UPDATES ON LABORATORY DIAGNOSIS OF TB

BY

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OUTLINE

- Introduction
- Updates on pre analytical area
- Smear and related tests
- Identification and drug sensitivity testing

INTRODUCTION

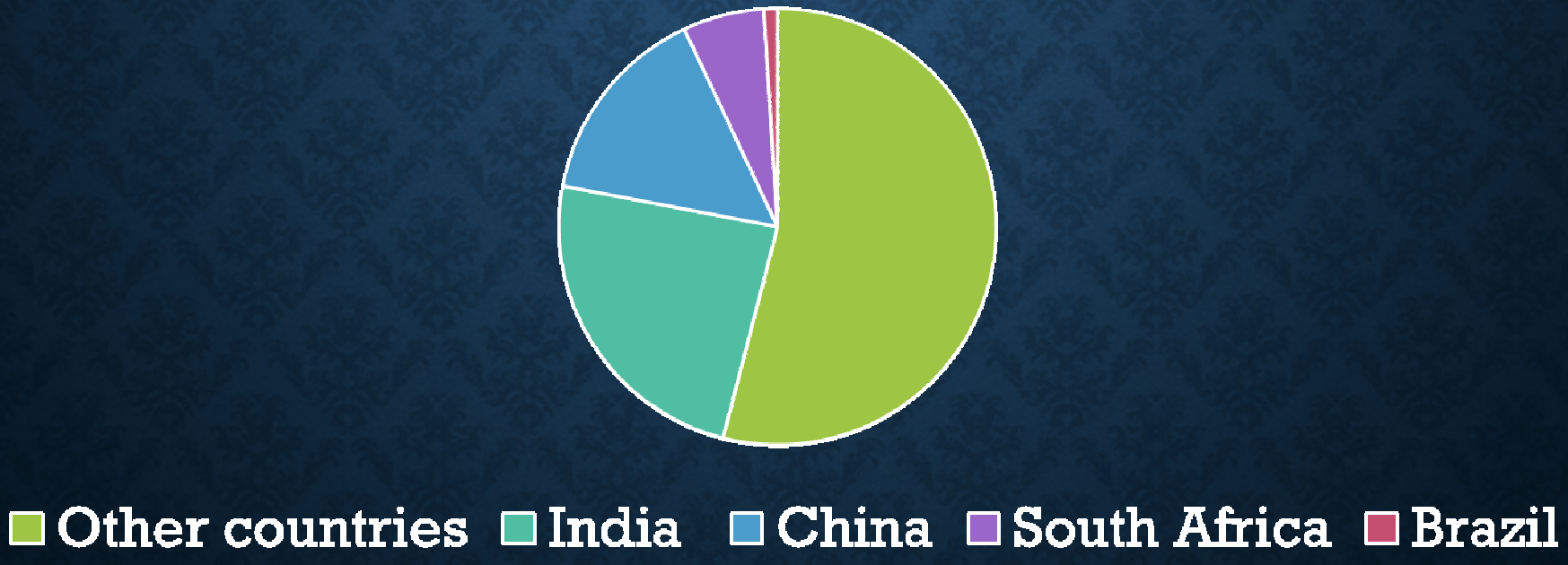
- TB is a global health problem
- 2013 9 million people had TB and and 1.5 million died.
- Estimated 480 000 developed multi drug resistant TB (MDR TB)
- Majority diagnosed by smear microscopy on sputum specimen at the peripheral micoscopy centres.
- Several diagnostic tests endorsed by WHO over the last eight years.

