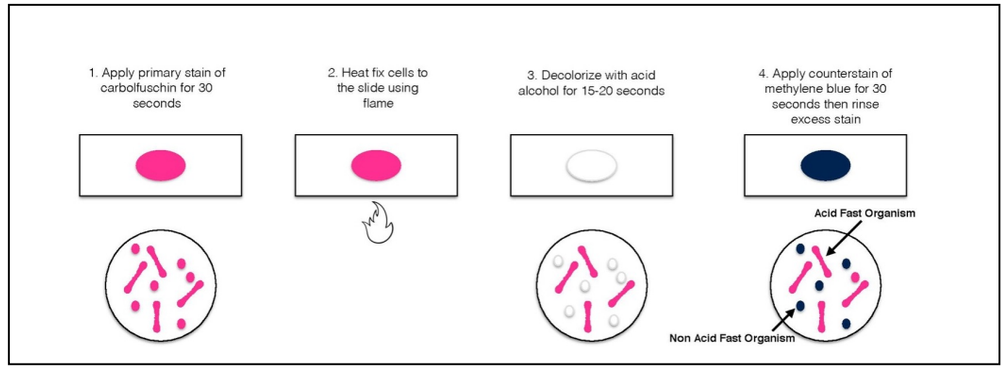
TB Diagnosis

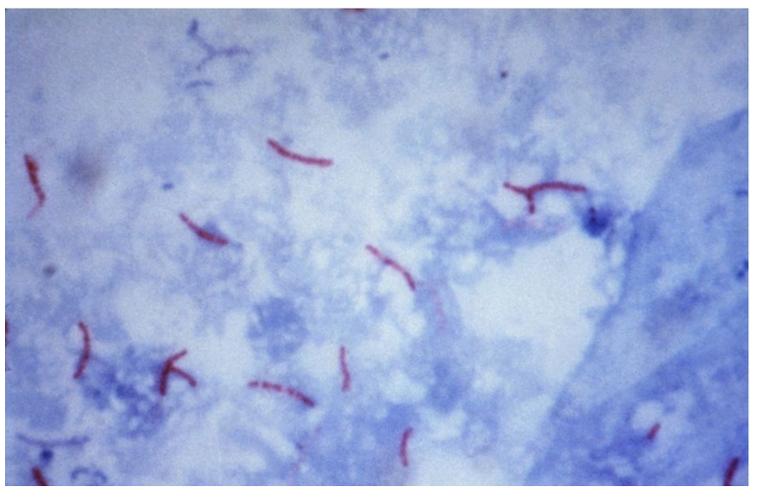
Passive versus active case finding

- Current guidelines recommend ACF only in high risk groups: HIV, HHCs, prisoners, silica exposed, urban slums, homeless, migrants.

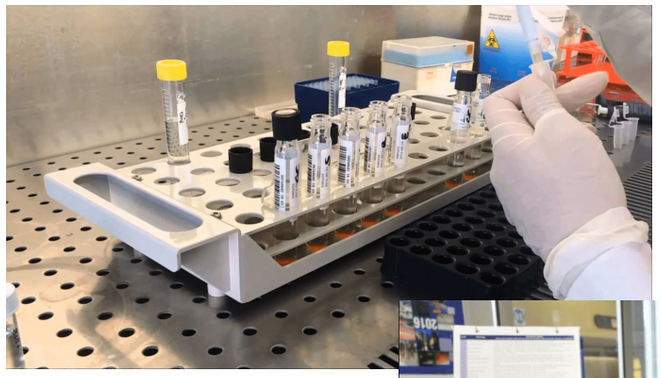
- Passive case finding in people who “present” with 2 weeks cough, fever, night sweats, weight loss.

- Sputum smear microscopy with Ziehl Neelson stain for acid fast bacilli.
- 2 samples per WHO
- Requires 10k bacilli/ml to visualize so low accuracy in HIV, children
- Does not distinguish atypicals from Mtb or DR from DS
- Sensitivity 46%, Specificity 99%





Mycobacterial culture and drug susceptibility testing

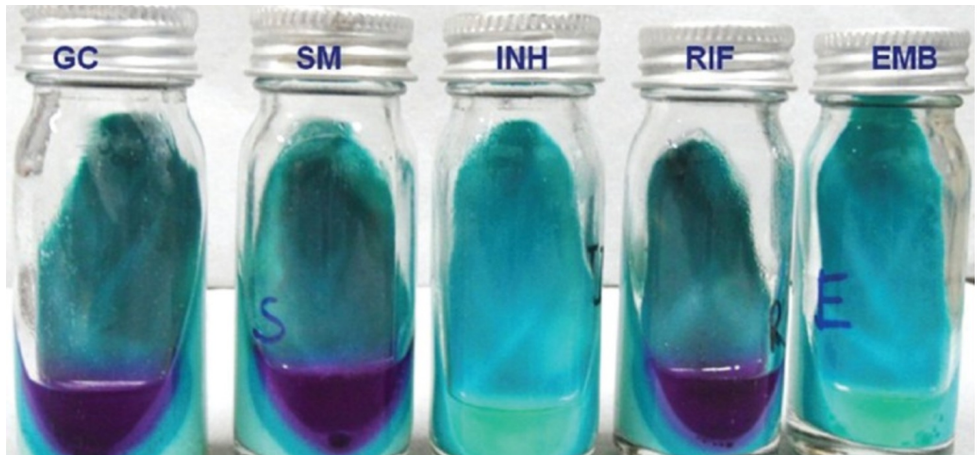


Done in National reference labs or tertiary facilities

Requires BSL3

Average time to growth:
4-6 weeks solid culture
1-2 weeks MGIT

“Gold standard” for diagnosis but “unculturable TB” may exist.



