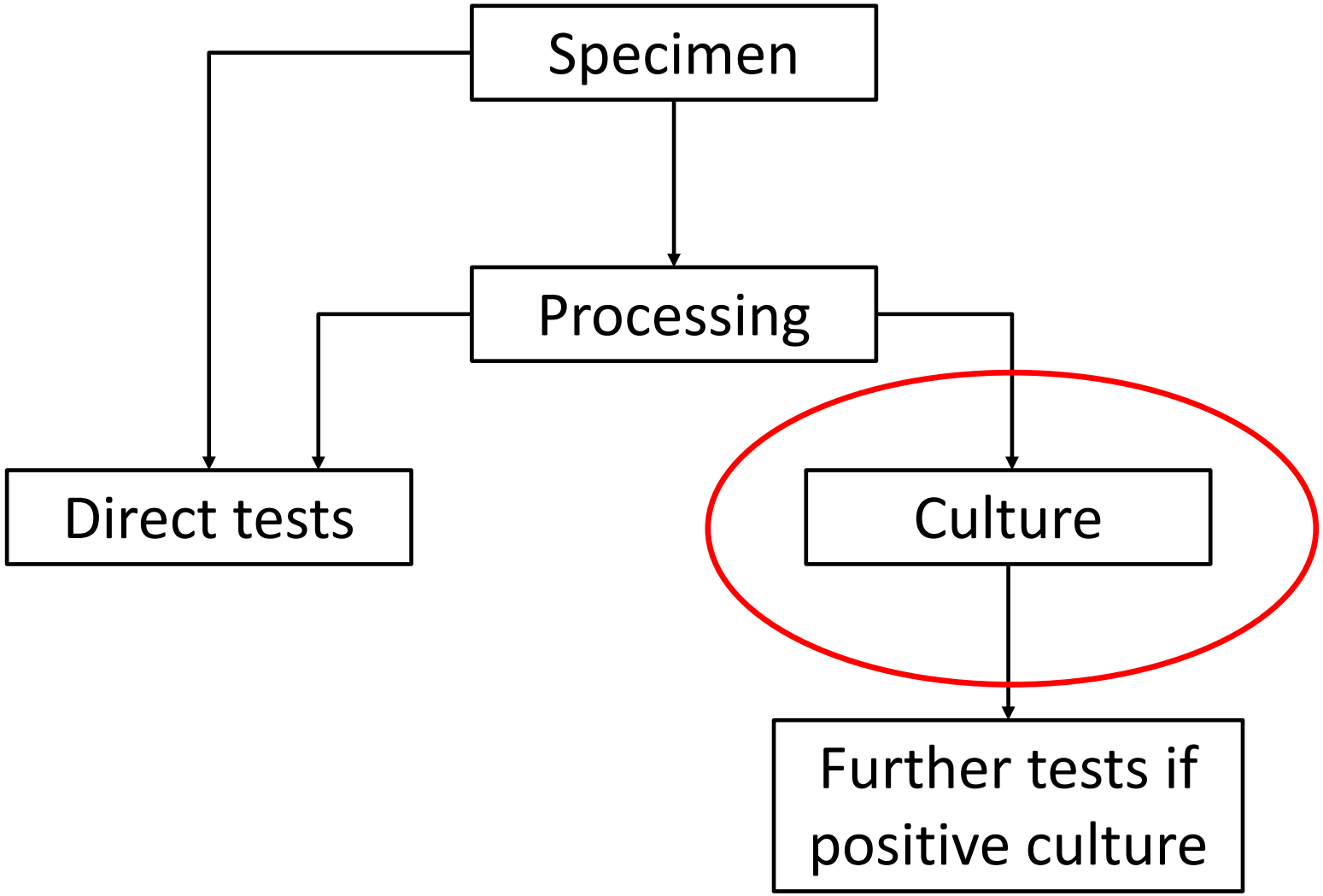




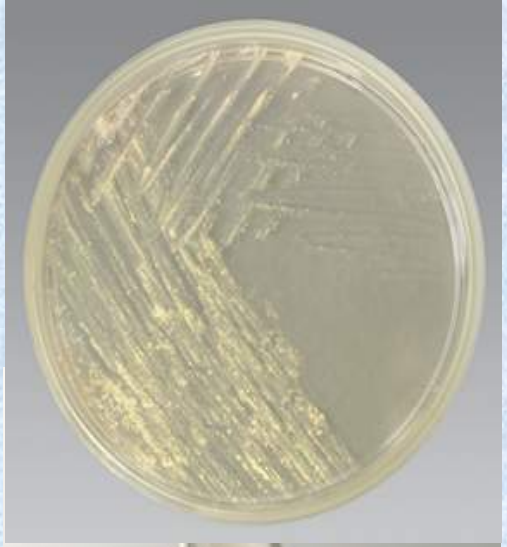
Test/Procedure	Processing (Decontamination-Digestion)
Type/Example	• NALC (N-acetyl L-cysteine)-NaOH (sodium hydroxide) • Oxalic Acid
Done	Directly on specimens from non-sterile sites
Principle	Decontamination (NaOH): • To eliminate contaminants as much as possible without affecting the viability of mycobacteria Digestion (NALC): • To release mycobacteria trapped in specimen mucus • To improve the decontamination process • To facilitate concentration of the specimen
Limitations	• Delicate procedure: if it is too harsh, the yield is affected, as mycobacteria are also killed; if too mild, specimens will be overgrown by other bacteria. Many manual steps. • Optimal method for specimens other than sputum? • Risk of *cross-contamination*
Result	N/A. Sediment or pellet obtained: used for smear, nucleic acid amplification test (NAAT) or culture
Quality indicator	*Contamination* rate: cultures overgrown by bacteria. Target: 3-5% (solid) and 8-10% (liquid)



Courtesy Frances Tyrrell, CDC


Specimen
Processing
Direct tests
Culture
Further tests if positive culture

Lowenstein-Jensen (LJ)



Middlebrook 7H10 or 7H11 agar (plate, tube)

Middlebrook 7H9 broth

MGIT tube:
Middlebrook 7H9 broth + <u>OADC enrichment</u>: oleic acid, albumin, dextrose, catalase + <u>PANTA antibiotic mixture</u> (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin)

MODS = Microscopic Observation Drug Susceptibility (Middlebrook 7H9 broth)

- Nutrients: egg, potato, etc.
- LJ: Malachite green to inhibit bacteria
- Antibiotics can be added

Thin Layer Agar (TLA): Microscopic on agar

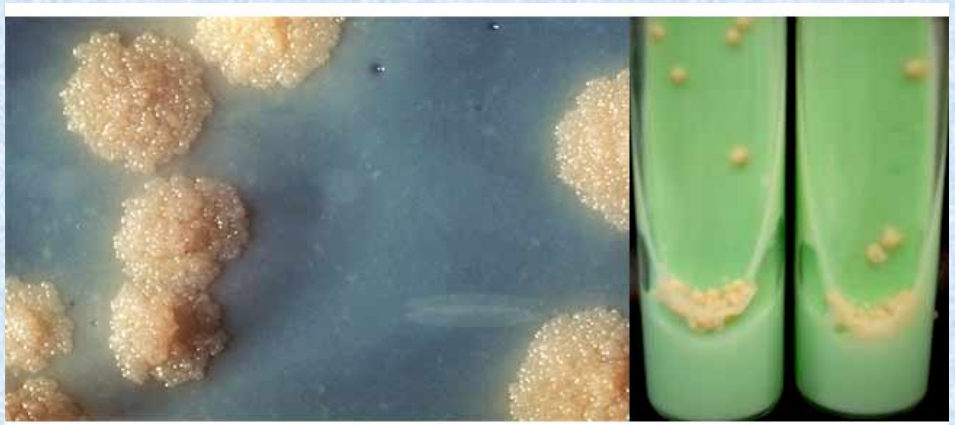
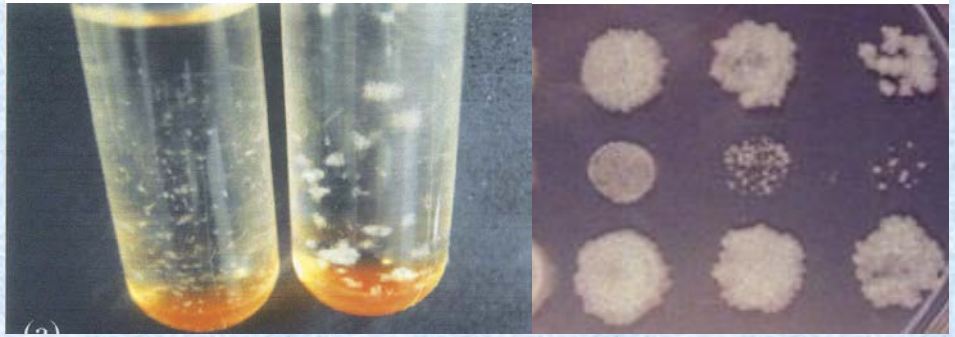
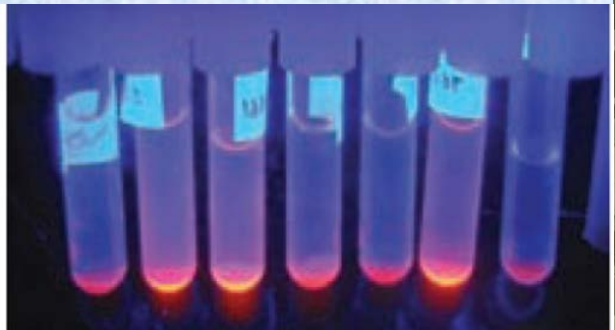


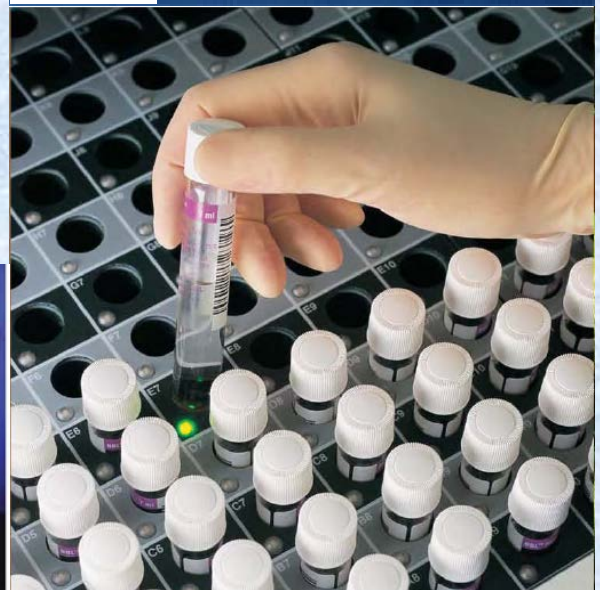
Fig: Cultural Characteristics of *Mycobacterium tuberculosis*