TB DIAGNOSTIC GAP

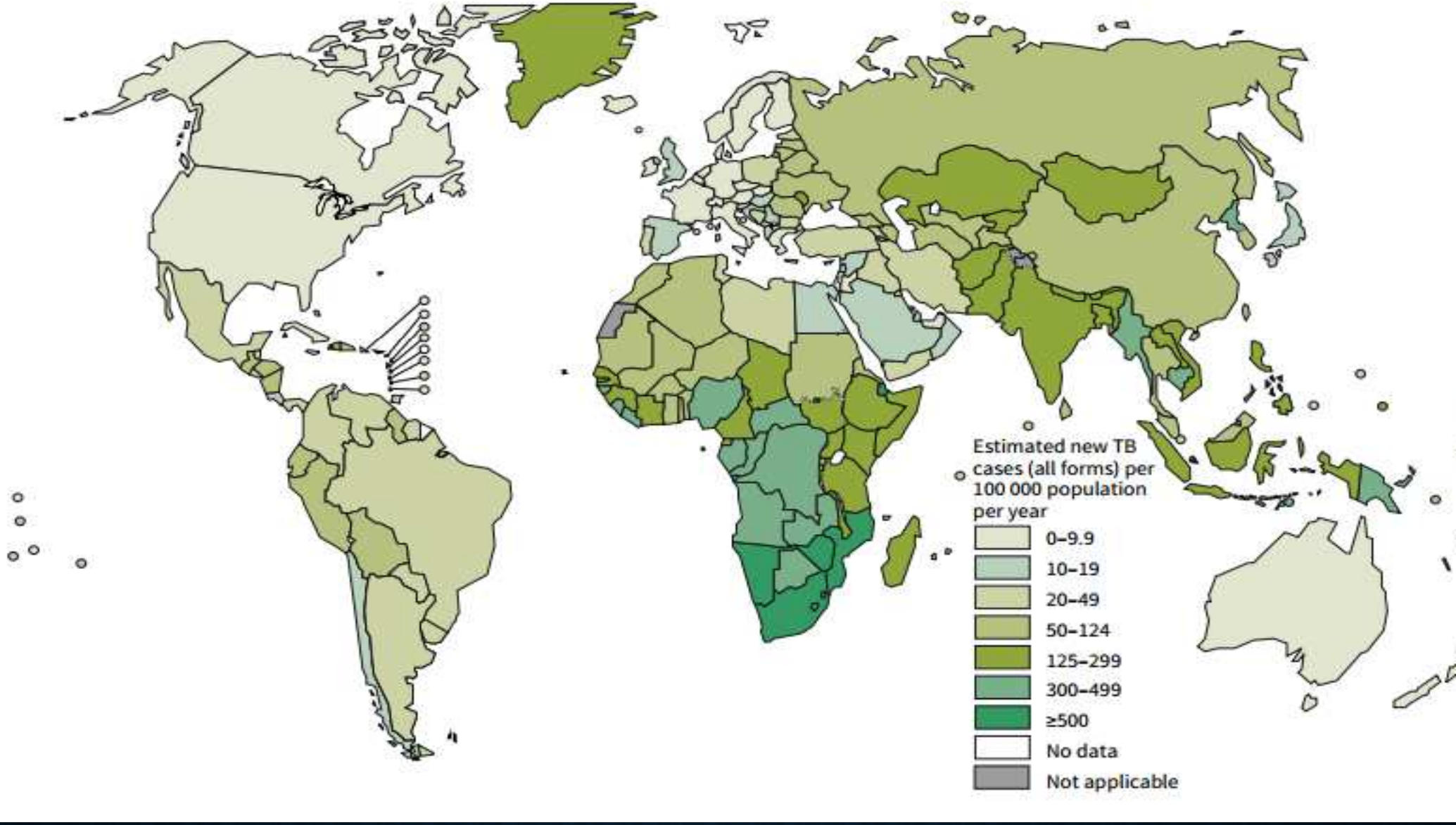
- More effective diagnostics available near patients' care.
- Growing pipeline of new TB diagnostics due to more funds from multinational donors and high burden countries with flourishing economies.
- Market reports showed more than UD$ 1 billion was spent on TB diagnostics in 2004.
- 83 million smear microscopy tests and 47 million chest X-rays
- US$480 million spent in 2013 on TB test alone in 4 countries…

COMMERCIAL INCENTIVES FOR NEW DIAGNOSTICS

Global TB report WHO 2013



Estimated TB incidence rates, 2013



PRIORITIES FOR NEW TB DIAGNOSTICS

- Rapid sputum based test to detect TB with/without drug sensitivity
- Rapid non-sputum based test to detect all forms of TB at point of care
- Community based triage test for use by first contact health care providers to identify persons who need further testing

CURRENT AVAILABLE TESTS IN MALAYSIA (MOH)

- Health clinics – sputum microscopy (ZN or Auramine stain)
- State or regional hospitals – Microscopy and culture (Gene Xpert)
- Regional Public Health Lab – Culture and Line Probe Assay
- National Public Health Lab – Culture, PCR, Line

DRUG SENSITIVITY TEST IN ONE LABORATORY ONLY??

- Dedicated infrastructure – biosafety level 3
- Because handling large bacterial load
- Dedicated and trained staff
- Fully maintained laboratory

LIGHT VS FLUORESCENT MICROSCOPY

- 5 minutes / slide
- 1000x magnification
- Light microscope
- 40-80% sensitivity
- 40 cents per slide

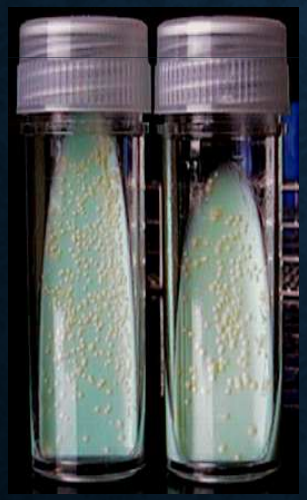
- 1 minute /slide
- 200x and 400x magnification
- LED microscope
- 10% increased in sensitivity
- RM4 per slide

SOLID CULTURE VS BROTH CULTURE (MGIT)

- Total incubation 8 weeks before NO GROWTH
- Positive growth observed after 3 weeks
- Manually observe tube for growth every week
- Contamination rate 5%
- RM 4 per bottle

- Total incubation 6 weeks
- Positive growth after 1 week
- Automated. Incubator will alert
- Higher risk of aerosolisation

