Advanced TB Diagnostic Research Course

After Gene Xpert: what does the future look like?

March 6-9 2012 (Cape Town)

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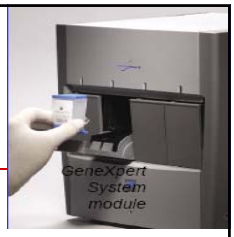
Conflict of interest: none

Diagnosis of TB

- Why do we need new tests and what do we need (DR-TB, EPTB)?
- General approach to TB diagnosis and emerging technologies
- Lack of suitable compartment-specific antigenic targets
- Point of care platforms: NAAT with POC detection
  Multiplex immunoassay including aptamers
  Optical readouts
  Biosensors
  Lateral flow assays
- Several proof of concept studies so why do they not readily work?
- Why is there limited development of new diagnostics
- Approach to evaluating new diagnostics (categorisation, study design, reference standards, rule-in vs rule-out etc)

Why do we need new tests?



- Xpert is accurate & cost-effective but very costly (even at $10 per cartridge consume about 25% of SA NTP budget)
  - stable power supply
  - suited to centralised rather than decentralised use
  - in up to a third of cases diagnosis cannot be made (sputum scarce, EPTB, smear negative TB undiagnosed)
- Large burden of undiagnosed TB (cost and access)
- Lack of a cheap same day test (human aspect and poverty)

What do we need?

TABLE 2. The ideal rapid test: ASSURED criteria

A = Affordable
S = Sensitive
S = Specific
U = User-friendly (simple to perform in a few steps with minimal training)
R = Robust and rapid (can be stored at room temperature and results available in <30 min)
E = Equipment-free or minimal equipment that can be solar-powered
D = Deliverable to those who need them

Peeling RW, Clinical Micro and Infection, 2010

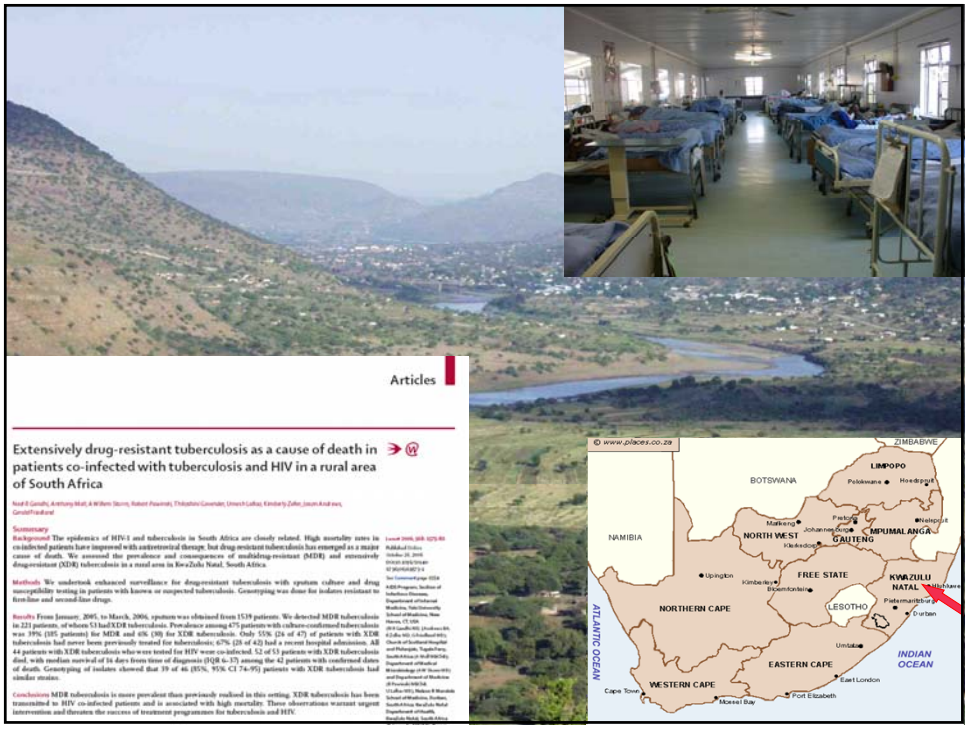
- Do we need a targeted approach? i.e. only in smear negative TB

  Theron and Dheda, ERJ, 2011
- Do we need rifampicin drug susceptibility results?

Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study

Keertan Dheda*, Karen Shean*, Alimuddin Zumla*, Motasim Badri*, Elizabeth M Streicher, Liesl Page-Shipp, Paul Willcox, Melanie-Anne John, Gary Reubenson, Darshini Govindasamy, Michelle Wong, Xavier Padanilam, Alicia Dziwiecki, Paul D van Helden, Sweetness Siwendu, Julie Jarand, Colin N Menezes, Avril Burns, Thomas Victor, Robin Warren, Martin P Grobusch, Martie van der Walt*, Charlotte Kvasnovsky*

- Treatment outcomes of 199 patients with XDR-TB
  Dheda K, Shean K, Warren R, Willcox P, Lancet, 2010
- Earliest cases of XDR-TB in 1992
  Symons G, Dheda K, SAMJ, 2011



ORIGINAL RESEARCH | Annals of Internal Medicine

High Incidence of Hospital Admissions With Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis Among South African Health Care Workers

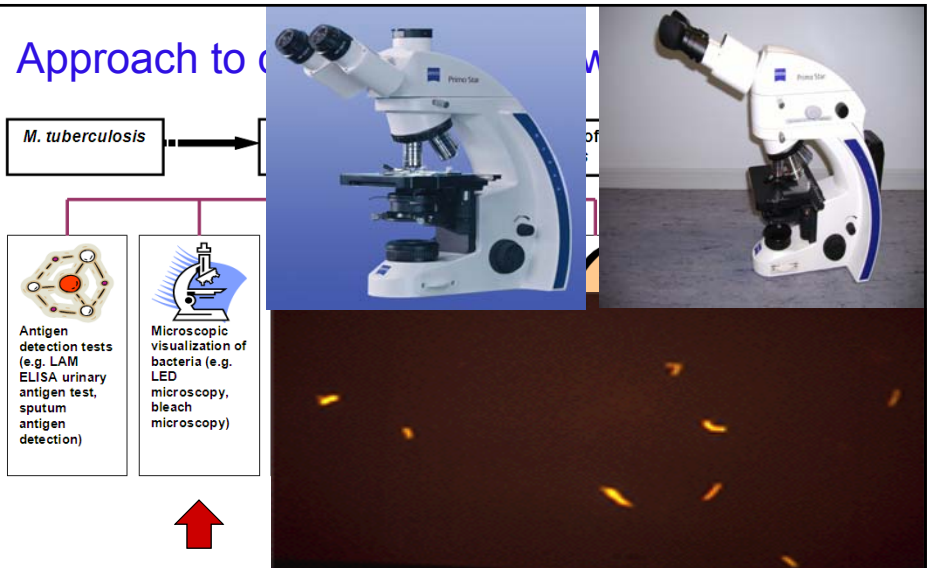
Max R. O'Donnell, MD, MPH; Julie Jarand, BSc, MD; Marian Loveday, BSc, MPhil; Nesri Padayatchi, BSc, MBChB, DCH, DTM+H, DHSM, DPH, MS(Epi); Jennifer Zelnick, MSW, ScD; Lise Werner, MSc; Kasavan Naidoo, MSc, BSc; Iqbal Master, MBChB; Garth Osburn, MBChB; Charlotte Kvasnovsky, MD, MPH; Karen Shean, MSc; Madhukar Pai, MD, PhD; Martie Van der Walt, PhD; Charles R. Horsburgh, MD, MUS; and Keertan Dheda, MBBCh, PhD

(23 XDR-TB and 208 MDR-TB HCWs in KZN)

	HCWs	General Population	Incidence Rate Ratio (95% C.I.)*
Annual MDR or XDR-TB Incidence	66.8/100,000	11.7/100,000	5.71 (4.96-6.69)
Annual MDR-TB Incidence	62.3/100,000	10.7/100,000	5.82 (5.03-6.87)
Annual XDR-TB Incidence	4.5/100,000	1.04/100,000	4.33 (2.69-8.18)