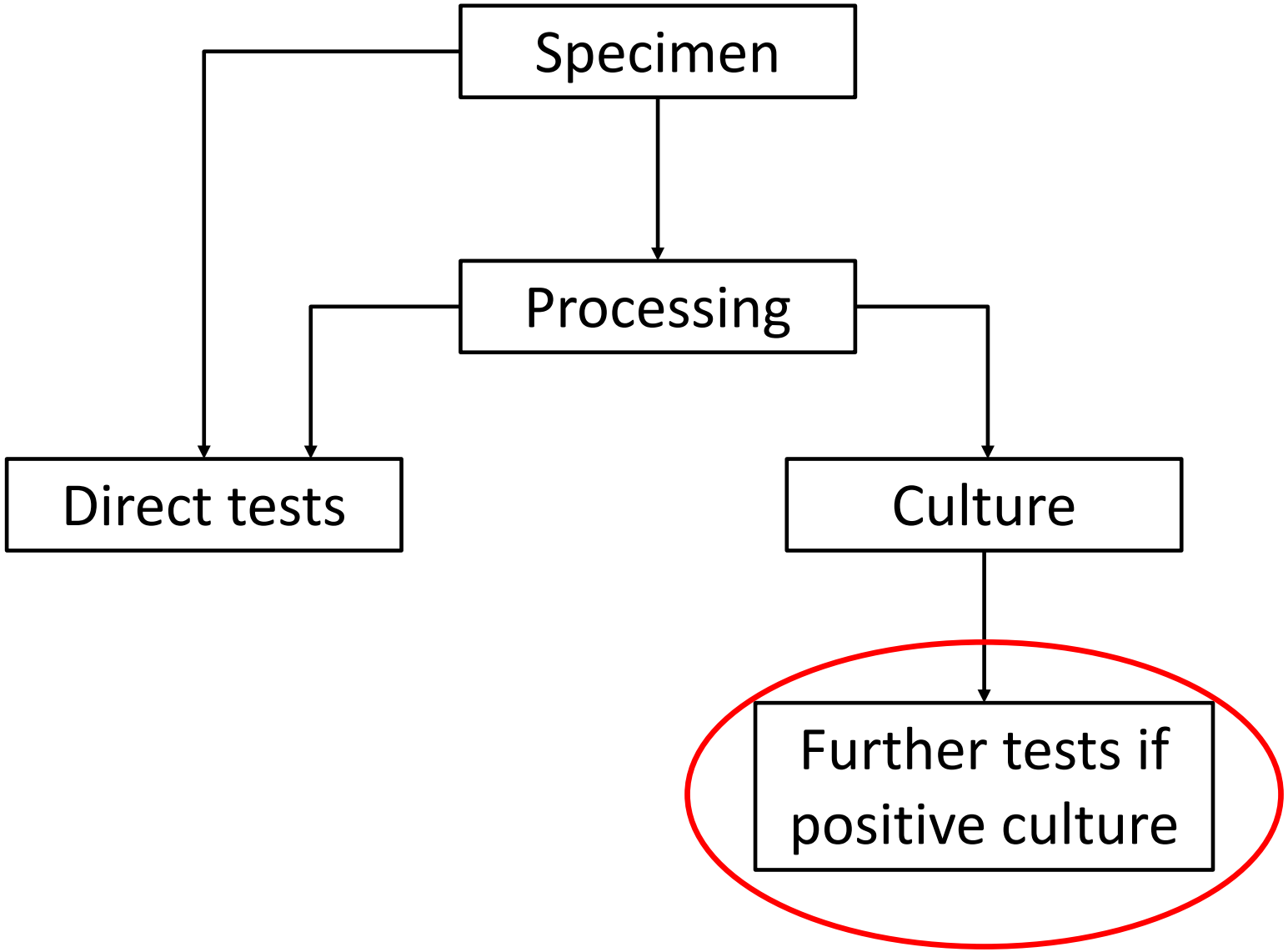
MGIT

Mycobacterial Growth Indicator Tube



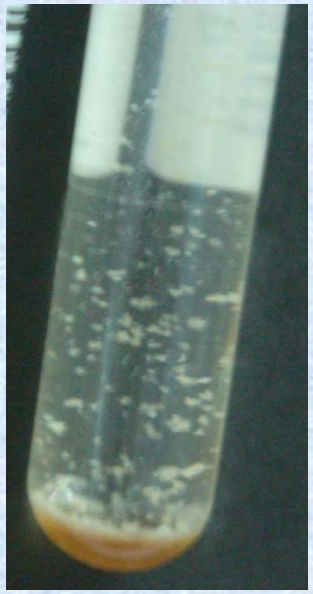


Test/Procedure	Culture
Type/Example	• Solid vs Liquid: Solid egg based: Lowenstein-Jensen (LJ), Ogawa Solid agar based: Middlebrook 7H10, 7H11 Liquid: Middlebrook 7H9 • Manual vs Automated (ex: MGIT 960,VersaTREK,MB/BacT ALERT) • Non-selective or selective (ex: 7H11S)
Done	• On processed sample for non-sterile sites • Directly on specimens for sterile sites
Sensitivity	• Considered gold standard (liquid and solid) • Detects 10 (liquid) to 100 (solid) TB bacilli / mL
Specificity	• Depends on identification method
Turn around time	• 8-10 days for smear-positive samples • 2–6 weeks for smear-negative samples • MGIT negative after 6 weeks (42 days). Solid often kept longer.
Limitations	• MGIT susceptible to contamination • Cost, expertise, biosafety, delays
Reporting	• Qualitative: positive/negative for MTBC, etc. • Quantitative: Solid: #colonies Liquid: time to detection (TTD)


Specimen
Processing
Direct tests
Culture
Further tests if positive culture

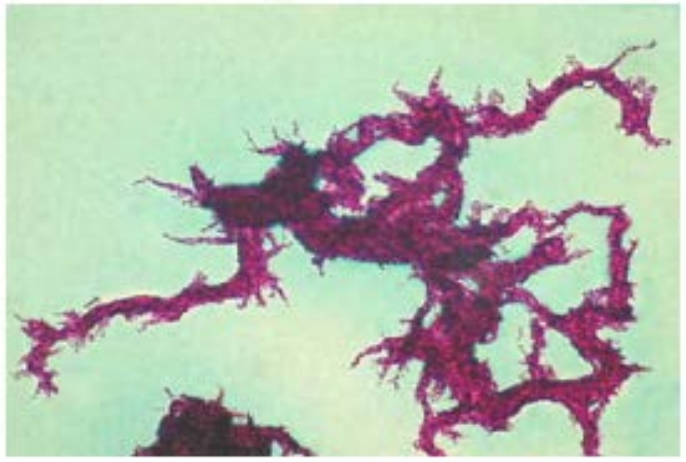
Identification of *M. tuberculosis* from liquid culture

Automated system but manual steps for identification



Identification test

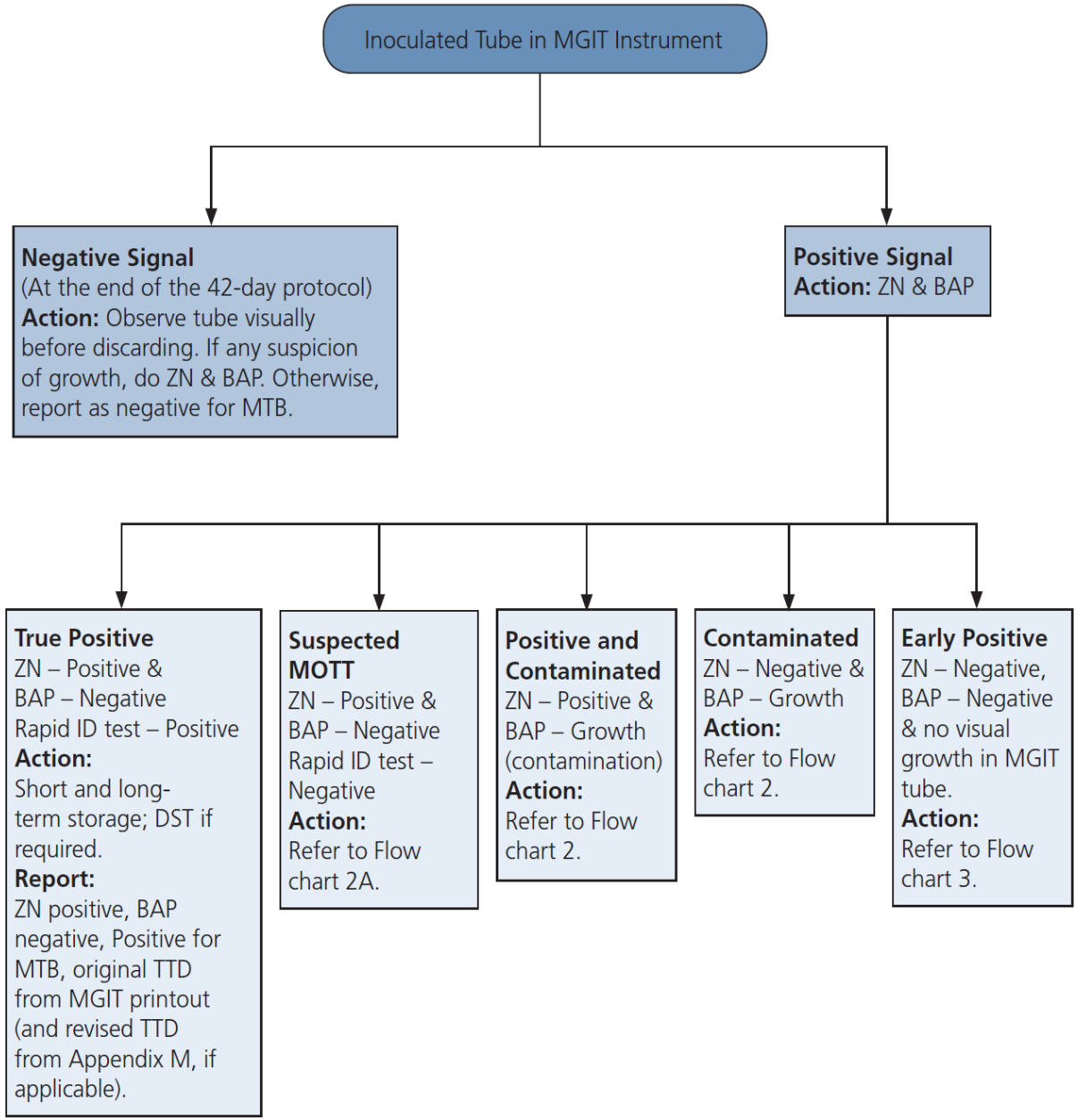
Ziehl-Neelsen



ZN: serpentine cords of varying length or district linear clumping

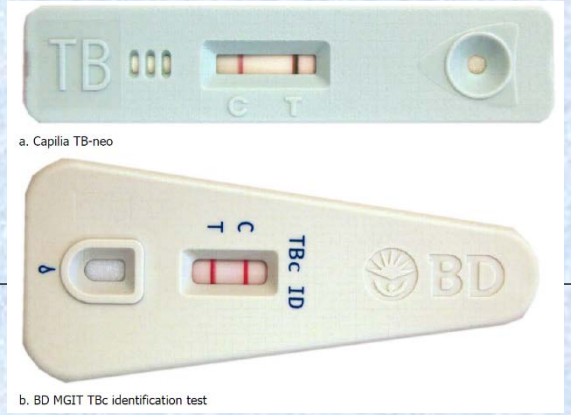
Blood agar plate

Flow Chart 1: General Algorithm MGIT 960 Cultures



http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf

Identification methods



a. Capilia TB-neo

b. BD MGIT TBc identification test

- Biochemical tests
  - Growth rate, morphology, pigmentation, combination of biochemical tests (nitrate, NAP, etc) → abandoned
  - No growth on medium with p-nitrobenzoic acid (PNB)
- High performance liquid chromatography (HPLC)
- Molecular methods
  - DNA probes (Accuprobe MTBC)
  - MPT64 antigen tests (lateral flow assays)
  - Nucleic acid amplification tests (NAAT)
    - Hain MTBDRplus done on positive culture
- Other methods

Test/Procedure	MPT64 antigen test (Immunochromatographic tests, lateral flow assay tests)
Type/Example	– Capilia TB-Neo [Tauns Laboratories;Japan] – SD Bioline's TB Ag MPT64 Rapid Test [South Korea] – Becton Dickinson's TBcID[Maryland, USA]
Principle	Detection of presence of the MTBC-specific protein MPT64 in culture isolates.
Done	• On culture if positive (solid or liquid). Also works on contaminated cultures
Sensitivity	• Detection limit ~ 10^5 CFU/ml: done on positive culture • High Se (92.4%–99.2%)
Specificity	• High specificity (99-100%) • Doesn't differentiate members of the MTBC • Some substrains of M. bovis BCG lack MPT64 and will be negative • Strains of microbes, such as S. aureus, that produce protein A may produce a false positive result
Turn around time	15 minutes
Reporting	N/A. Part of the identification of positive cultures

Identification of mycobacteria

- Usually, in the context of TB drug trials:
  - MTBC or not
  - NTM may or may not be identified to species level
- Differentiation within MTBC
  - Not differentiated by Xpert MTB/RIF, Hain MTBDRplus
  - Specialised testing
    - LPA for MTBC differentiation, PCR Region of Differences
  - Could be useful in the context of BCG studies