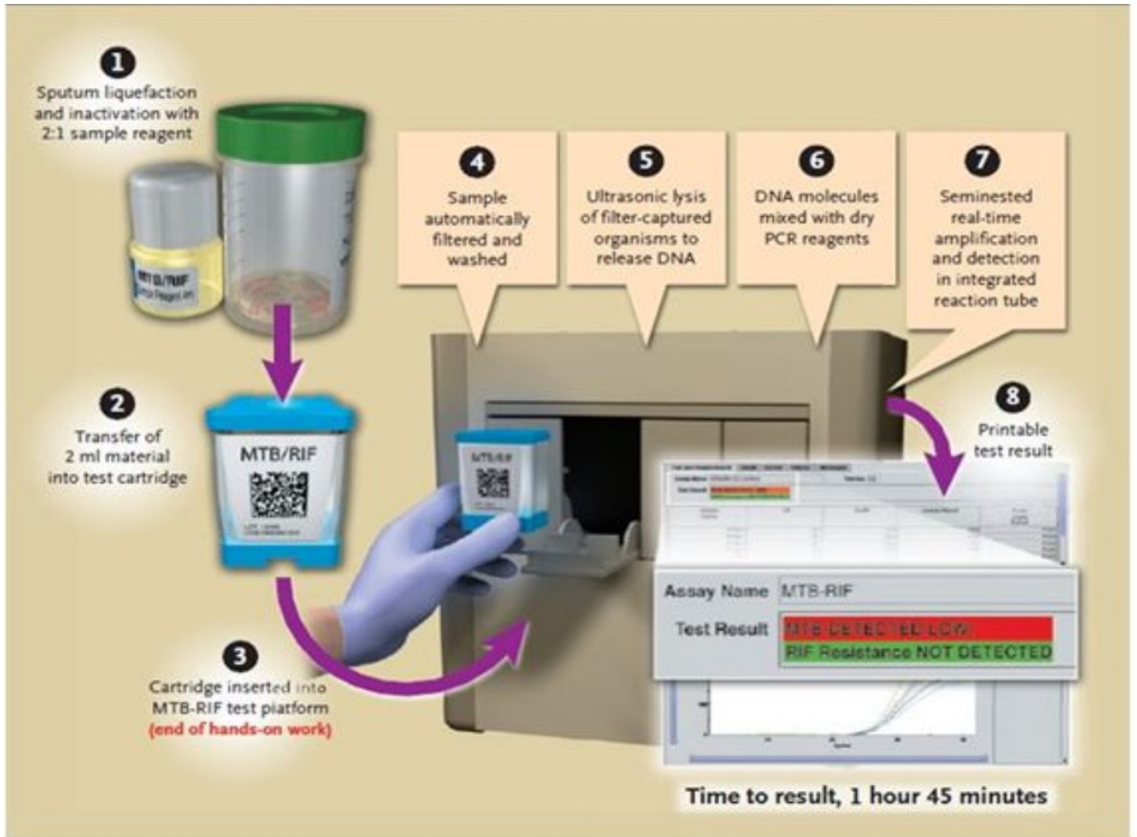
Gene Xpert

Sensitive and specific, fully automated, commercial nucleic acid amplification test for use with sputum and other body specimens.

Closed cartridge system of rt-PCR to detect TB marker and rifampicin resistance (RpoB).

Minimal processing, 2 hour turn-around time.

As of 2013, recommended by WHO as initial test for all TB.