O' Donnell, Padayachi, Dheda; Annals Intern Med; 2010
Jarand J & Dheda K, TMIH, 2010

Approach to d



Visualise the bug- LED microscopy, various techniques to concentrate the bugs (AB, magnets, spin filters)

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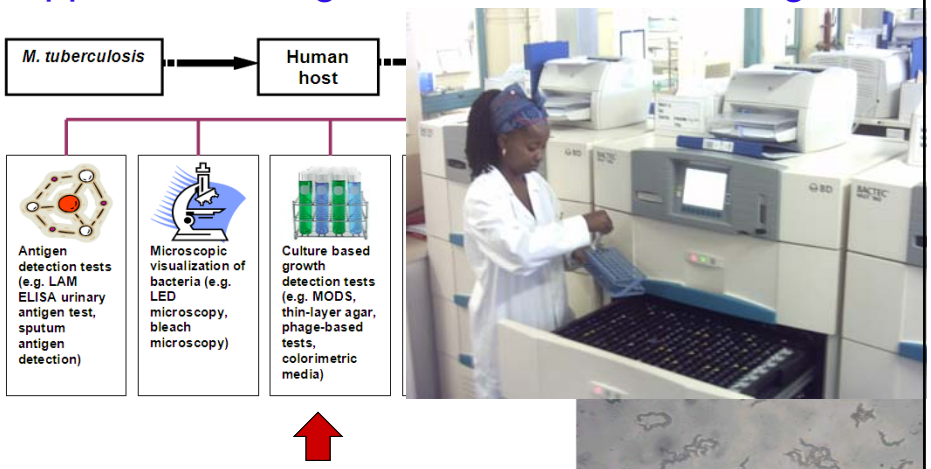
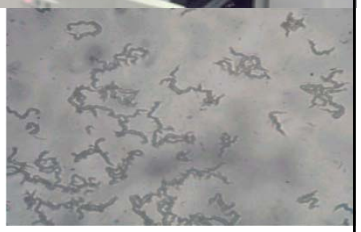
Pai et al. Sem Resp Crit Care Med 2008