SOLID CULTURE VS LIQUID CULTURE



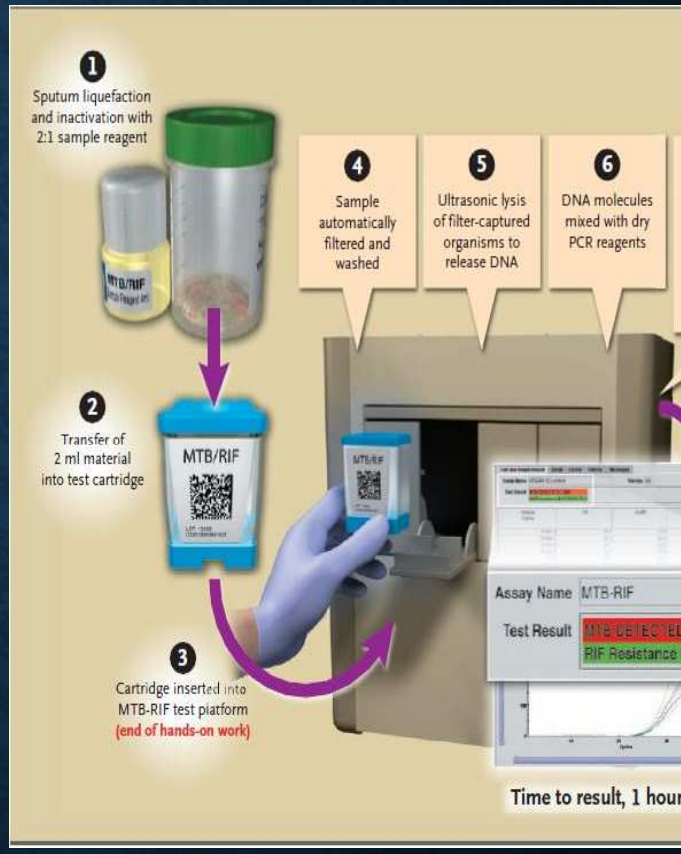
COMMERCIAL NAAT: GENE XPERTS

- 14 Gene Xperts in MOH hospitals
- WHO endorsed IN 2010
- Use limited to

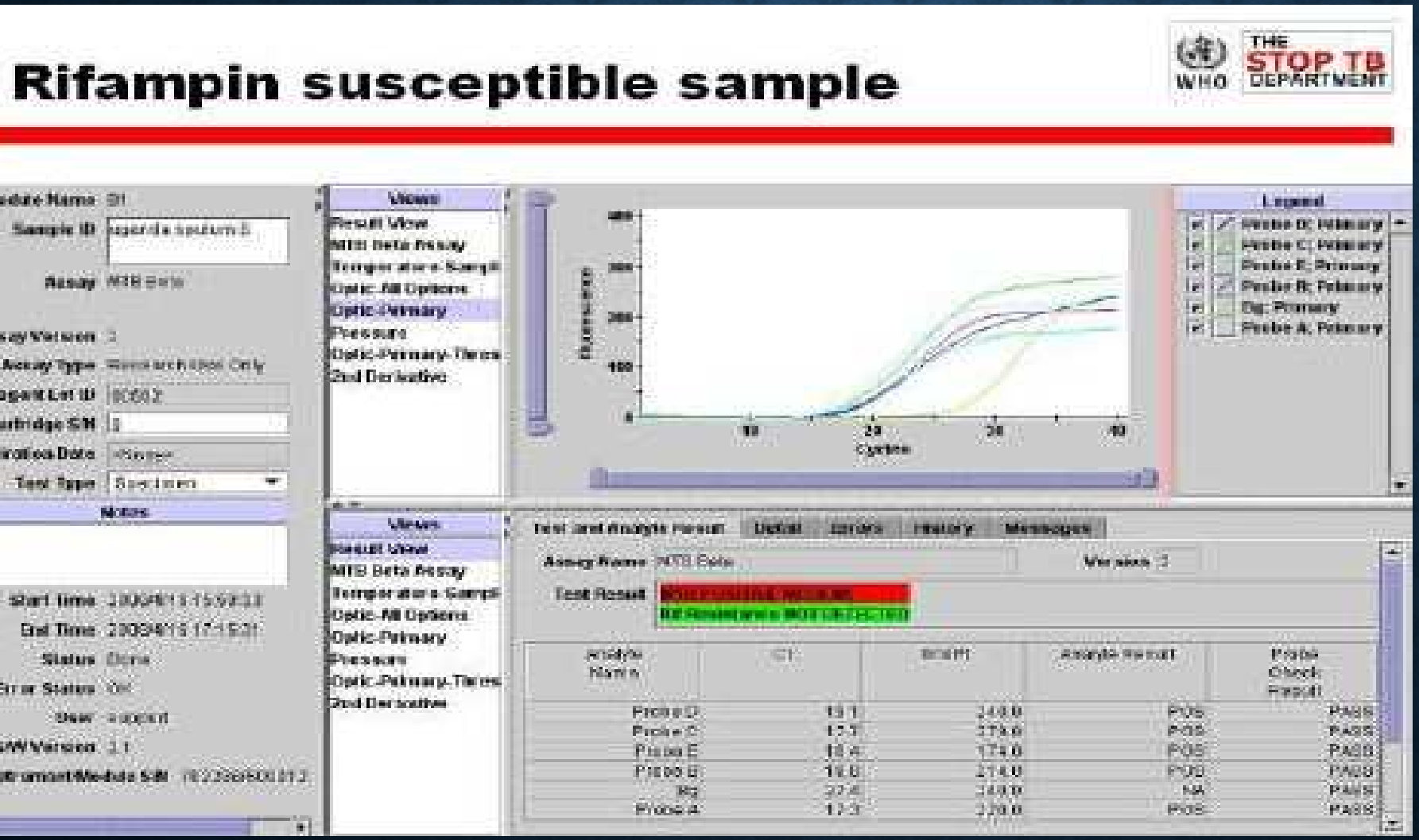
1. ?MDR
2. Contact of MDR patient
3. Paediatrics TB patient
4. HIV with ?TB
5. Extrapulmonary sample