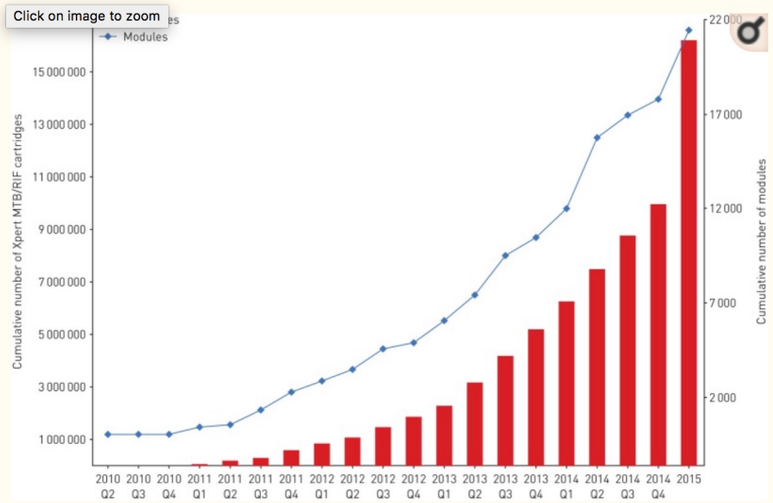


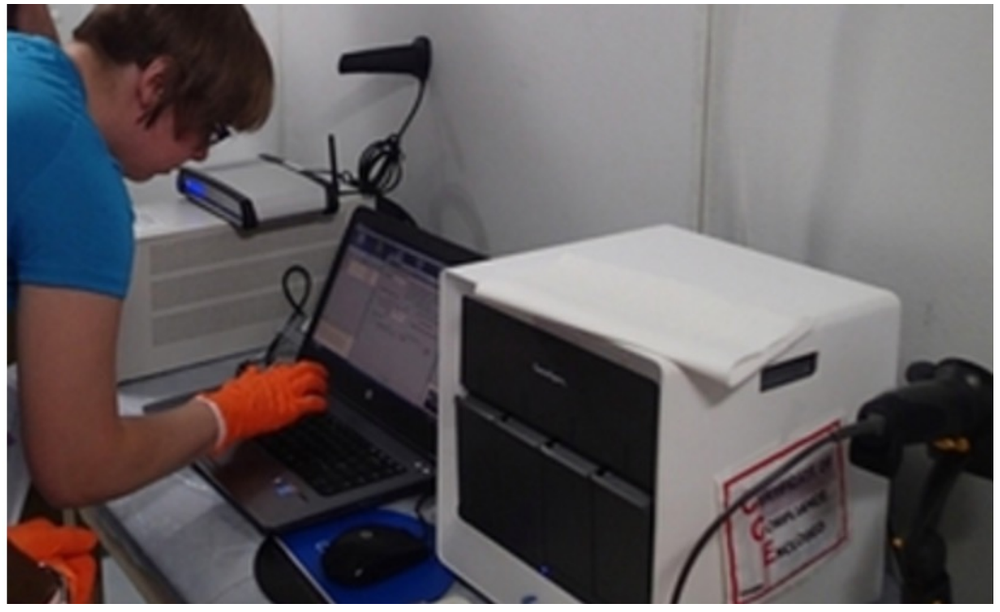
FIGURE 2

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

	All	All		HIV+	
		Smear +	Smear -	Smear +	Smear -
GenXpert MTB/Rif	90/98%	98/99	67/99	97/99	61/99
GenXpert Omni					
GenXpert Ultra	88/98		63/98	90/98	

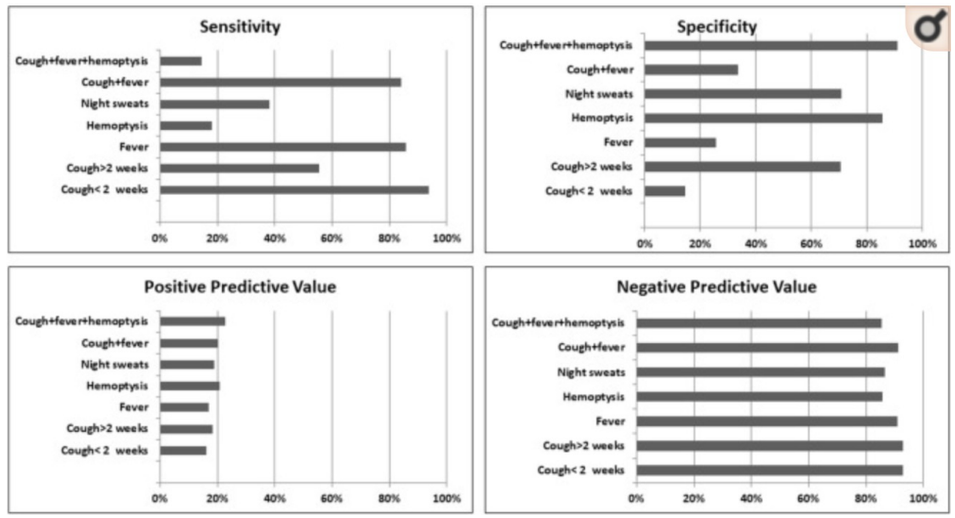
Issues with Xpert

- Pricing
  - 17k for 4 module instrument
  - 10$ for concessionary price for cartridges
- Training
  - 1-2 days officially, 1-2 months by report
- Infrastructure
  - Targeted to district health facilities. Requires stable power, temperature, humidity, no dust.
- Maintenance
  - Higher than expected rates of module failure, up to 10%
- Supply Chain
  - Stock outs of cartridges
  - Expiration of cartridges
- IT/Reporting