Approach to diagnosis and new technologies

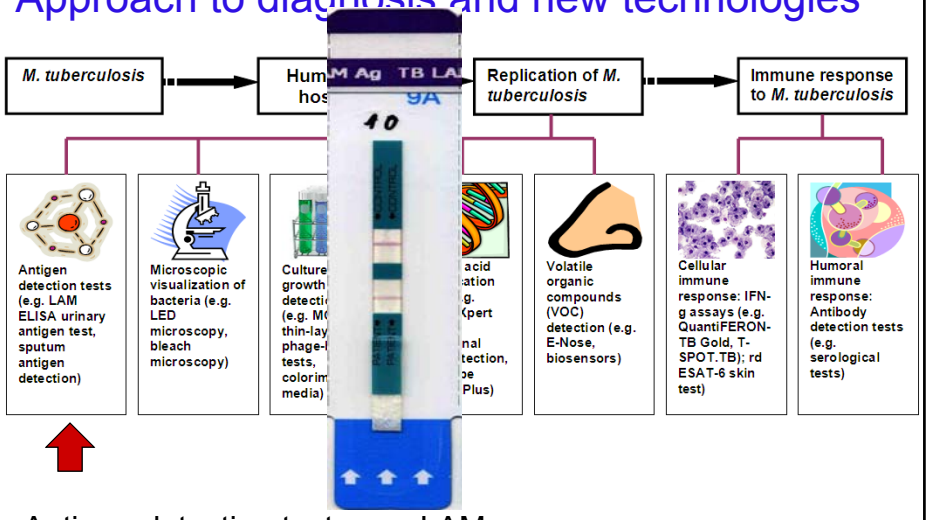




Grow the bug- liquid culture, MODS

Pai et al. Sem Resp Crit Care Med 2008

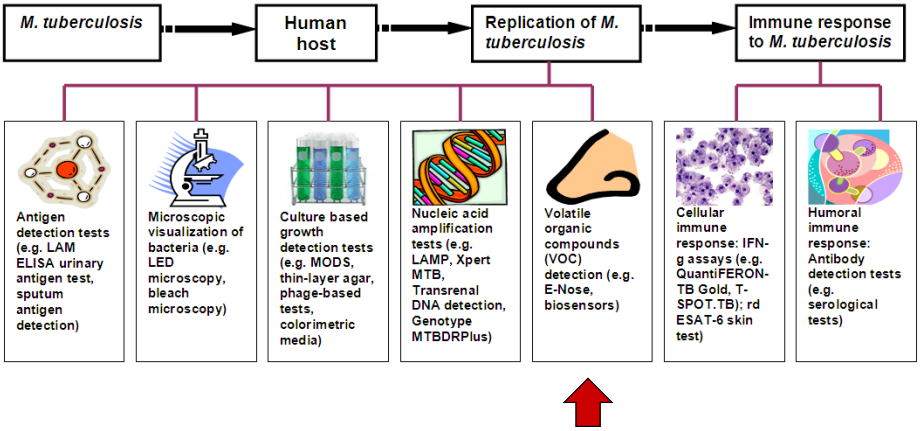
Approach to diagnosis and new technologies



Antigen-detection tests e.g. LAM

Pai et al. Sem Resp Crit Care Med 2008

Approach to diagnosis and new technologies



Volatile organic compounds

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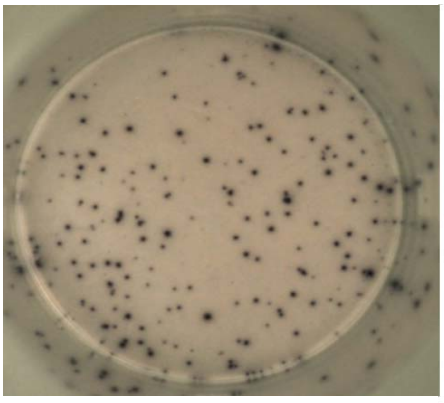
Pai et al. Sem Resp Crit Care Med 2008

Approach to diagnosis and new technologies



IGRAs (no role in active TB)
Serological tests (no role) but resurgence in interest about multiplex approaches

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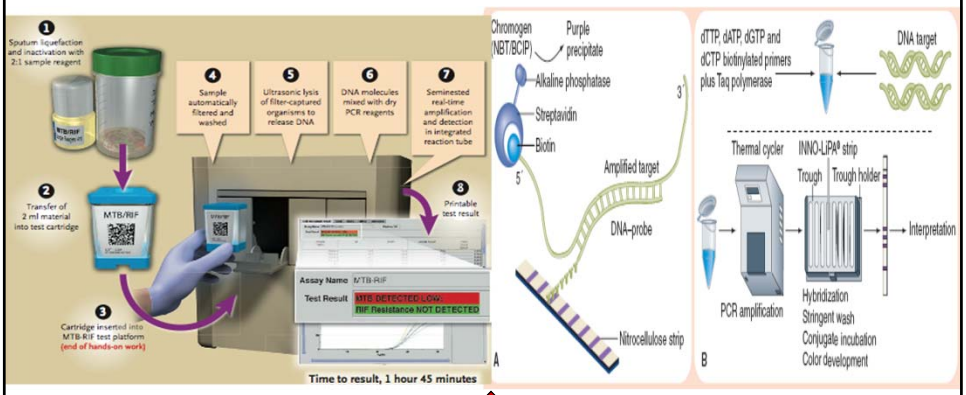
Pai et al. Sem Resp Crit Care Med 2008

Approach to diagnosis and new technologies



NAATs- urine, sputum, EPTB
Gene Xpert, LPA, Roche, SDA (BD)