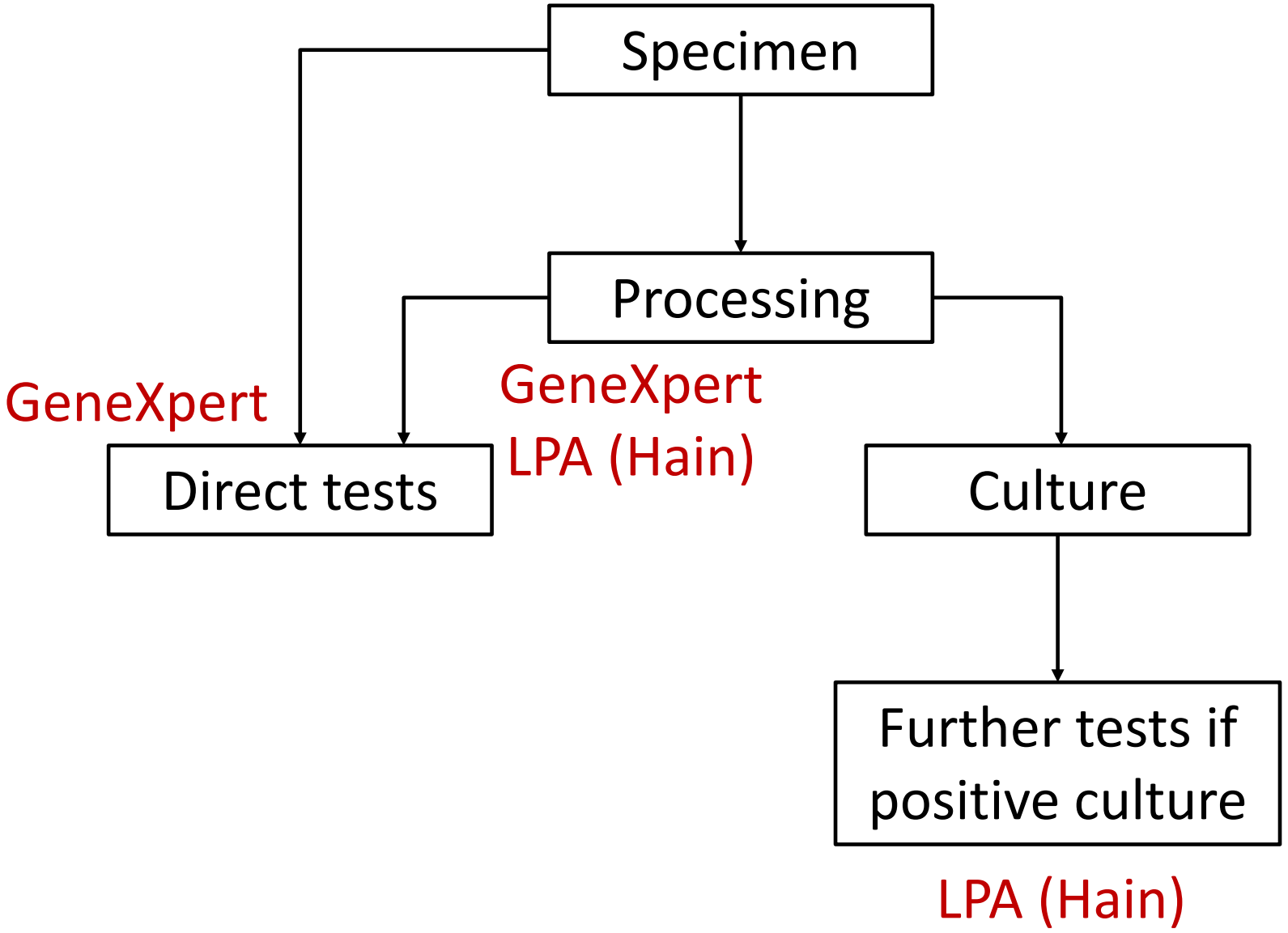
Nucleic acid amplification tests (NAAT)

- Many commercially available
- Most used TB high burden countries:
  - GeneXpert
  - Line probe assays (LPA) e.g. Hain MTBDRplus
- Detection of MTB
- Detection of drug resistance

Drug susceptibility testing (DST)

- Genotypic (molecular) DST
- Based on detecting the presence of wild-type sequences or mutations in genes known to be associated with antibiotic susceptibility or resistance.

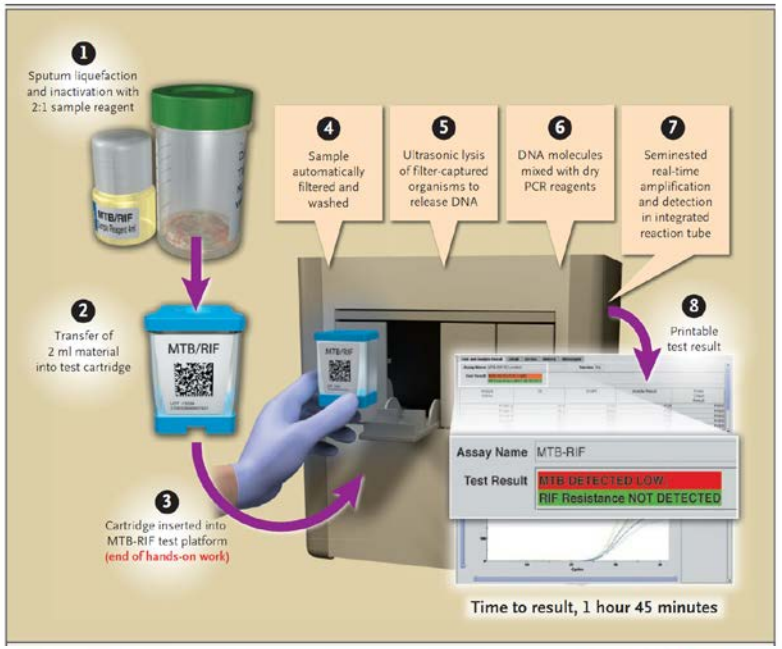
- Phenotypic (culture based) DST
- Based on whether or not the organism can grow in the presence of the antibiotic
- Currently regarded as the gold standard
- Slow


Specimen
Processing
GeneXpert
GeneXpert
LPA (Hain)
Direct tests
Culture
Further tests if positive culture
LPA (Hain)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance



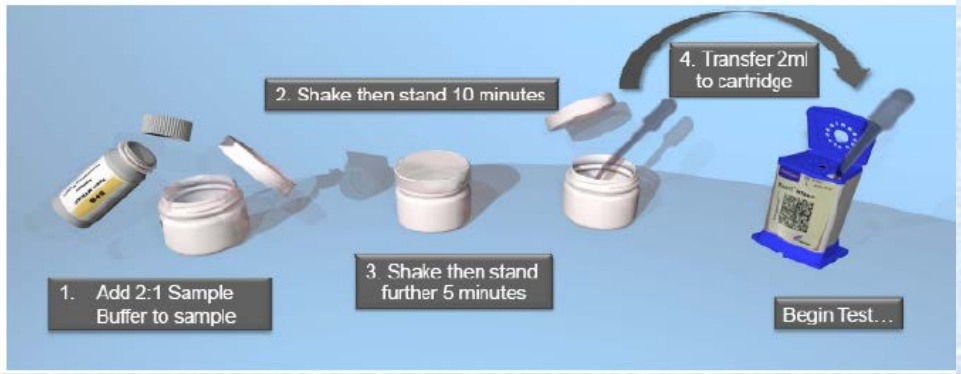


Figure 2. GeneXpert instruments with 1, 2, 4 and 16 modules

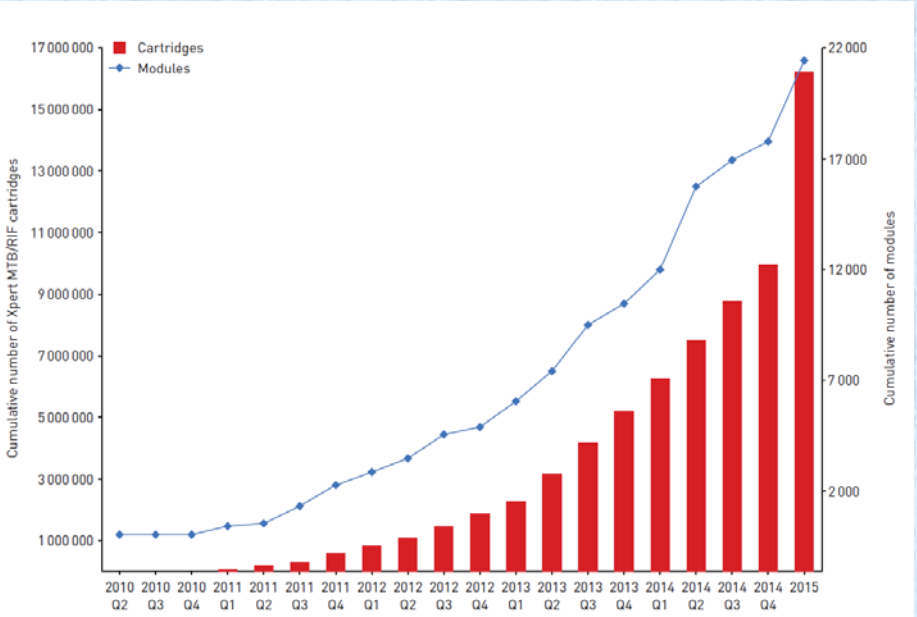
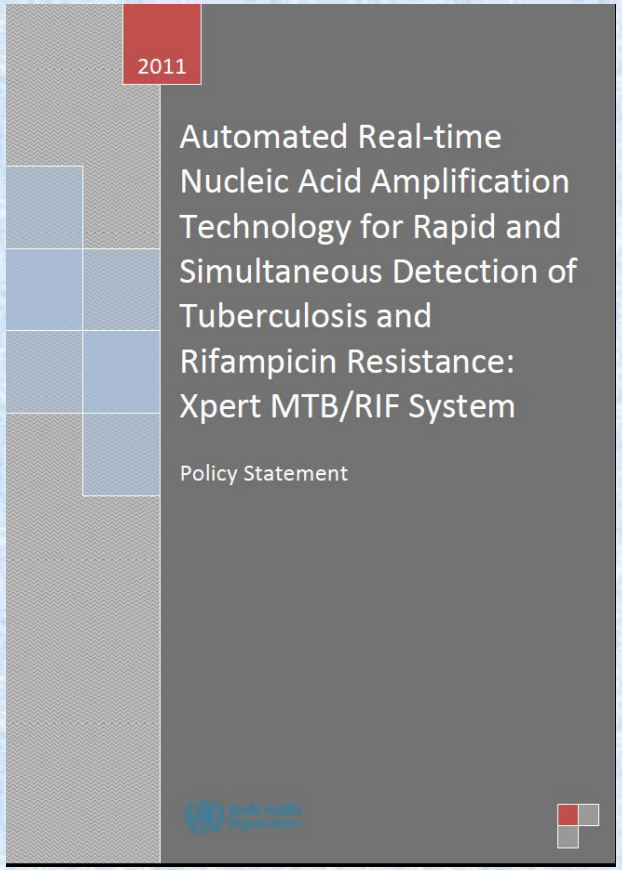
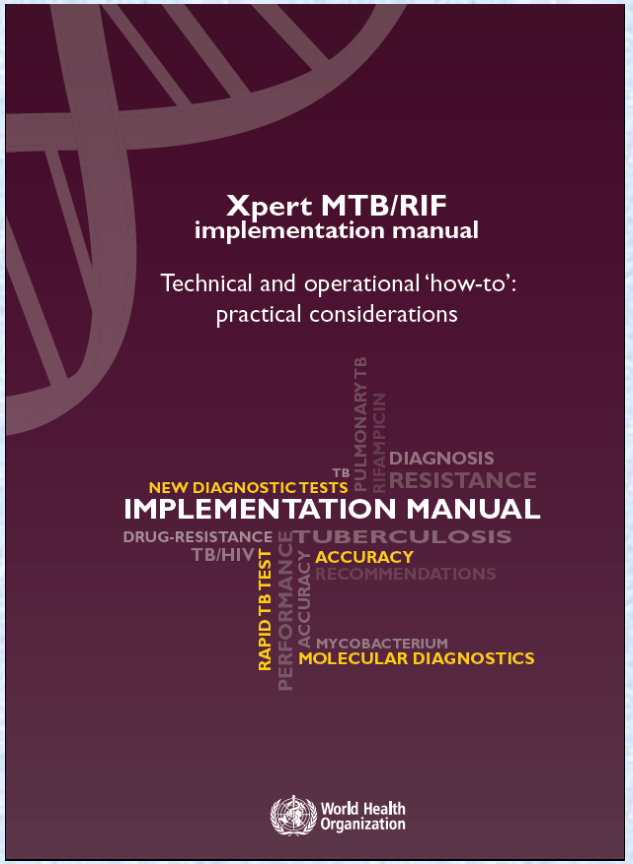


FIGURE 2 Procurement of Xpert MTB/RIF modules and cartridges under concessionary pricing by quarter (Q) in 2010–2015 (Cepheid data).