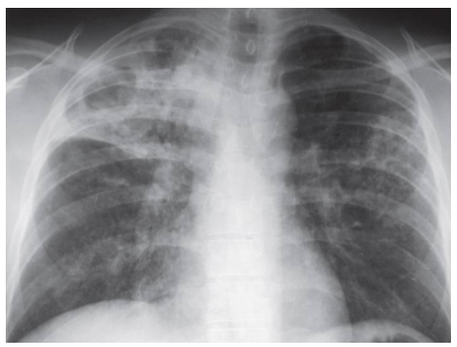
Chest Radiography

- CXrs
- Digital CXRs
- Chest CT
- PET/CT



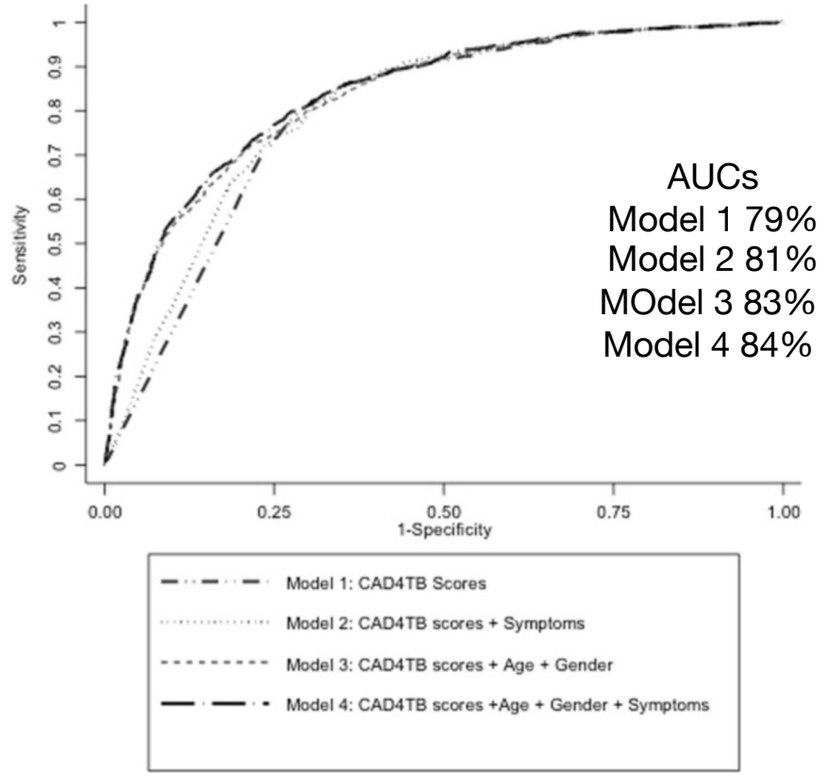
- Myriad possible presentations
- High sensitivity, low specificity
- Role for AI?

Consolidation



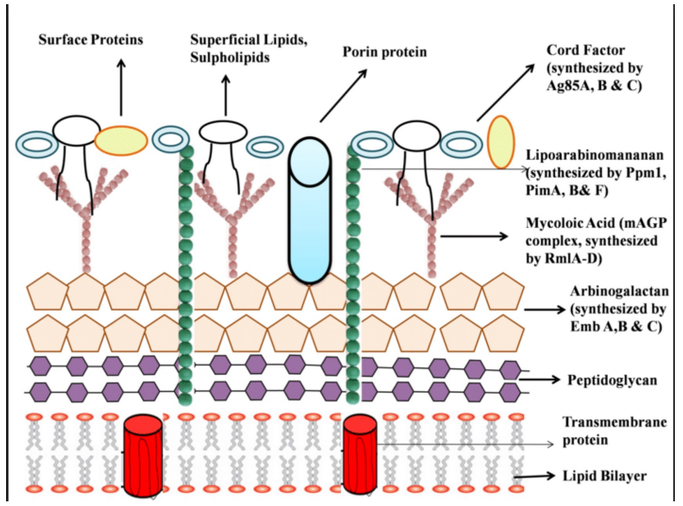
Cavity

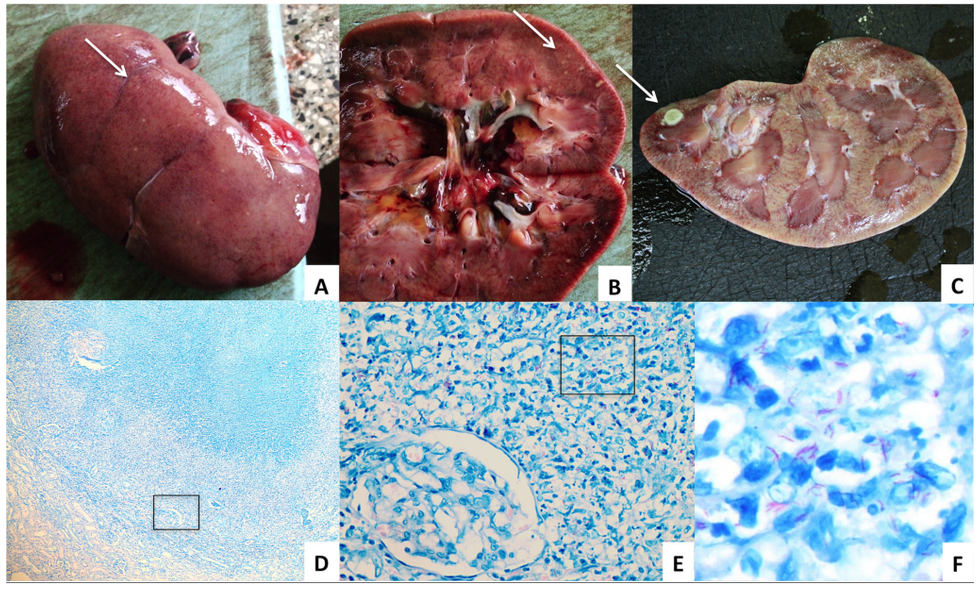
Miliary



Urine LAM

- Lipoaribinomannan, Cell wall liposaccharide specific to Mtb.
- Measured in urine with ELISA or *Determine TB-LAM test strip*
  - 3.50$, 3 minutes
  - High Specificity in HIV, Specificity increases as CD4 decreases:
    1. Cd4<200 39%
    2. Cd4<100 51.7%
    3. Cd4<50 66.7%
- Renal TB explains most positive results.