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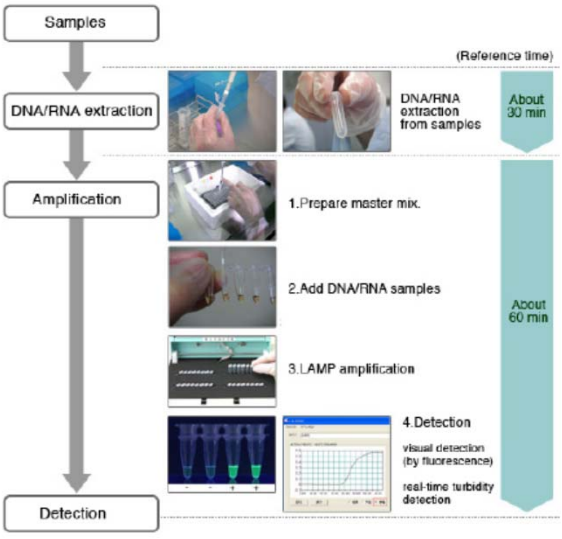
Pai et al. Sem Resp Crit Care Med 2008

Simplified NAAT: LAMP (loop mediated isothermal amplification)



Rapid (1 hour), isothermal, high through-put, clinical samples

Feasible in high burden settings (hospital or central laboratories)

Sens in sputum smear negative TB 49%

Limited data in HIV+

Cheaper

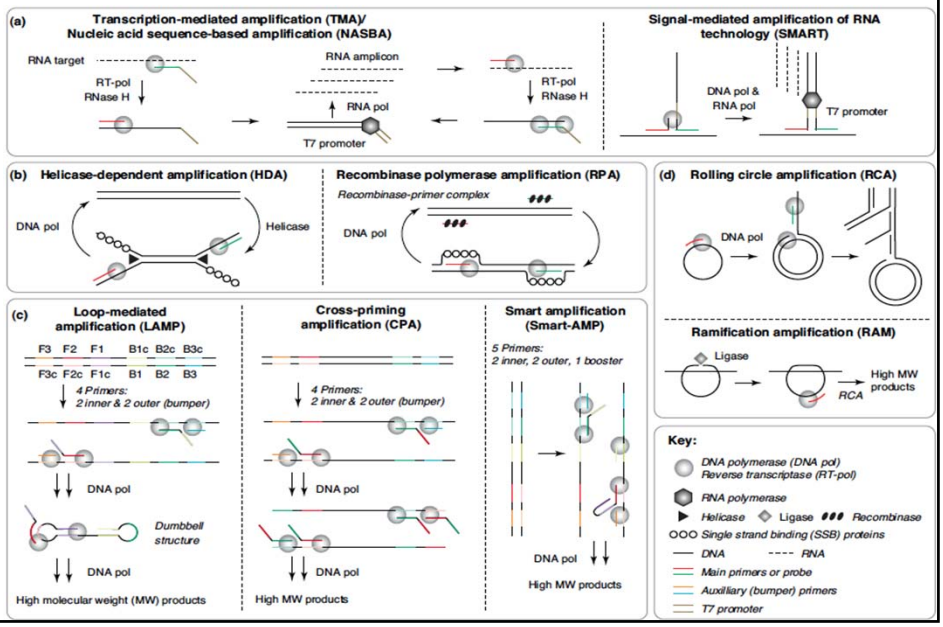
Cannot multiplex

Boehme CC, J Clin Micro, 2007

USTAR- LAMP

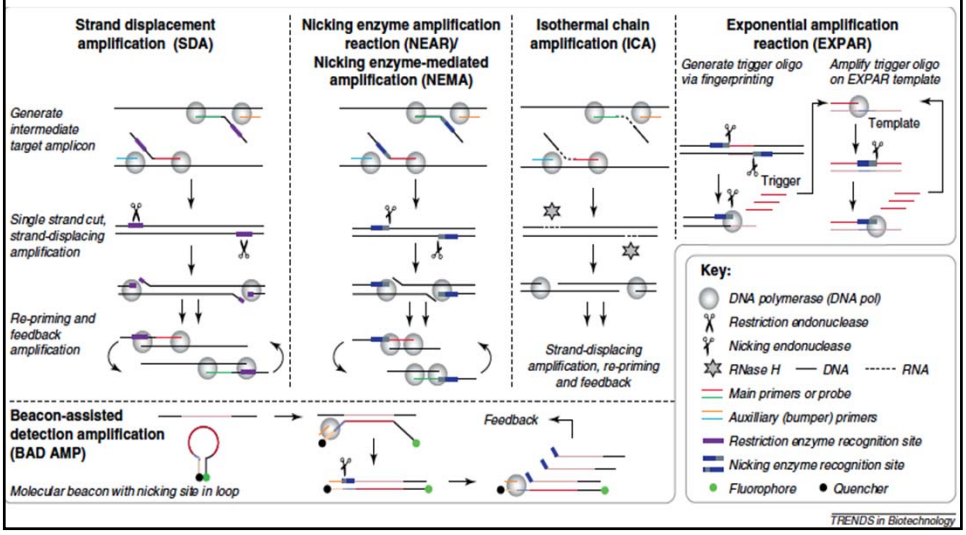
http://www.bioustar.com/en/product_show.aspx?id=39