Eur Respir J 2016;





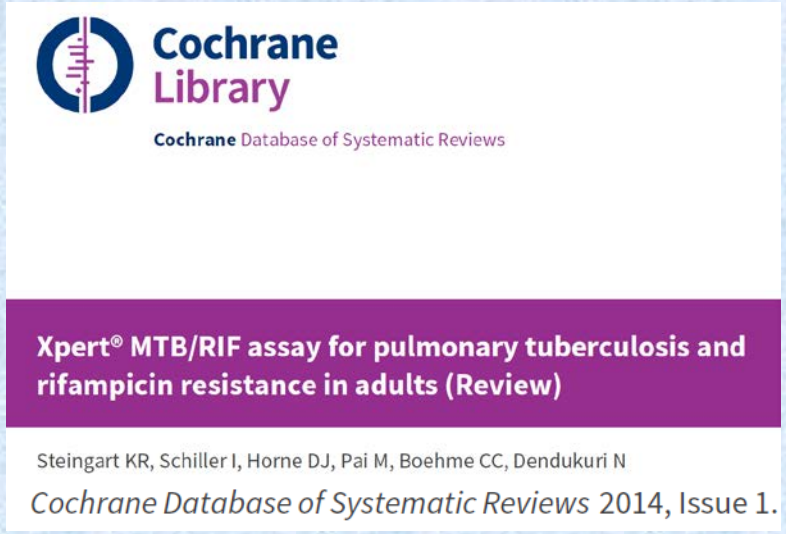
http://www.who.int/tb/publications/diagnosis/en/

Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis

Anne K Detjen, Andrew R DiNardo, Jacinta Leyden, Karen R Steingart, Dick Menzies, Ian Schiller, Nandini Dendukuri, Anna M Mandalakas

Lancet Respir Med 2015; 3: 451–61

Test/Procedure	Nucleic acid amplification test
Type/Example	GeneXpert MTB/RIF (Cepheid)
Principle	Cartridge-based assay that integrates sample preparation, amplification, and detection of DNA. Real time PCR using molecular beacons (5 probes). Designed to identify RIF resistance mutations in an 81-bp region of rpoB gene: "Rifampicin-resistance-determining region" (RRDR)
Done	• On direct specimen or processed specimen (sediment)
Sensitivity Specificity	• Cochrane adults 2014: For TB detection as smear replacement: Se 89% Sp 99% As add-on test after negative smear: Se 67% Sp 99% For RIF R detection: Se 95% Sp 98% • Lancet Resp Med 2015 SR MA children: Compared to culture: Se 62% sputum 66% GL; Sp 98% For RIF R detection: Se 86% Sp 98%
Limitations	Initial high Se and Sp for detecting TB and DR, but afterwards concerns for false pos R→ other test to confirm DR (LPA, phenotypic). Also false negatives
Turn around time	2h



For RIF R detection: Se 95% Sp 98%
If pooled accuracy are applied to a hypothetical cohort of 1000 individuals where:

15% of those with symptoms are rifampicin resistant: Xpert®MTB/RIF would
correctly identify 143 individuals as RIF R and miss 8 cases
correctly identify 833 individuals as RIF S and misclassify 17 individuals as R

5% of those with symptoms are rifampicin resistant, Xpert® MTB/RIF would
correctly identify 48 individuals as RIF R and miss 3 cases
correctly identify 931 individuals as RIF S and misclassify 19 individuals as R

Xpert Ultra

- Improved limit of detection
  - 16 CFU/ml vs 114 CFU/ml Xpert MTB/RIF
  - Culture ~10 - 100
  - Smear microscopy ~10 000
- An additional semi-quantitative category (‘trace’) to take into account the higher sensitivity
  - Lowest bacillary burden for TB detection
  - Updated categories: high, medium, low, very low and trace

Ultra

- Ultra vs Xpert in 1520 adults
  - Se Ultra 5% higher than Xpert
    - Highest in smear negative patients and HIV infected
  - Sp Ultra 3.2% lower than Xpert
    - Lowest in patients with history of TB. Detects non-viable bacilli. Problem in high endemic setting.
    - The impact of increased sensitivity results in decreased specificity for TB detection…and becomes a trade-off between increased diagnosis and overtreatment
  - As good accuracy for RIF detection

http://www.finddx.org/wp-content/uploads/2017/03/Ultra-WHO-report_24MAR2017_FINAL.pdf

Ultra

- For EPTB and paediatric TB: ↑ Se due to trace-call
  - Se Ultra 95% vs 45% Xpert in TBM
  - Se Ultra 71% vs 47% Xpert resp specimens children
  - Positive Xpert Negative Culture


REPORT FOR WHO

A multicentre non-inferiority diagnostic accuracy study of the Ultra assay compared to the Xpert MTB/RIF assay

Version 1.8 / February 2017

FIND
Because diagnosis matters

- The interpretation of Ultra results for MTB detection are the same as for Xpert MTB/RIF with the exception of “trace calls”.
- Ultra has high sensitivity for MTB detection and incorporates a new semi-quantitative category “trace call” that corresponds to the lowest bacillary burden for MTB detection. Interpret “trace calls” as follows:
    - Among persons with HIV, children and extrapulmonary specimens “trace calls” should be considered to be true positive results for use in clinical decisions and patient follow-up;

- Ultra has both high sensitivity and specificity for rifampicin resistance detection.

http://who.int/tb/publications/2017/XpertUltra/en/

- All persons with rifampicin resistance, identified by Ultra should undergo further testing as per current WHO policy guidance to determine if there is additional resistance to the class of fluoroquinolones and/or the group of second-line injectable drugs.
- Ultra can be used on all GeneXpert instrument platforms and is suitable for use at central or national reference laboratory level, regional and district levels. GeneXpert has the potential to be

Population-level projection using TB prevalence of 20%

OUTCOME	NUMBER OF RESULTS PER 1,000 INDIVIDUALS TESTED (200 WITH TB, 800 WITHOUT TB)		
	XPERT MTB/RIF SENS = 83% SPEC = 98%	ULTRA SENS = 88% SPEC = 95%	ULTRA WITHOUT TRACE[b] SENS=85% SPEC=97%
True positives (TPs) (individuals with TB)	166	176	170
False negatives (FNs) (individuals incorrectly classified as not having TB)	34	24	30
False positives (FPs) (individuals incorrectly classified as having TB)	16	42	24
True negatives (TNs) (individuals without TB)	784	758	776
FPs per 10 TPs	1.0	2.4	1.4
Incremental FP/TP ratio[a]	—	2.6	1.8

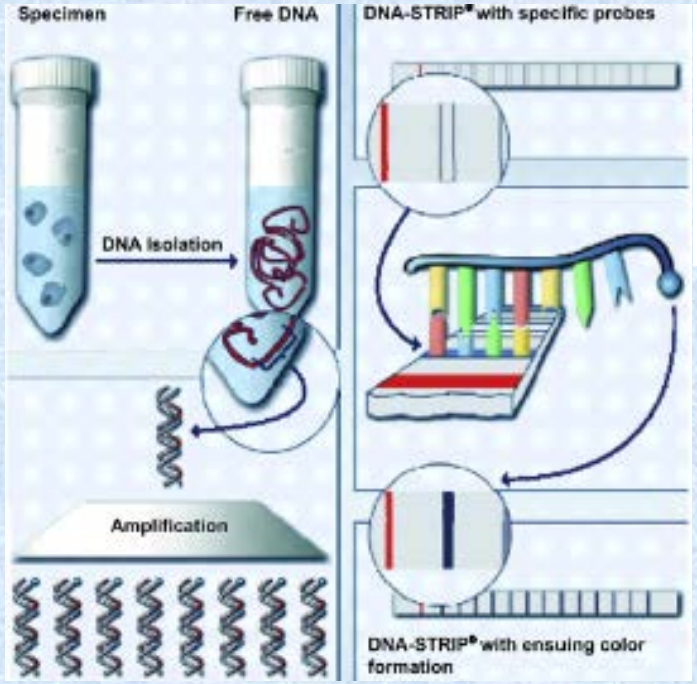
Note: Accuracy estimates are based on a 30% proportion of smear-/culture+ among TB cases, and a 21% proportion of having a prior TB episode (as in FIND study)

[a] Computed as (# Ultra FPs - # Xpert FPs)/(# Ultra TPs - # Xpert TPs). Can be interpreted as "How many additional FPs do I get per additional TP detected with Ultra **over and above** Xpert MTB/RIF?"

[b] For the Ultra without trace analysis, 'MTB detected trace' results were considered as negative results

http://www.stoptb.org/wg/gli/assets/documents/GLI_ultra.pdf

Test/Procedure	Nucleic acid amplification test: Line Probe Assays
Type/Example	• Hain MTBDRplus to detect MDR-TB by detecting mutations in rpoB gene (RIF) and katG and inhA (INH) • Hain MTBDRsl to detect XDR-TB by detecting mutations in genes for susceptibility to FQs (ofloxacin, moxi, levo) and second line injectable drugs (SLIDs; amikacin, kanamycin,and capreomycin)
Principle	Hybridization of labeled amplicons (amplified by PCR from M. tuberculosis DNA present in patient specimens) to oligonucleotide probes arranged on a membrane strip
Done	• On processed specimen (sediment) from smear positive or negative patient OR on positive culture
Sensitivity Specificity	See next slides
Limitations	• Open tube format: possible cross contamination • Requires appropriate laboratory infrastructure and equipment • Reading strips: possible subjectivity • LPAs are less sensitive for the detection of isoniazid resistance
Turn around time	24-48h



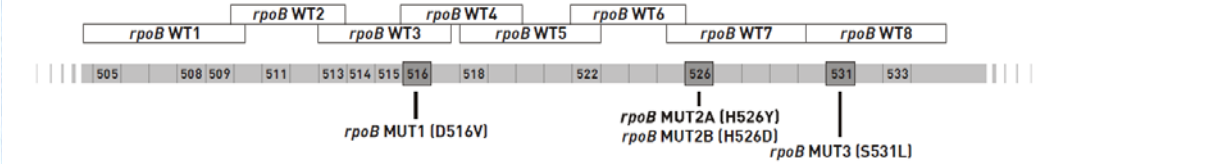
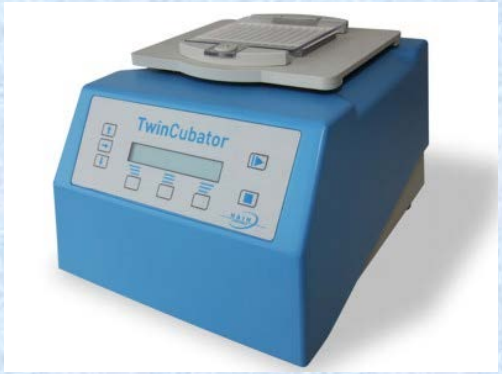
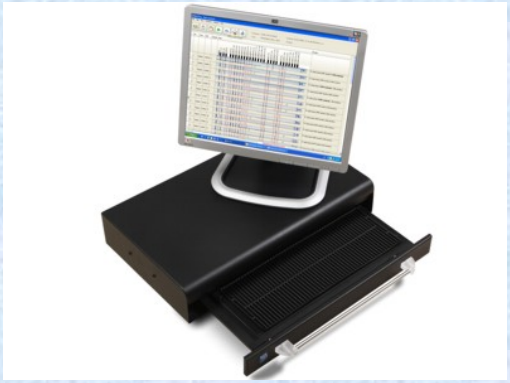
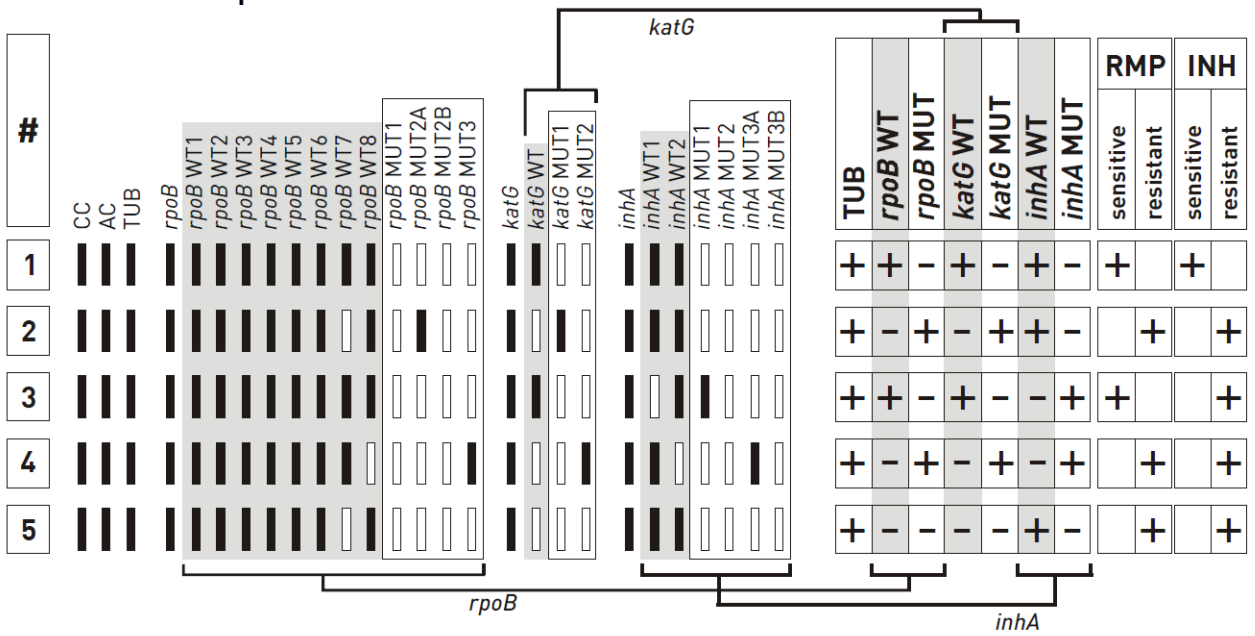