TB LAMP

- LAMP = loop mediated isothermal amplification

- Isothermal nucleic acid amplification technique. (in contrast to PCR which is carried out with a series of alternating temperature steps or cycles, requiring a thermal cycler.

- Typically, 4 different primers used to identify 6 regions on the target gene, improving specificity. "loop primers" further accelerate the reaction. Amount of DNA produced in LAMP than PCR based amplification.

- Detection of amplification product determined via photometry for turbidity caused by an increasing quantity of magnesium pyrophosphate precipitate in solution as a byproduct of amplification, allowing easy visualization by the naked eye. Dyes can be used to create a visible color change - Dye molecules directly label the DNA and can be correlated to the number of copies initially present.

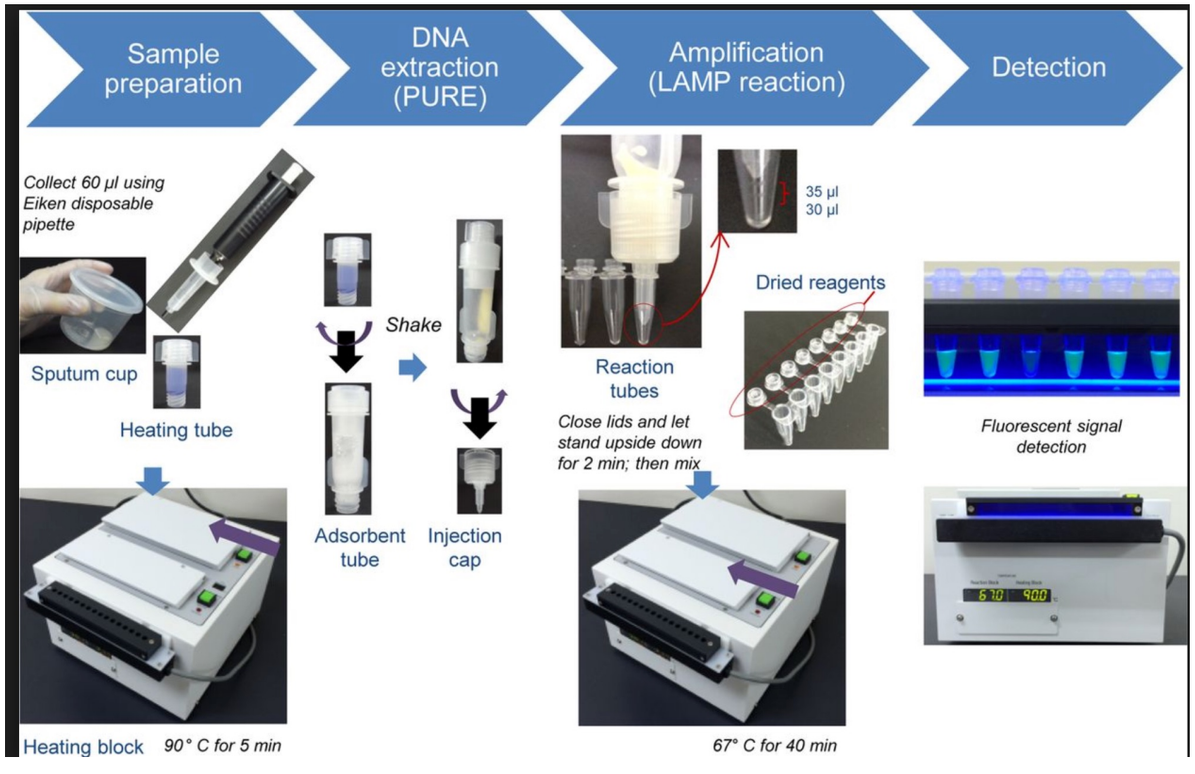


Table 1. Comparison of TB-LAMP positive rates according to smear grade

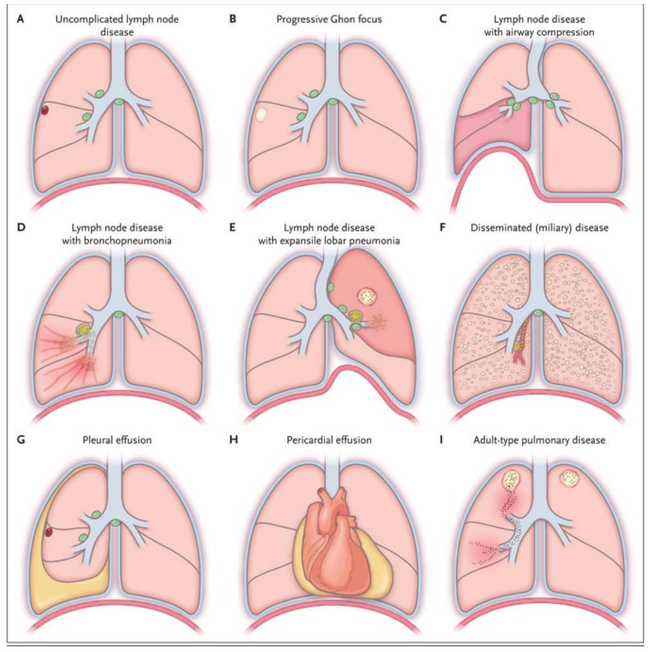
Smear	TB-LAMP		Total	% Agreement (95% CI)	κ (95% CI)