- Minimum sample handling
- Need continuous air conditioned room

GENEXPERT


1
Sputum liquefaction and inactivation with 2:1 sample reagent
2
Transfer of 2 ml material into test cartridge
MTB/RIF
3
Cartridge inserted into MTB-RIF test platform
(end of hands-on work)
4
Sample automatically filtered and washed
5
Ultrasonic lysis of filter-captured organisms to release DNA
6
DNA molecules mixed with dry PCR reagents
Assay Name MTB-RIF
Test Result
RIF Resistance
Time to result, 1 hour

GENEXPERT


Rifampin susceptible sample
WHO
THE STOP TB DEPARTMENT
Views
Result View
MTB Beta Assay
Optic-All Options
Optic-Primary
Pressure
Optic-Primary-Trace
2nd Derivative
Cycles
Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
Rg; Primary
Probe A; Primary
Notes
Test and Analysis Result
Details
Errors
History
Messages
Assay Name
Test Result
Assay Name
CT
Result
Analysis Result
Probe Check Result
Probe D
Probe C
Probe E
Probe B
Rg
Probe A
POS
PASS
NA

LINE PROBE ASSAY (HAIN)

- Need positive smear
- WHO endorsed
- Manual and automated
- From patient sputum samples or culture isolates

1. Detection of MTB complex
2. Detection of MTB complex with rif / isoniazid resistance
3. Detection of MTB complex with resistance to fluroquinolones, aminoglycoside, cyclic peptides, and ethambutol

LINE PROBE ASSAY

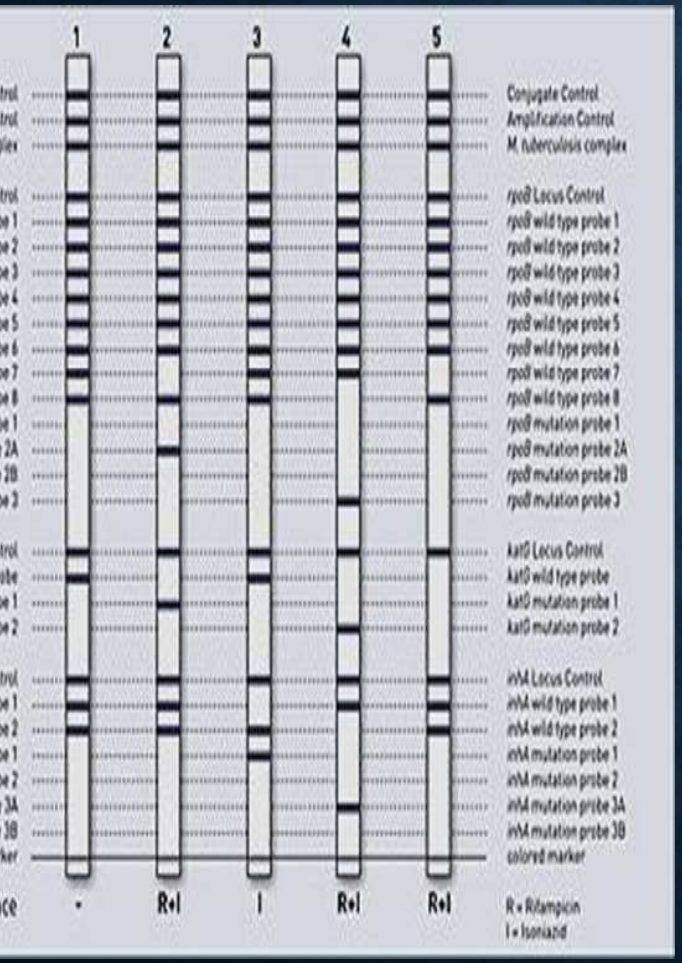
- From Mycobacterium cultures

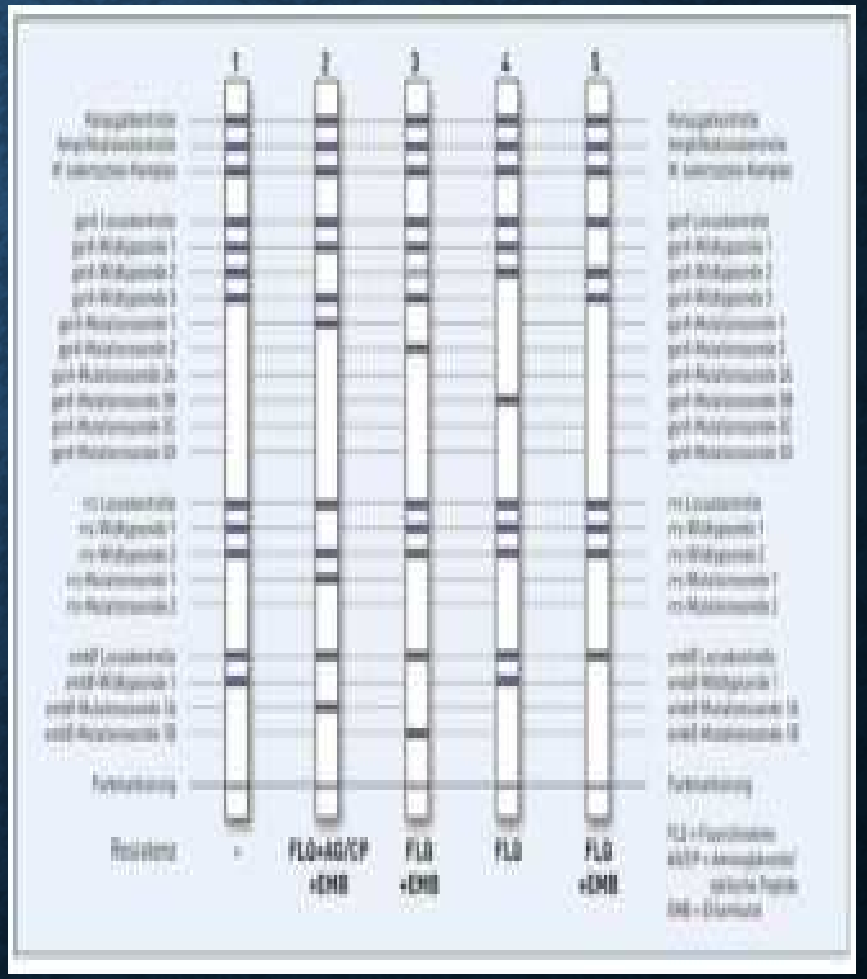
1. Identification of MTB complex
2. Identification of MTB complex and 27 Non Tuberculous Mycobacterium (NTM)
3. Identification of further 19 NTMs