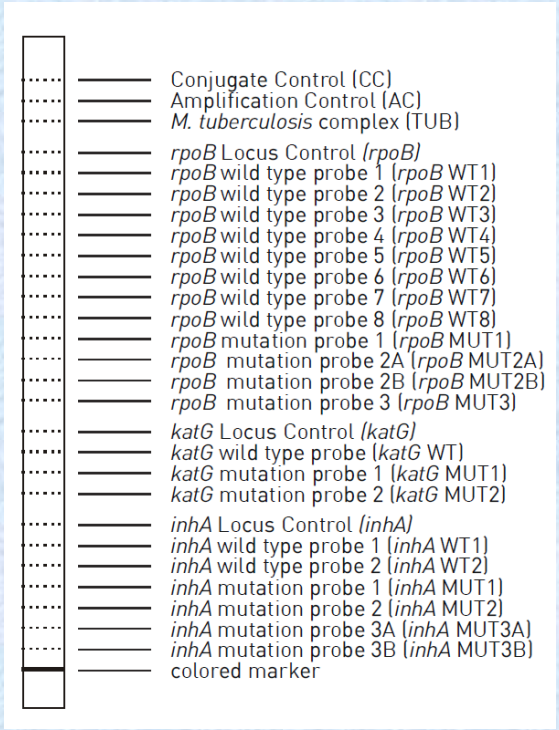
Figure 1: RMP resistance region of the *rpoB* gene
rpoB WT1-8: *rpoB* wild type probes; *rpoB* MUT1-3: *rpoB* mutation probes. The numbers specify the positions of the amino acids (codons) for all mutations listed in the table. The codons for which mutation probes were designed are highlighted.

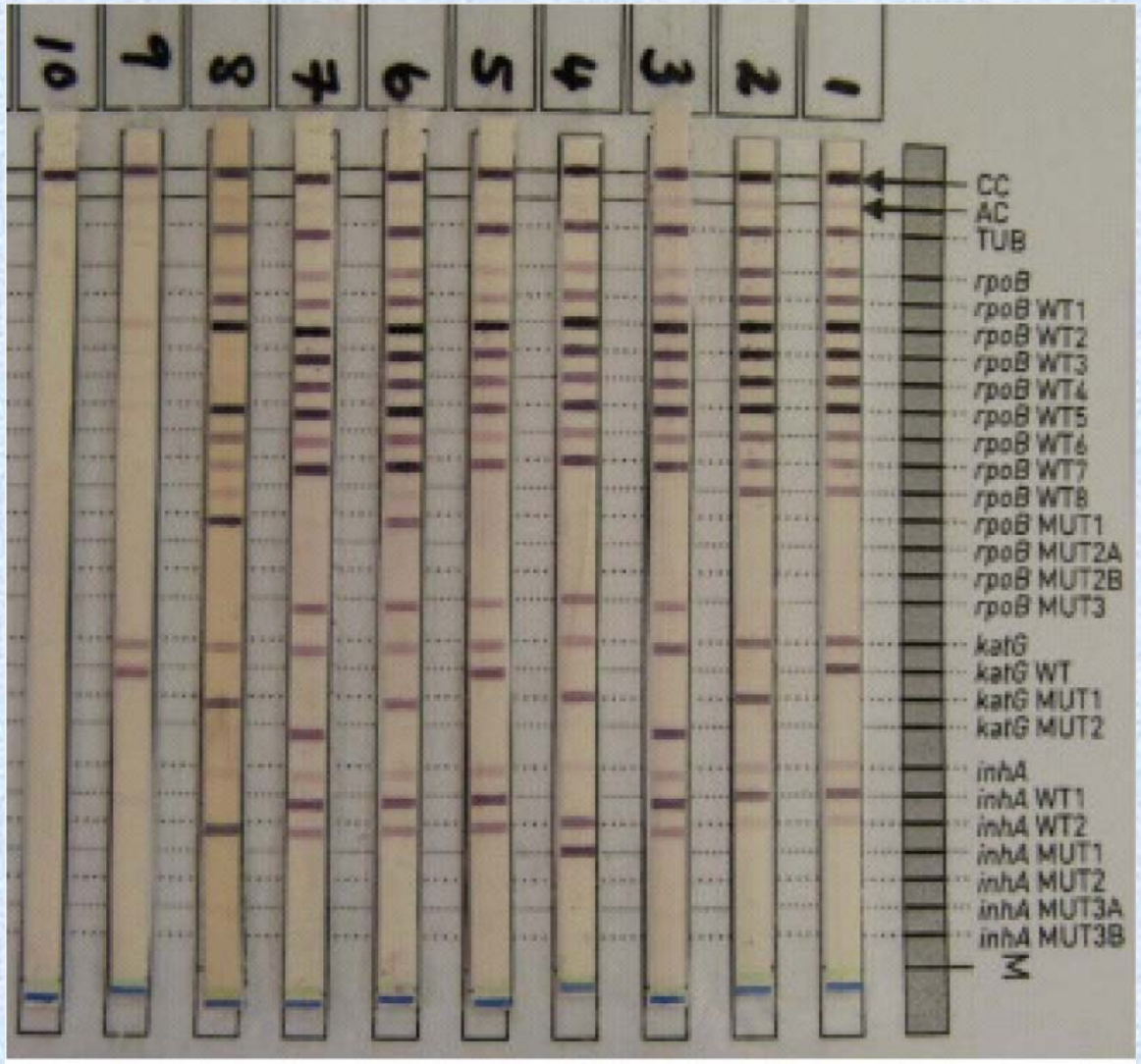




Evaluation Examples




Conjugate Control (CC)
Amplification Control (AC)
M. tuberculosis complex (TUB)
rpoB Locus Control (rpoB)
rpoB wild type probe 1 (rpoB WT1)
rpoB wild type probe 2 (rpoB WT2)
rpoB wild type probe 3 (rpoB WT3)
rpoB wild type probe 4 (rpoB WT4)
rpoB wild type probe 5 (rpoB WT5)
rpoB wild type probe 6 (rpoB WT6)
rpoB wild type probe 7 (rpoB WT7)
rpoB wild type probe 8 (rpoB WT8)
rpoB mutation probe 1 (rpoB MUT1)
rpoB mutation probe 2A (rpoB MUT2A)
rpoB mutation probe 2B (rpoB MUT2B)
rpoB mutation probe 3 (rpoB MUT3)
katG Locus Control (katG)
katG wild type probe (katG WT)
katG mutation probe 1 (katG MUT1)
katG mutation probe 2 (katG MUT2)
inhA Locus Control (inhA)
inhA wild type probe 1 (inhA WT1)
inhA wild type probe 2 (inhA WT2)
inhA mutation probe 1 (inhA MUT1)
inhA mutation probe 2 (inhA MUT2)
inhA mutation probe 3A (inhA MUT3A)
inhA mutation probe 3B (inhA MUT3B)
colored marker


10
9
8
7
6
5
4
3
2
1
CC
AC
TUB
rpoB
rpoB WT1
rpoB WT2
rpoB WT3
rpoB WT4
rpoB WT5
rpoB WT6
rpoB WT7
rpoB WT8
rpoB MUT1
rpoB MUT2A
rpoB MUT2B
rpoB MUT3
katG
katG WT
katG MUT1
katG MUT2
inhA
inhA WT1
inhA WT2
inhA MUT1
inhA MUT2
inhA MUT3A
inhA MUT3B
M

RIF resistance

- WHO Policy update LPA INH RIF 2016
- Test accuracy LPA for direct testing compared with phenotypic RIF DST (done on sputum):
  - Sensitivity: 0.96 (95% CI:0.95-0.97)
  - Specificity: 0.98 (95% CI: 0.97-0.99)
- Test accuracy LPA for indirect testing compared with phenotypic RIF DST (done on culture):
  - Sensitivity: 0.97 (95% CI: 0.95-0.98)
  - Specificity: 0.99 (95% CI: 0.99-1.00)

http://www.who.int/tb/publications/molecular-test-resistance/en/

INH resistance

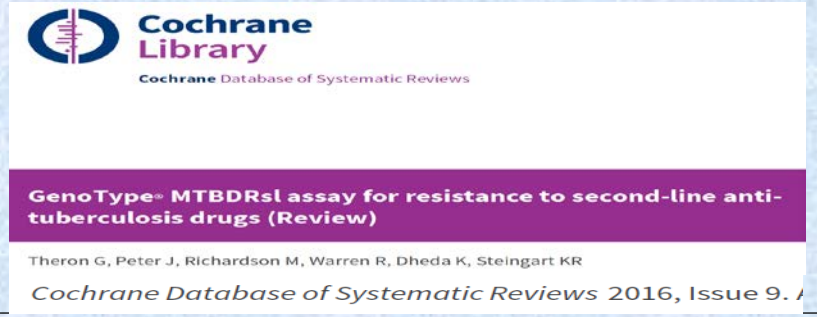
- Resistance-conferring mutations in inhA and katG genes account ~ 90% of INH R detected by phenotypic DST
- Test accuracy LPA for direct testing compared with phenotypic INH DST (done on sputum):
  - Sensitivity: 0.89 (95% CI: 0.86–0.92)
  - Specificity: 0.98 (95% CI: 0.97–0.99)
- Test accuracy LPA for indirect testing compared with phenotypic INH DST (done on culture):
  - Sensitivity: 0.91 (95% CI: 0.89–0.93)
  - Specificity: 1.00 (95% CI: 0.99–1.00)
- Conventional culture-based DST should be used in the follow-up evaluation of patients with a high risk for INH R and a negative LPA result, especially in settings with a high pre-test probability of INH R
  - A 90% prevalence of INH R likely in a population of MDRTB patients when a patient is diagnosed by the Xpert MTB/RIF assay

Test result	Number of results per 1000 patients tested (95% CI)			Number of participants (studies)	Quality of the Evidence (GRADE)
	5% prevalence	10% prevalence	15% prevalence		
True positives (patients with isoniazid resistance)	45 (43–46)	134 (129–138)	803 (772– 827)	3 576 (46)	Moderate
False negatives (patients incorrectly classified as not having isoniazid resistance)	5 (4-7)	16 (12-21)	97 (73-128)		
True negatives (patients without isoniazid resistance)	935 (926–940)	836 (829–841)	98 (97–99)	6 896 (46)	Moderate
False positives (patients incorrectly classified as having isoniazid resistance)	15 (10–24)	14 (9–21)	2 (1–3)		