	Positive (%)	Negative (%)			
Negative	14 (7.5)	172 (92.5)	186	83.8 (79.1–87.6)	0.63 (0.54–0.73)
Scanty	3 (17.6)	14 (82.4)	17		
1+	39 (73.6)	14 (26.4)	53		
2+	13 (81.3)	3 (18.8)	16		
3+	16 (88.9)	2 (11.1)	18		
Total	85 (29.3)	205 (70.7)	290		

For assessing agreement, the smear results were divided into positive and negative regardless of grade.

Diagnosis in Children

Issues

- Several different child TB phenotypes:
  - Age 2-3 and under, Age 4-10 or 11, Age >11
- Kids can’t expectorate sputum easily.
- Often have LN disease rather than parenchymal consolidation.
- More pleural disease in adolescents.
- Relies heavily on CXR, Chest CT



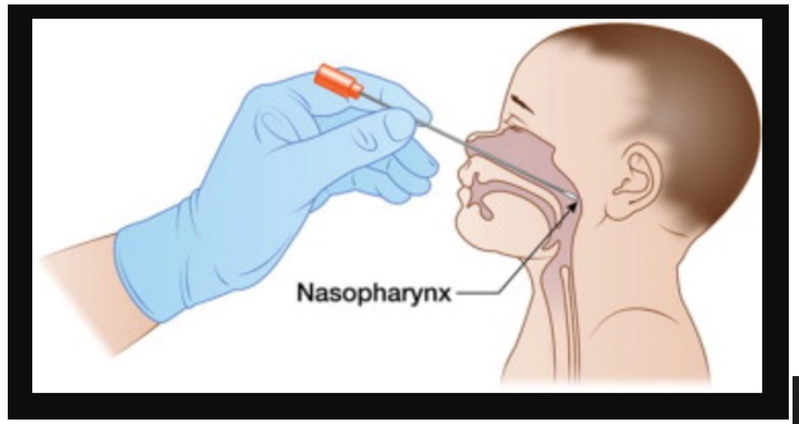
Tests

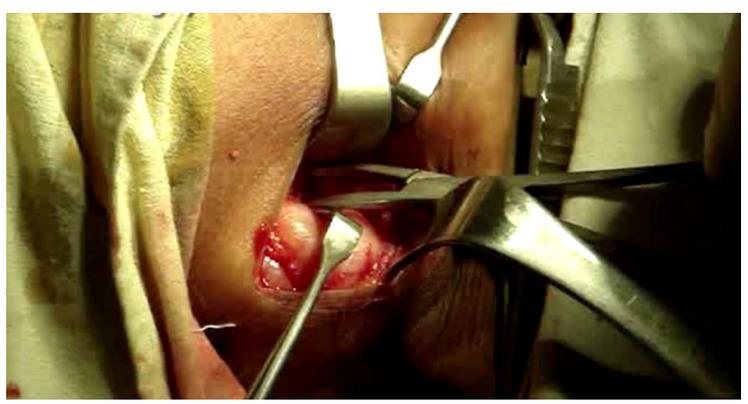
Host based tests
- Radiography
- Biomarkers

Bug based tests

- Gastric aspiration
- Sputum induction
- Naso-pharngeal swab
- String test

- Stool
- Urine
- Biopsy of LN or pleura





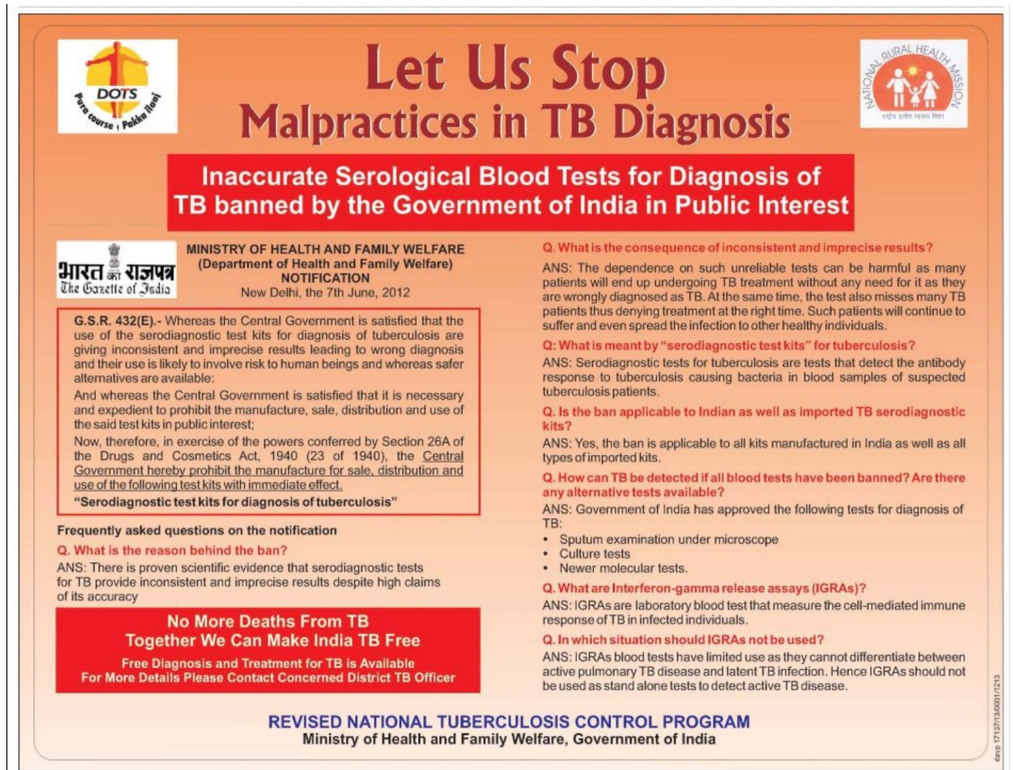
Tests that should not be used to diagnose active TB