Performance Comparisons

	LPA	GeneXpert
Require 3 rooms	Yes	No ✓
Dedicated equipment	Yes	Yes ✓
Stock control	Complex	Simple ✓
Need for BSC	Yes	??No ✓
TAT	≈ 1-2 days	≈2 hrs ✓
Test complexity	Very	Simple ✓
Requires processed sputum	Yes	No ✓
Sensitivity in Sm-/Cult+	≈20%	≈75% ✓
Recommended for Sm- specimens	No	Yes ✓
Recommended for extrapulmonary TB	No	Limited ✓
INH resistance	Yes ✓	No
Second-line DST	Yes ✓	No
Interpretation of results	Challenging	Computerised ✓

LINE PROBE ASSAY (LPA)





WHAT IS NEW?

Global TB diagnostic pipeline

DIAGNOSTIC TESTS RECENTLY ENDORSED BY WHO

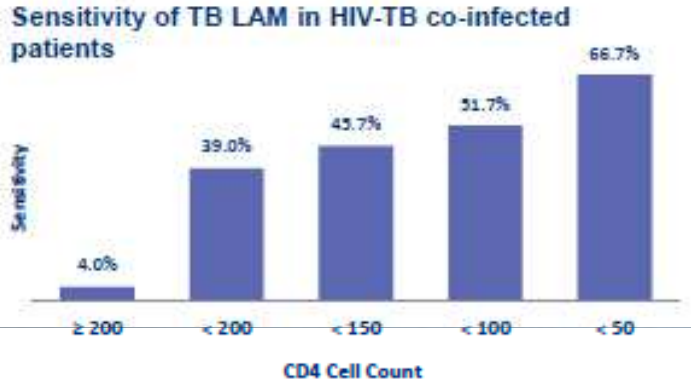
- Urine LAM (Alere) in 2015
- TB LAMP (Eiken) in 2016

LAM

- Antigen test for urine
- Lipoarabinomannan (LAM) found in growing mycobacterium cell wall
- Released in blood and subsequently in urine
- Sensitivity of the test correlates with low CD4 count.
- Only useful in AIDS cases

Urinary LAM (Alere Determine) as a screening test for TB in HIV positives with low CD4 cell counts?

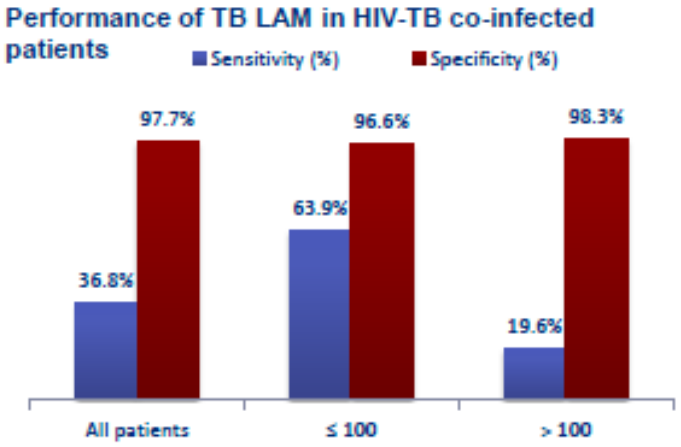
Lawn, SD et al 2012



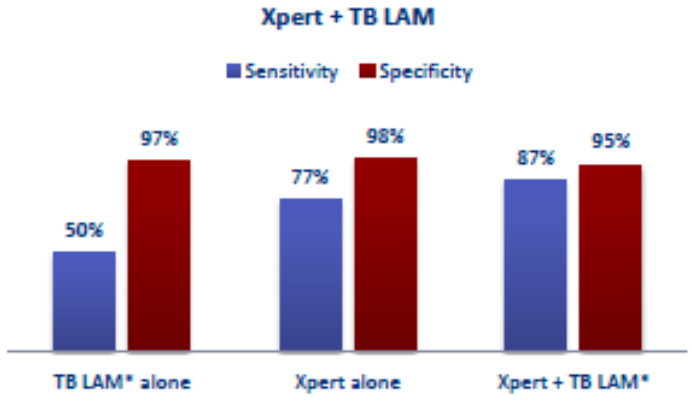
Peter, JG et al 2012

Against composit RS	Sensitivity (%)	Specificity (%)
All patients	45	96
CD4 > 200	29	100
CD4 ≤ 200	52	94