Hain MTBDRsl

Table 1. Characteristics of Genotype MTBDRsl versions 1.0 and 2.0 as per manufacturer

Detection	**Version 1.0** *M. tuberculosis* complex and resistance to fluoroquinolones, SLIDs and ethambutol	**Version 2.0** *M. tuberculosis* complex and resistance to fluoroquinolones and SLIDs
Samples	Smear-positive specimens and culture isolates	Smear-positive and smear-negative specimens and culture isolates
Fluoroquinolone resistance	Mutations in resistance-determining region of the *gyrA* gene	Mutations in resistance-determining regions of the *gyrA* and *gyrB* genes
SLID resistance	Mutations in resistance determining region of the *rrs* gene	Mutations in resistance determining region *rrs* gene and the *eis* promoter region
Ethambutol resistance	Mutations in the *embB* gene	Not included



VERSION 1 (compared to culture based DST)
26 studies

- *FQ R:* direct testing, MTBDR*sl*

Se 86.2% Sp 98.6% smear-positive specimen

- *FQ R: in*direct testing, MTBDR*sl*

Se 85.6% Sp 98.5% smear-positive specimen

- *SLID R:* direct testing, MTBDR*sl*

Se 87.0% Sp 99.5% smear-positive specimen

- *SLID R: in*direct testing, MTBDR*sl*

Se 76.5% Sp 99.1% smear-positive specimen

- *XDR-TB:* direct testing, MTBDR*sl*

Se 69.4% Sp 99.4% smear-positive specimen

- *XDR-TB: in*direct testing, MTBDR*sl*

Se 70.9% Sp 98.8% smear-positive specimen

VERSION 2 (compared to culture based DST)
1 study

- *FQ R:* direct testing, MTBDR*sl*

Se 97% Sp 98% smear-positive specimen
Se 80% Sp 100% smear-negative specimen

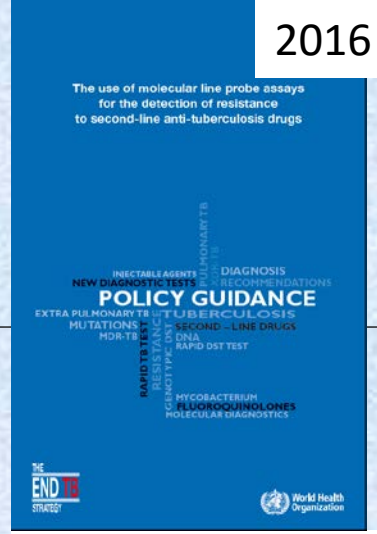
- *SLID R:* direct testing, MTBDR*sl*

Se 89% Sp 90% smear-positive specimen
Se 80% Sp 100% smear-negative specimen

- *XDR-TB:* direct testing, MTBDR*sl*

Se 79% Sp 97% smear-positive specimen
Se 50% Sp 100% smear-negative specimen

2016



For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones

(Conditional recommendation; moderate certainty in the evidence for test accuracy for direct testing of sputum specimens; low certainty in the evidence for test accuracy for indirect testing of *Mycobacterium tuberculosis cultures*).

For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the second-line injectable drugs

(Conditional recommendation; low certainty in the evidence for test accuracy for direct testing of sputum specimens; very low certainty in the evidence for test accuracy for indirect testing of *Mycobacterium tuberculosis* cultures).

http://www.who.int/tb/publications/molecular-test-resistance/en/

Table 1. 2016 Tuberculosis Diagnostics Pipeline: Products in Later-Stage Development or on Track for Evaluation by the WHO with New Published Data or Policy Updates Since the *2015 Pipeline Report*

Test	Type	Sponsor	Status	Comments
MOLECULAR/NAAT				
BD MAX MTB assay	qPCR for MTB in automated BD MAX	BD	In 16 *M. tuberculosis* samples, 100% sensitivity, 97.1% specificity[6]	
Genedrive MTB/RIF	Portable RT-PCR for MTB + RIF resistance	Epistem	Worse sensitivity than smear [!] in 2016 study[7]	Marketed in India
GenoType MTBDR*plus*	Line probe assay for RIF + INH resistance	Hain Lifescience	WHO now recommends based on FIND evaluation[8]	WHO guidance pending
GenoType MTBDR*sl*	Line probe assay for FQ + SLID resistance	Hain Lifescience	WHO now recommends[9]	FIND's multicountry evaluation of MTBDR*sl* version 2.0 from 2015 still unpublished
MeltPro	Closed-tube RT-PCR	Zeesan Biotech	New study from China of 2,057 smear-positive TB patients shows sensitivity of detecting resistance to rifampin 94.2%, isoniazid 84.9%, ofloxacin 83.3%, amikacin 75.0%, kanamycin 63.5%[10]	
NTM+MDRTB Detection Kit 2	Line probe assay for RIF + INH resistance	Nipro	WHO now recommends based on FIND evaluation[11]	WHO guidance pending
RealTime MTB/ TB MDx m2000	Automated RT-PCR for MTB; can be added to HIV RNA platform	Abbott	Sensitivity 100%, 95% CI: 98.6–99.9 in smear-positive samples, similar to GeneXpert MTB/RIF[12]	
Truenat MTB	Chip-based NAAT with RT-PCR on handheld device for MTB	Molbio Diagnostics, Bigtec Labs	FIND and ICMR studies underway	
Xpert MTB/RIF Ultra	Next-generation cartridge-based detection of MTB + RIF resistance	Cepheid	FIND study results anticipated end 2016	
Xpert Omni	Single-cartridge mobile platform that can use single MTB/RIF or Ultra cartridge	Cepheid	FIND study pending but delayed	
Xpert XDR	NAAT	Cepheid	FIND study anticipated 2018	

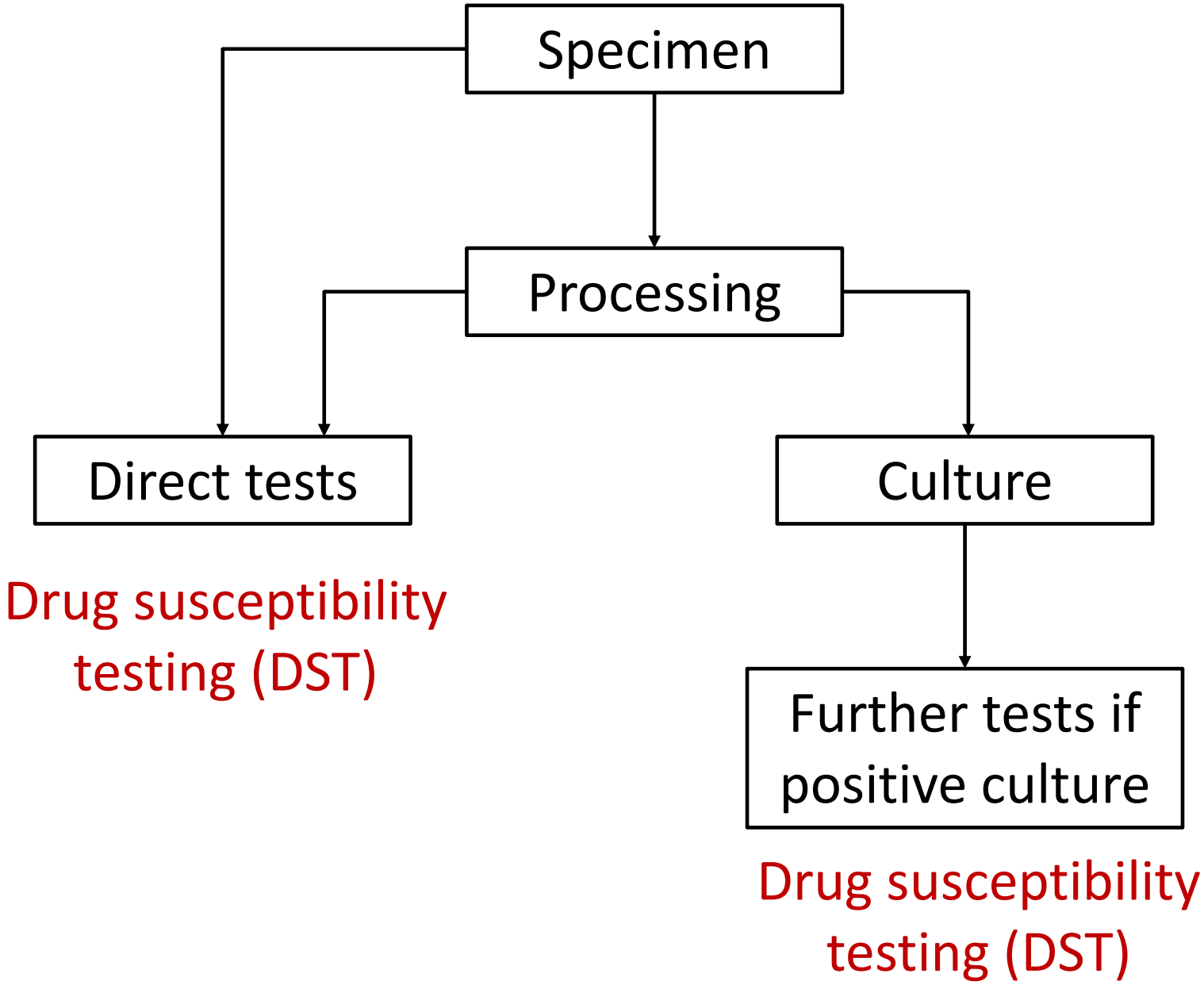
http://pipelinereport.org/home

NAAT in TB drug trials

- Adult TB drug trials
  - Screening tests for patient enrolment
    - e.g. r/o MDR if drug susceptible TB study
    - e.g. confirm FQ susceptibility in MDR-TB study giving FQ
  - Always confirmed by culture
- Paediatric TB
  - Xpert part of TB confirmed definition
  - Xpert usually done on specimen and Hain done on culture

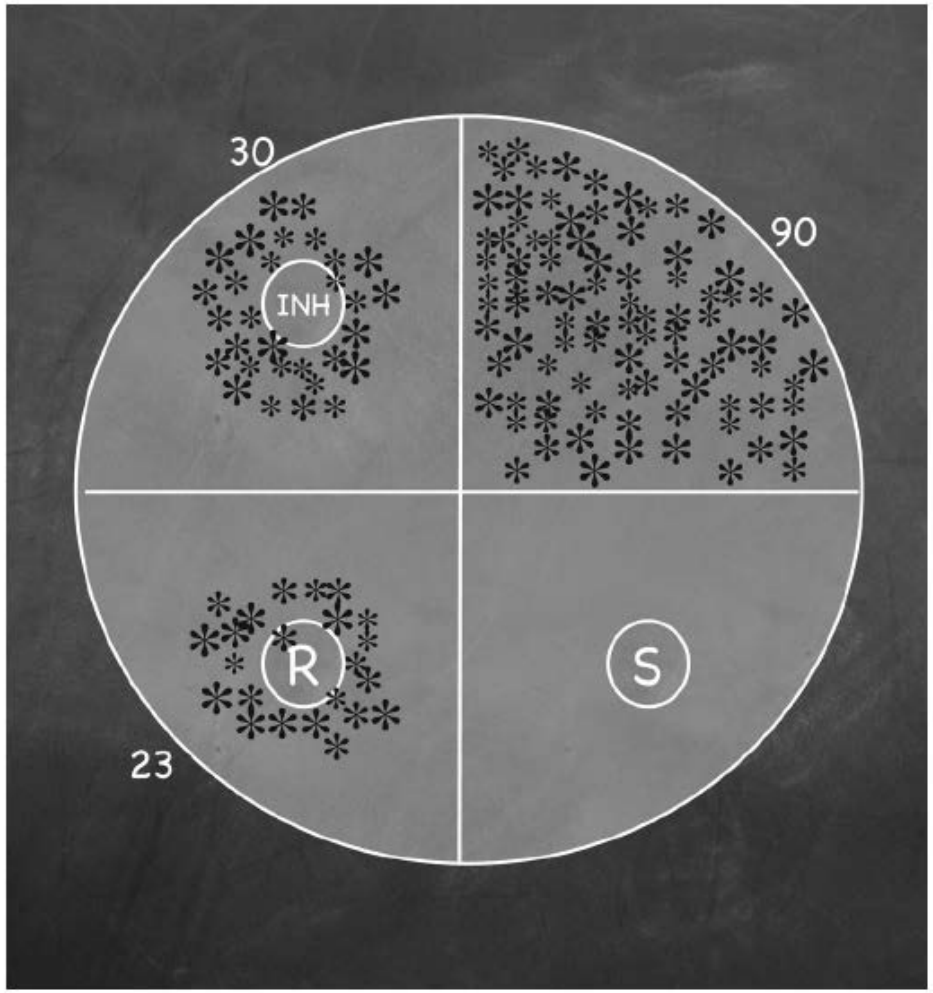
Drug susceptibility testing (DST)

- Molecular: done on sample or on culture
  - GeneXpert
  - LPA: Hain MTBDRplus and MTBDRsl
  - Whole genome sequencing
- Phenotypic (culture-based)
  - On sample (direct) or on culture (indirect)
  - On liquid culture or on solid culture
  - Methods: proportion method (most used), absolute concentration method, and resistant ratio method


Specimen
Processing
Direct tests
Culture
Drug susceptibility testing (DST)
Further tests if positive culture
Drug susceptibility testing (DST)

Agar proportion method for drug-susceptibility testing.