- Serology (antibody tests)
- TST
  - Can be negative in very sick people
  - Can lead to necrosis with strong positive response.
- IGRA

TABLE 4.

Overall sensitivities, specificities, and likelihood ratios for antigens evaluated for serodiagnosis of pulmonary TB with assays detecting IgG and/or IgA antibodies

Type of compound	Antigen name	Rv designation	No. of studies	Smear status	HIV status	Sensitivity (%)[a]	Specificity (%)[a]	Likelihood ratio positive[a]	Likelihood ratio negative[a]
Recombinant	38 kDa	0934	12	Positive	+/−	47 (39-55)	94 (86-98)	8.22 (3.41-24.85)	0.56 (0.48-0.65)
	Malate synthase	1837c	8	Positive	+/−	73 (58-85)	98 (95-100)	40.78 (14.43-155.7)	0.27 (0.16-0.42)
	MPT51	3803c	5	Positive	−	59 (38-76)	94 (77-99)	10.50 (2.70-69.69)	0.44 (0.26-0.67)
	MPT51	3803c	4	Positive	+	58 (30-82)	97 (84-100)	19.03 (3.73-172.3)	0.44 (0.19-0.73)
	CFP-10	3874	6	Positive	+/−	48 (29-68)	96 (83-99)	12.11 (3.20-64.63)	0.55 (0.35-0.73)
	TbF6[b]		4	Positive	−	70 (37-90)	93 (69-99)	9.61 (2.23-53.99)	0.33 (0.13-0.66)
	TbF6, DPEP[c]		4	Positive	−	75 (50-91)	95 (86-99)	14.97 (5.43-56.66)	0.26 (0.10-0.53)
Native protein	38 kDa	0934	13	Positive	+/−	49 (37-61)	97 (94-99)	15.73 (8.84-31.55)	0.53 (0.41-0.65)
	38 kDa	0934	7	Negative	−	31 (15-52)	97 (92-99)	9.13 (3.88-24.05)	0.72 (0.51-0.87)
	Ag 85B	1886c	4	Positive	−	53 (20-83)	95 (78-99)	9.36 (2.52-53.81)	0.51 (0.20-0.84)
	Ag 85B	1886c	4	Positive	+	62 (19-92)	97 (89-99)	17.83 (4.04-62.32)	0.39 (0.08-0.84)
	α-Crystallin	2031c	6	Positive	+/−	54 (32-75)	96 (83-99)	13.23 (3.52-66.61)	0.48 (0.28-0.71)
Lipid	DAT		7	Positive	+/−	63 (45-78)	81 (50-96)	3.32 (1.32-13.35)	0.47 (0.30-0.74)
	TAT		4	Positive	+/−	81 (21-99)	44 (24-67)	1.44 (0.42-2.31)	0.42 (0.03-1.71)
	SL-I		4	Positive	+/−	80 (56-93)	59 (8-96)	1.94 (0.89-20.90)	0.34 (0.14-2.22)
	Cord factor		5	Positive	+/−	69 (28-94)	91 (78-97)	7.03 (2.44-20.65)	0.35 (0.06-0.80)