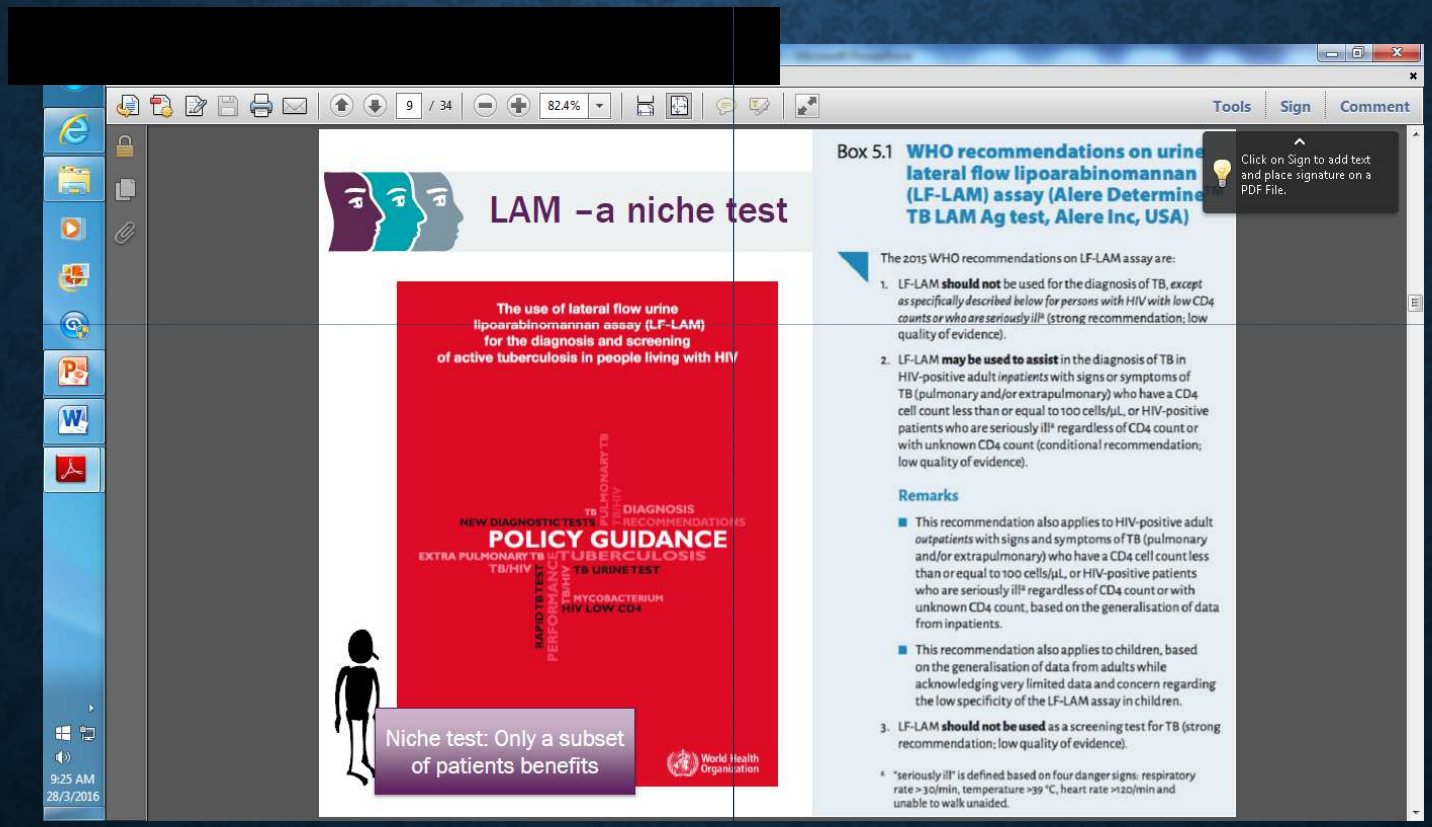
Dorman, S et al 2012



Shah, M et al 2012



LAM – URINE TEST



TB LAMP (EIKEN)

- Manual molecular test
- Feasible to be implemented in peripheral microscopy lab
- Relatively high throughput
- Do not require sophisticated instrumentation
- Biosafety requirements similar to those performing sputum smear microscopy
- May be used as replacement to smear microscopy or adjunct to smear microscopy in smear negative samples
- Available in China, South Korea, Taiwan, Thailand and Japan (August 2016)

LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

- Amplification and detection on gene completed in a single step by incubating mixture of sample, primers, DNA polymerase with strand displacement activity and substrates at constant temperature 69˚C
- Reagents located at the cap of reaction tubes
- Detection by fluorescence dye

Sample preparation

DNA extraction (PURE)

Amplification (LAMP reaction)

Detection

l using sable

up

Heating tube

ock *90° C for 5 min*

Adsorbent tube

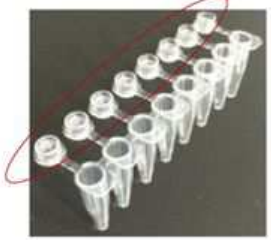
Shake

Injection cap

35 µl
30 µl

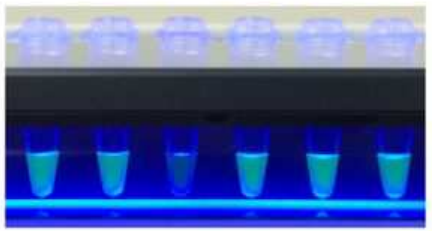
Reaction tubes

Dried reagents



Close lids and let stand upside down for 2 min; then mix

67° C for 40 min



Fluorescent signal detection

DEVELOPMENTS IN OTHER AREAS?