Quadrant plate—Inoculum of *M. tuberculosis* growth from liquid media has been inoculated into each of the 4 quadrants with the following results:



Control quadrant: 90 colonies

Isoniazid (INH) quad: 30 colonies

Rifampin (R) quad: 23 colonies

Streptomycin (S) quad: 0 colonies

Isoniazid 30/90 = 33% resistant

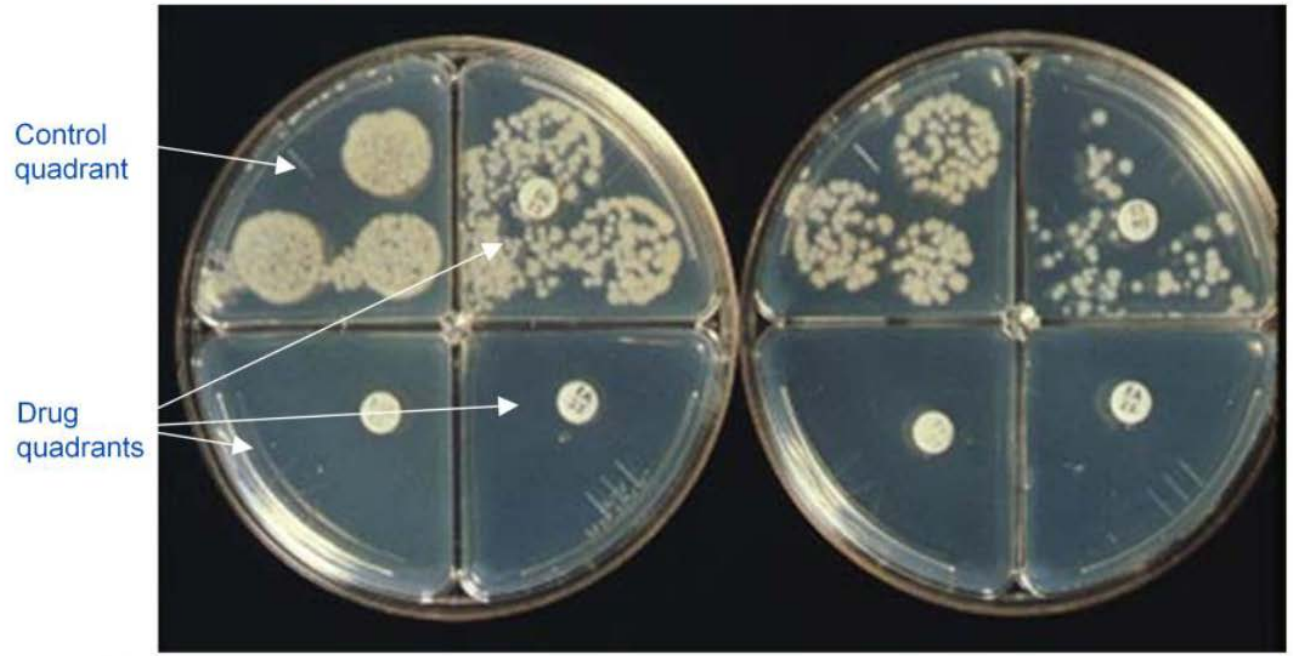
Rifampin 23/90 = 25% resistant

Streptomycin 0/90 = susceptible

This is an MDR-TB isolate.

The isolate is considered resistant if the number of colonies in the drug quadrant is equal to or more than 1% of that in the control quadrant

Drug Susceptibility Testing on Solid Medium Indirect Proportion Method



Organism is resistant to drug A in the upper right compartment (>1% of inoculum shown by upper left control quadrant is growing in presence of drug). Organism is susceptible to drugs B & C in the lower quadrants. Control quadrant in upper left contains no drugs.

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- MGIT DST is a modified proportion method
- Results in 4-14 days after the test is set up

- The method is based on the fluorescence produced from reduced oxygen in the MGIT medium due to microbial growth.

- The fluorescence generated is then converted to “growth units” (GU). In general, more GU indicates more growth.

- When the growth control generates GU to 400 within 4-14 days, the DST is valid for interpretation:
  - If a drug-containing MGIT tube yields GU<100, the organism is interpreted as being susceptible
  - If GU is ≥100, the organism is considered resistant.

Growth Control tube
STR
INH
RIF
EMB

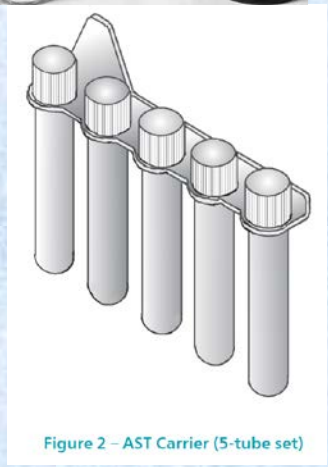


Figure 2 – AST Carrier (5-tube set)

GLOBAL TB PROGRAMME

Updated critical concentrations for first-line and second-line DST (as of May 2012)

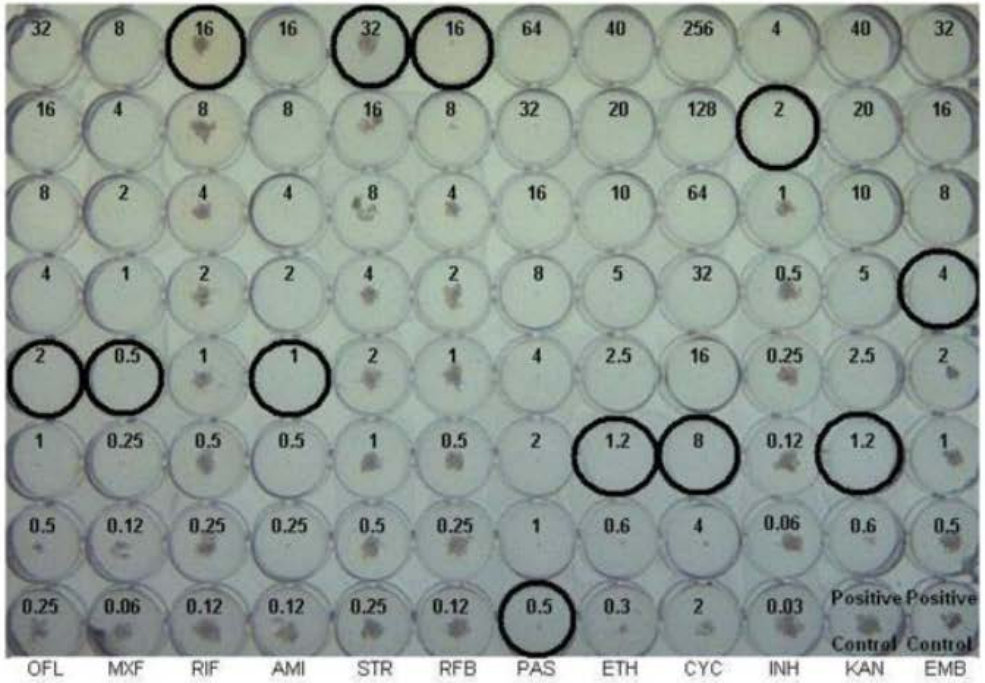
World Health Organization

Drug group[a]	Drug	DST method available	DST critical concentrations (µg/ml)			
			Löwenstein-Jensen[b]	Middlebrook 7H10[b]	Middlebrook 7H11[b]	MGIT960
Group 1 First-line oral anti-TB agents	Isoniazid Rifampicin[c] Ethambutol[d] Pyrazinamide	Solid, liquid Solid, liquid Solid, liquid Liquid	0.2 40.0 2.0 -	0.2 1.0 5.0 -	0.2 1.0 7.5 -	0.1 1.0 5.0 100.0
Group 2 Injectable anti-TB agents	Streptomycin[e] Kanamycin Amikacin Capreomycin	Solid, liquid Solid, liquid Solid, liquid Solid, liquid	4.0 30.0 30.0 40.0	2.0 5.0 4.0 4.0	2.0 6.0 - -	1.0 2.5 1.0 2.5
Group 3 Fluoroquinolones	Ofloxacin[f] Levofloxacin Moxifloxacin[g] Gatifloxacin[h]	Solid, liquid Solid, liquid Solid, liquid Solid	4.0 - - -	2.0 1.0 0.5/2.0 1.0	2.0 - - -	2.0 1.5 0.5/2.0 -
Group 4[i] Oral bacteriostatic second-line anti-TB agents	Ethionamide Prothionamide Cycloserine *P*-aminosalicylic acid	Solid, liquid Solid, liquid Solid Solid, liquid	40.0 40.0 30.0 1.0	5.0 - - 2.0	10.0 - - 8.0	5.0 2.5 - 4.0
Group 5[i] Antituberculosis agents with unclear efficacy (not recommended by WHO for routine use in MDR-TB patients)	Clofazimine Amoxicillin/clavulanate Clarithromycin Linezolid	Liquid None None Liquid	- - - -	- - - -	- - - -	- - - 1.0

[a] WHO *Guidelines for the programmatic management of drug-resistant tuberculosis.*
[b] Indirect proportion method recommended. Other solid media methods (resistance ratio) have not been adequately validated for second-line drugs. Concentrations for the absolute concentration method were not evaluated.
[c] Rifampicin borderline resistance more frequently missed by MGIT. Prevalence and geographical distribution of borderline resistance not clear, final LJ interpretations should be made after 6 weeks
[d] Ethambutol 5ug/ml in MGIT is not equivalent to other methods. Ethambutol testing in 7H11 not equivalent to 7H10. There is insufficient evidence to recommend a change in concentration for any method
[e] Streptomycin has a bimodal distribution of MIC values. Insufficient evidence to recommend a change.
[f] Ofloxacin concentration in LJ media increased to 4.0ug/ml. Insufficient data to extrapolate change in 7H10 or 7H11 methods.
[g] Moxifloxacin. Two concentrations proposed. In programmes using both ofloxacin/levofloxacin and moxifloxacin , possible testing is for moxifloxacin only at both concentrations OR test ofloxacin/levofloxacin and moxifloxacin at higher concentration. In programmes using ofloxacin/levofloxacin only test only these drugs. In programmes using only moxifloxacin test at higher concentration of moxifloxacin only.
[h] Gatifloxacin only to be used in exceptional circumstances.
[i] Routine DST for group 4 and 5 drugs is not recommended. Linezolid suitable for testing in MGIT only.

http://www.stoptb.org/wg/gli/assets/documents/Updated%20critical%20concentration%20table_1st%20and%202nd%20line%20drugs.pdf

New Method for *Mtb* Drug Susceptibility Testing – MIC Plate



- Broth microdilution method
- Multicenter studies supporting FDA-submission completed[2]
- Rapid (14 days)
- Contains INH, RIF, EMB, and 9 second-line drugs
- Test first- and second-line drugs simultaneously with same inoculum
- Provides MIC endpoint – helpful for isolates with MIC near critical concentration (CC) breakpoint that give fluctuating results w/CC method

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Drug susceptibility testing (DST)

- Discordants
  - Different genotypic tests
  - Genotypic and phenotypic tests
  - Genotypic and/or phenotypic tests and clinical response to treatment

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REVIEW ARTICLE

Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement

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C. Lange;###***††† for the TBNET and RESIST-TB networks

Gold standard

13. If the results of molecular and culture-based drug susceptibility testing differ, what is the gold standard?

The level of discordance between molecular and culture-based DST depends on the drug and the genomic region evaluated. Despite the fact that results of phenotypic methods do not always correspond to response to clinical treatment, culture-based methods are still regarded by most experts involved in this document as the gold standard for DST.