Promising approaches

Holy Grail = Urine dipstick test

Breath test for volatiles

Blood for biomarkers, Mtb

Urine for lipids, glycolipids, proteins or Mtb DNA

Stool for Mtb or microbiome profile consistent with TB

Oral or buccal swab for Mtb

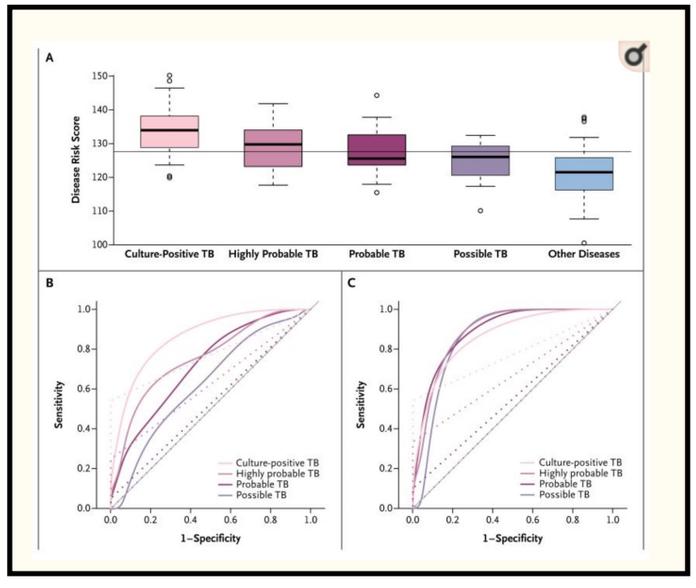
Breathalyser detects tuberculosis

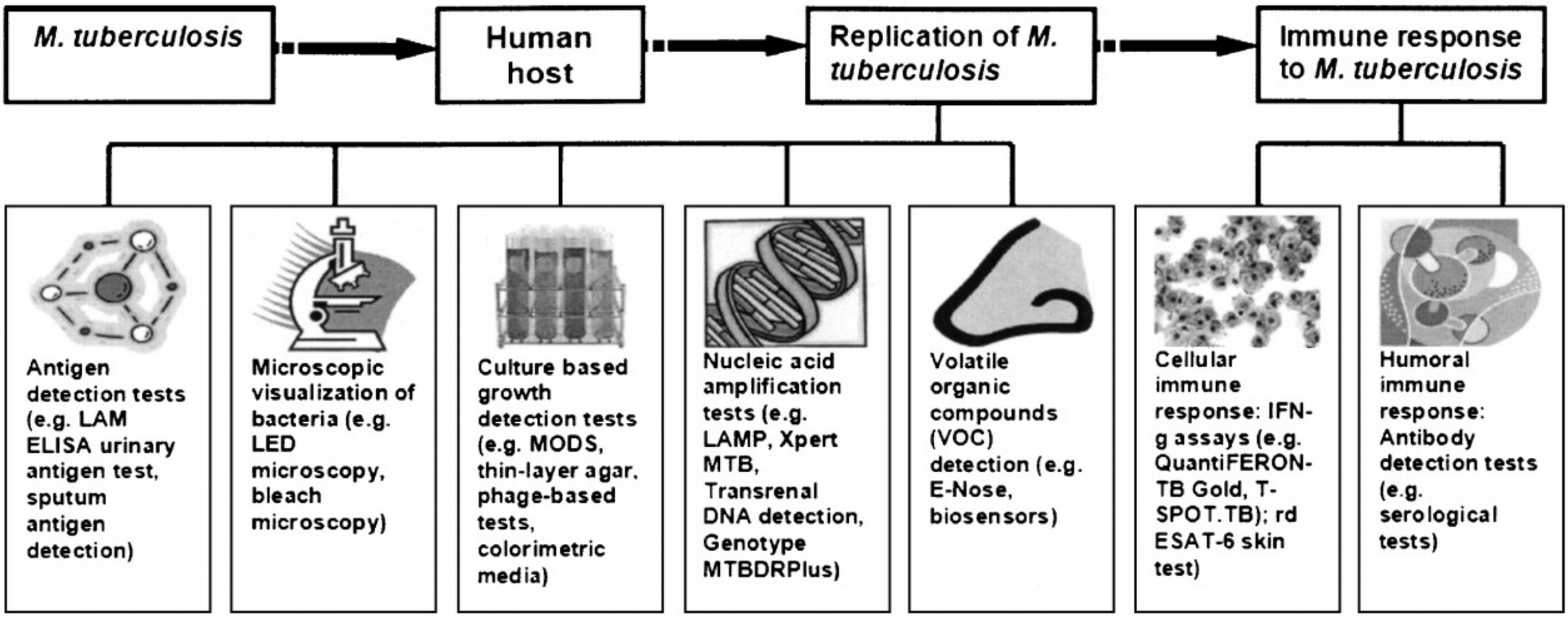
Coughing into a breathalyser could be the new way to detect the most common form of tuberculosis.

The portable device, developed by Rapid Biosensor Systems, would be quicker and easier to use than the current screening method, called the Heaf test.

The Heaf test involves an injection of tuberculin into the skin. The patient has to wait one week to see if a reaction develops, which would indicate exposure to infection.

A simple breath test could detect tuberculosis




M. tuberculosis
Human host
Replication of M. tuberculosis
Immune response to M. tuberculosis
Antigen detection tests (e.g. LAM ELISA urinary antigen test, sputum antigen detection)
Microscopic visualization of bacteria (e.g. LED microscopy, bleach microscopy)
Culture based growth detection tests (e.g. MODS, thin-layer agar, phage-based tests, colorimetric media)
Nucleic acid amplification tests (e.g. LAMP, Xpert MTB, Transrenal DNA detection, Genotype MTBDRPlus)
Volatile organic compounds (VOC) detection (e.g. E-Nose, biosensors)
Cellular immune response: IFN-g assays (e.g. QuantiFERON-TB Gold, T-SPOT.TB); rd ESAT-6 skin test)
Humoral immune response: Antibody detection tests (e.g. serological tests)