STILL UNDERGOING VALIDATION…

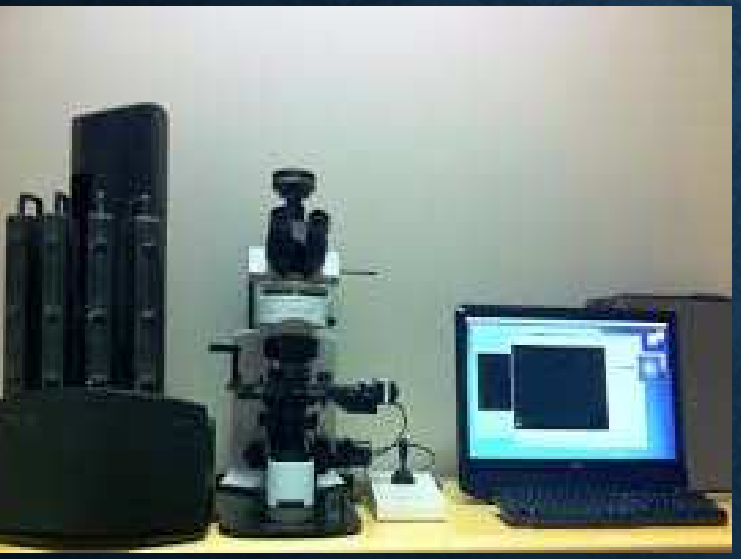
IMPROVEMENT IN SAMPLE COLLECTION

- Major persistent problem with collection of adequate specimen when patient unable to provide sufficient volume of specimen
- Sputum induction may need additional effort, cost and increased risk to the medical personnel
- New product: Lung Flute from Medical Acoustics (USA)
- Non-invasive, user holds a small plastic device to their lips and exhale into it. This creates vibrations in the lungs that help to loosen and liquefy sputum in the alveolar cavities.
- Sensitivity hypertonic saline vs Lung flute is 78.4% and 84.3%

MAINTAINING SPECIMEN IN OPTIMAL CONDITION

- Product are being developed to liquefy and decontaminate sample allowing it to be transported without cold chain
- Allow transport of samples up to 8 days without cold chain
- Significant reduction in culture contamination fom 13% to 0%

AUTOMATED MICROSCOPY

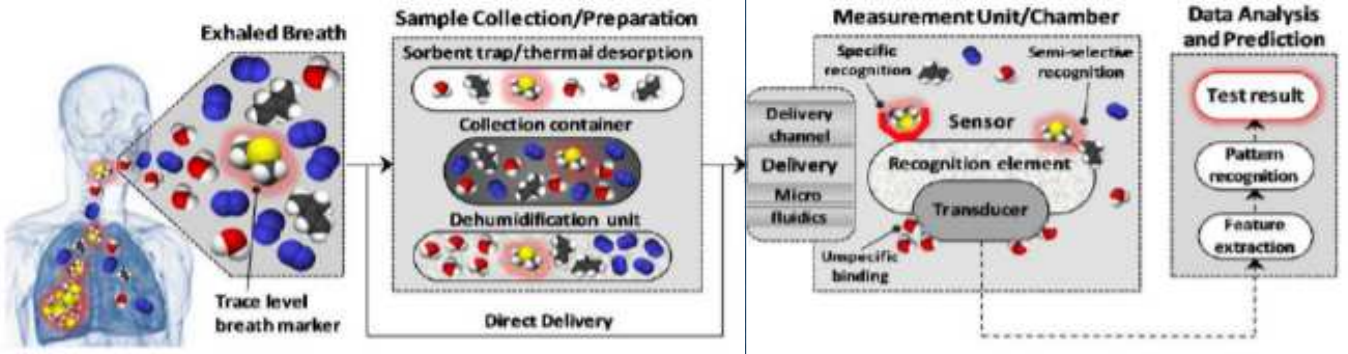


Automating smear microscopy

RAPID SCREENING OF TB INFECTION AT POINT OF CARE: VOLATILE COMPOUNDS

- Proven by giant pouch rats which can accurately predict active PTB by sniffing sputum specimens
- Still in early developments
- eNose undergoing validation studies in Bangladesh, Indonesia Kenya, South Africa and Venezuala

Overview of the processes involved in breath testing.