Agreed: 13; disagreed: 0; abstained: 0.

Drug susceptibility testing (DST)

- In TB drug trials
  - Screening using NAATs
  - Hain can help to guide OBR
  - MGIT DST
  - DST for new TB drugs or MICs sent to reference lab
- Complex and evolving field
  - Role for both phenotypic and genotypic methods

Whole genome sequencing

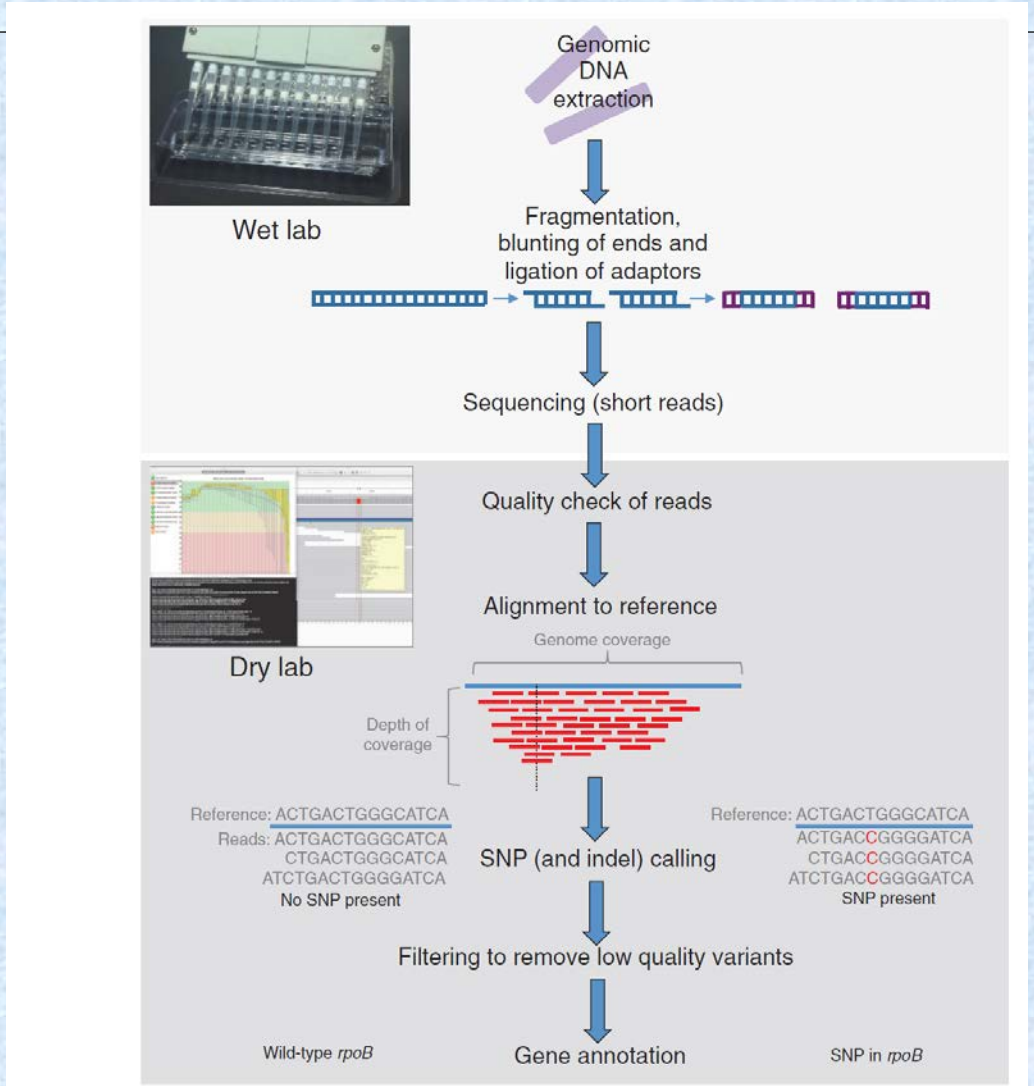


Figure 1. WGS workflow for *Mycobacterium tuberculosis*.

	Advantages	Disadvantages	Applications
IS6110 restriction fragment length polymorphism[44]	High discriminatory index	Requires culture and DNA extraction; cannot differentiate between drug-sensitive and drug-resistant strains	Identification of transmission chains, mechanism leading to primary resistance, and temporal changes in the strain population
Spoligotyping[66]	Direct genotyping of clinical specimens; global reference database; relatively inexpensive; requires fewer laboratory resources	Low discriminatory index; undergoes homoplasy; cannot differentiate between drug-sensitive and drug-resistant strains	Classification of strains according to lineages, re-infection, and strain migration
Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR)[67-69]	Direct genotyping of clinical specimens; high discriminatory index; global reference database	Undergoes homoplasy; cannot differentiate between drug-sensitive and drug-resistant strains	Identification of transmission chains and mechanisms leading to primary resistance
Targeted gene sequencing (Sanger)[58,70]	Direct genotyping of clinical specimens; relatively inexpensive	Information limited to nucleotide variants in a selected set of genes; no strain type information	Identification of mutations conferring resistance
Targeted deep sequencing[71-73]	Direct genotyping of clinical specimens	Information limited to nucleotide variants in a selected set of genes; no strain type information; more expensive; requires high-level laboratory infrastructure	Identification of mutations conferring resistance and heteroresistance
Whole-genome sequencing[74-78]	Comprehensive analysis of the genome of the pathogen	Requires culture (or specimen enrichment); more expensive; might be computationally demanding or complex	Identification of transmission chains, mutations conferring resistance, heteroresistance (low resolution), mixed infections, specimen heterogeneity, and intrapatient evolution

Table 1: **Molecular epidemiological genotyping methods**

Lancet Respir Med 2017; 5: 291–360

Phases of laboratory testing

Pre-Analytical	Analytical	Post-Analytical

Lab report

- Important to understand what tests are done
- Verify results for all specimens collected
- Verify final reports
- Contact laboratory if unclear

PARTIAL FINAL LABORATORY REPORT

MICROBIOLOGY

Tel: 021 417 9360/1

Specimen received: Sputum (Suspect new : 0mths)
Tests requested: GeneXpert, TB mic, TB cult, TB antigen

Real time PCR for M. tuberculosis (GeneXpert):	
PCR result	Mycobacterium tuberculosis complex detected
Rifampicin	Resistant

This patient has presumptive MDR-TB. Please refer URGENTLY to an appropriate treatment facility. Send a 2nd sample for microscopy, TB culture and further susceptibility testing for confirmation.

Auramine O Stain:	
Result (concentrated)	Negative (No AFB/100 immersion fields)

Smear

TB Culture:	
Culture result	Culture positive. AFBs observed.
Incubation time	20 days

MGIT

Time to detection in days

Mycobacterial Identification - Antigen:	
Result	Mycobacterium tuberculosis complex

Identification of positive culture

Hain MTBDRplus

Molecular resistance testing for first line agents for TB:

Test performed on:	Cultured isolate
PCR/Line Probe Assay Result	Mycobacterium tuberculosis complex
Isoniazid (INH)	Resistant
Rifampicin	Resistant

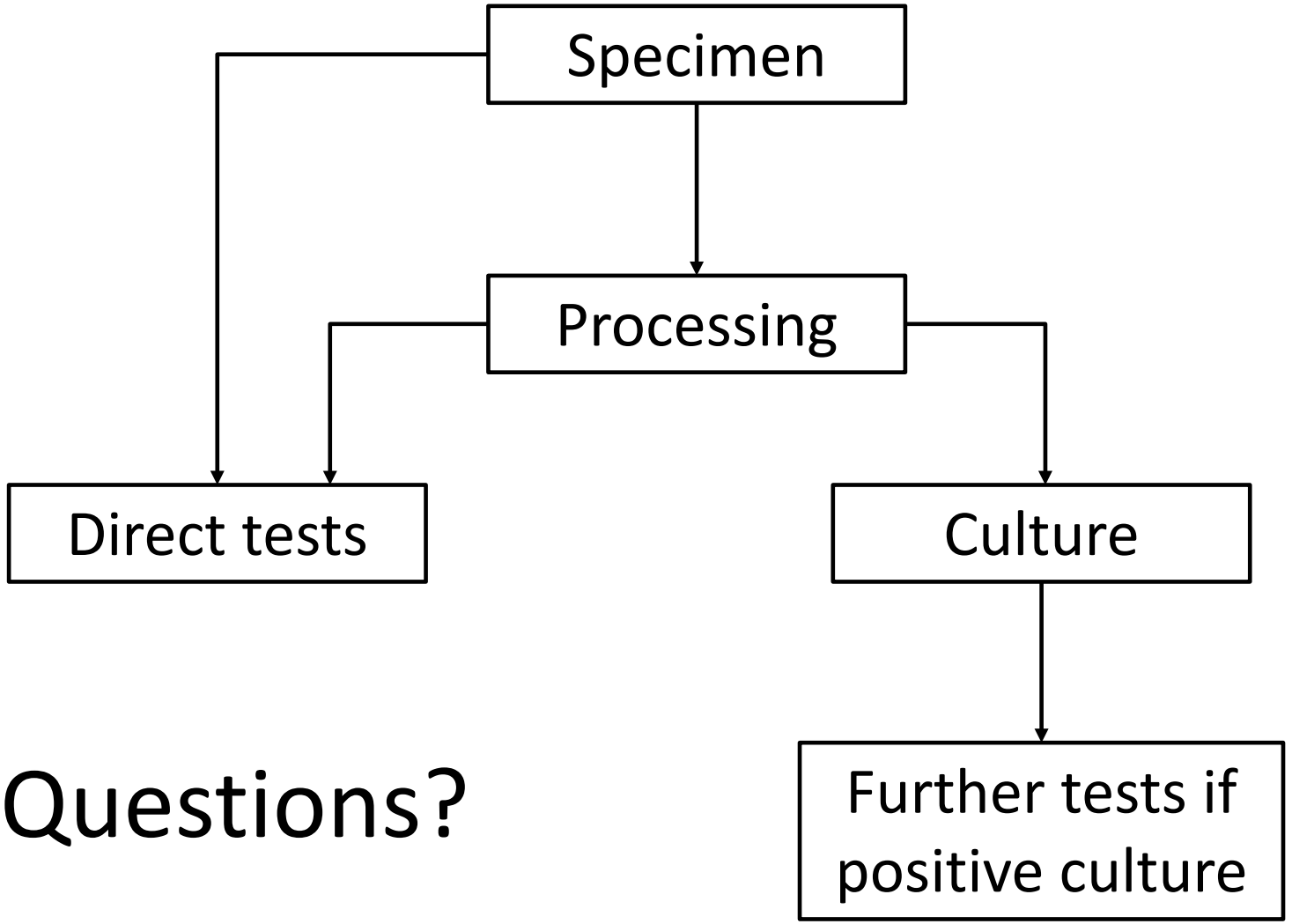
This patient has multi-drug resistant tuberculosis. Please ensure that this patient has been referred to an appropriate treatment facility. 2nd line susceptibility testing will follow.

This isolate has a mutation in the inhA gene, which has been shown to correlate with ethionamide resistance. This may also represent low-level INH resistance, and addition of INH in high doses may be useful.

Antimycobacterial Drug Sensitivity Testing:

Second Line Drugs - Agar Culture Based:

Amikacin	Sensitive
Ofloxacin	Sensitive


Specimen
Processing
Direct tests
Culture
Further tests if positive culture

Questions?

References

- 100 mg of sodium carbonate (as per Manual of Clinical Microbiology. 10th. Washington, DC.: ASM Press; 2011 or Clinical Microbiology Procedures Handbook. 3rd Edition ed. Washington, D.C: American Society of Microbiology; 2010)
- Bicarbonate solution (as per WHO Guidance for national tuberculosis programmes on the management of tuberculosis in children, Second Edition, WHO/HTM/TB/2014.03 2014. Available from: http://www.who.int/tb/publications/childtb_guidelines/en/
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