[Konvalina and Haick 2014]

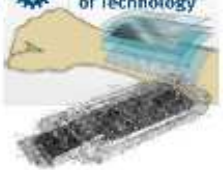
Metal-oxide-based olfactory sensor [Bruins et al. 2013]

Cough/aerosol collection combined with immunoassay based antigen detection [McNerney et al. 2010]

Portable GC coupled to surface acoustic wave (SAW) detector [Phillips et al. 2013]

^{13}C-urea is converted to $^{13}CO_2$ in the patients lung if bacteria is present and detected in a spectrometer [Jassal et al. 2010]

New sensors based on array of chemical films: VOC leads to changes in electrical conduction



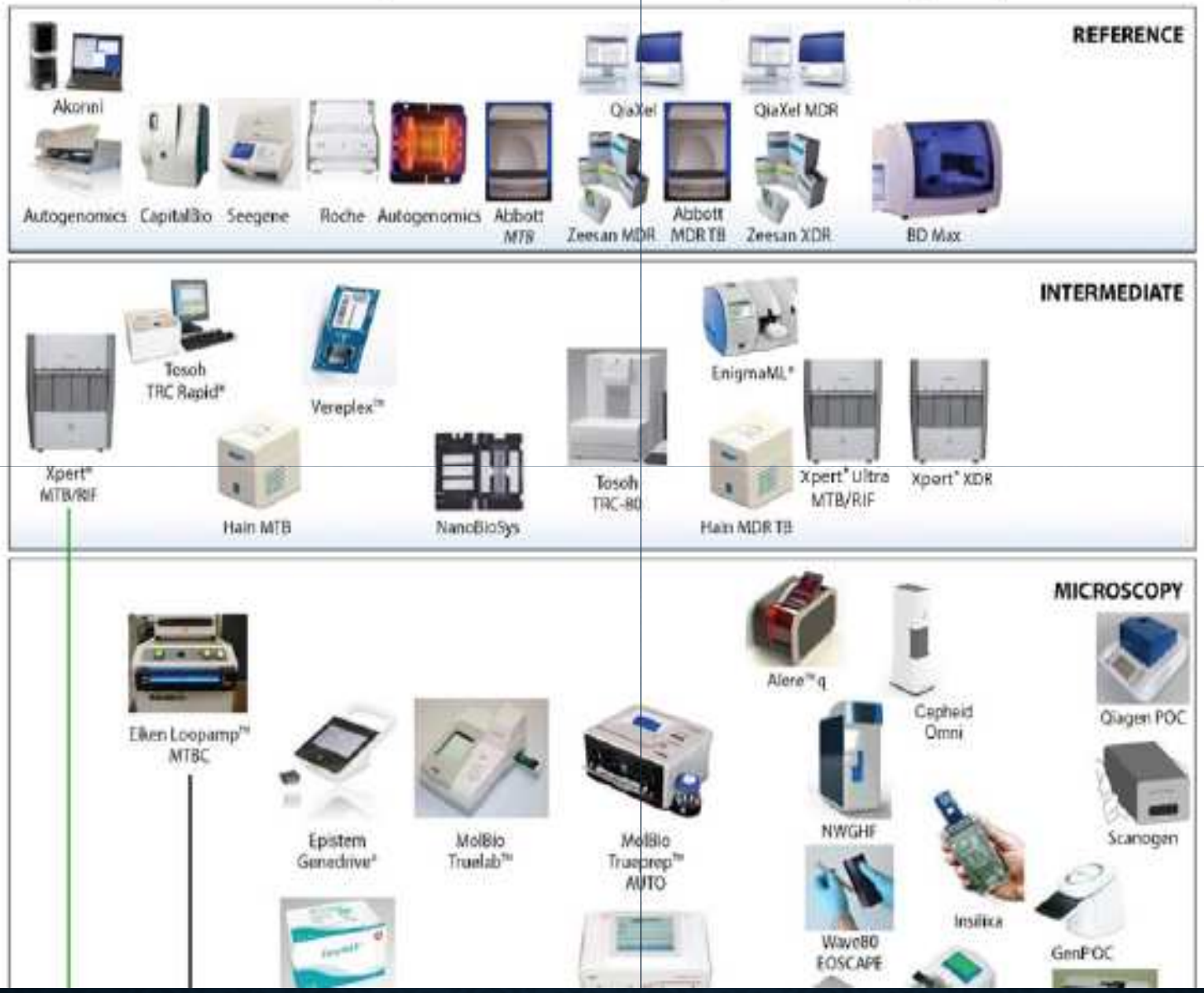
Ligand coated monolayer gold nanoparticles to analyze breath (in an adhesive patch)

Colorimetric sensor array for odour visualization.

Proof-of-principle data from feasibility studies available for some technologies (performance thus far not meeting TPP, limited independent study data)

SMELL THE TB

Intended laboratory location and release date (actual or anticipated)



MOLECULAR DETECTIONS /DST

- Genedrive (Epistem)
- Truelab(Molbio)
- Expert Ultra

All are in late or completed development and available in some countries

Awaiting WHO endorsement

MICROARRAY-BASED PLATFORMS

- Oligonucleotide capture probes are printed as clearly defined spots on a surface
- Their smaller size enables more spots to be printed to detect amplified target DNA
- Offer greater discriminatory power to a wider variety of drug resistance markers in a single test format
- Many are used in research purposes as yet.

CONCLUSION

- The last two decades showed tremendous progress in TB diagnostic tests
- Simpler, faster and cheaper test are needed and being developed to diagnose TB and MDR TB at the point of care testing
- TB has been here for a very long time and we will never win IF

WE KEPT BRINGING A KNIFE TO A GUNSHOT FIGHT

THANK YOU