Overview of isothermal NAAT reactions- differ by enzyme type and number, restriction or nicking sites, RNA or DNA-based, temperature, type of primer complex etc



Isothermal methods based on polymerase-based extension

Niemz A, Trends in Biotech, 2011



Several new commercial platforms that lend themselves to POC NAAT detection are now available

http://www.iquum.com/products/analyzer.shtml

Cartis

Integrates all the diagnostic steps needed to provide true sample-in to result-out functionality, providing a significantly shorter turnaround time than current MDx technologies while requiring minimal hands-on time and training.

http://www.biocartis.com/cms/index.php?page=molecular-diagnostics-platform

Enigma

Enigma's first clinical instrument, the ML (mini laboratory), is a fully automated molecular test platform ideally situated to life in both the clinical laboratory and at the point-of-care including clinics, doctor's surgeries and pharmacies.

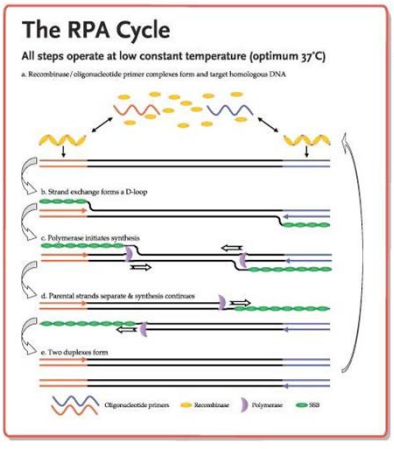
http://www.enigmadiagnostics.com/template2.php?page=instruments.php

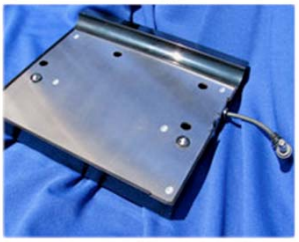
Idahotech RAPID System

The Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.) is a portable real-time PCR system designed to identify biological agents.

http://www.idahotech.com/RAPID/index.html

TWISTDX



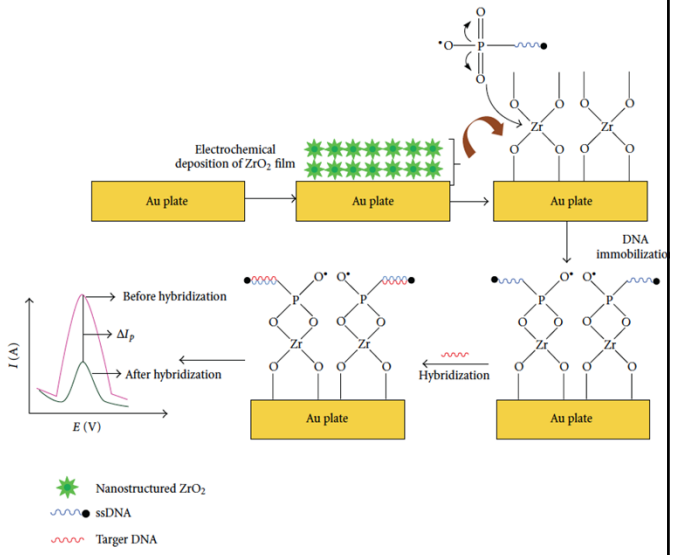


http://www.twistdx.co.uk/products/twista/

OptiGene- amplification and detection platform for LAMP

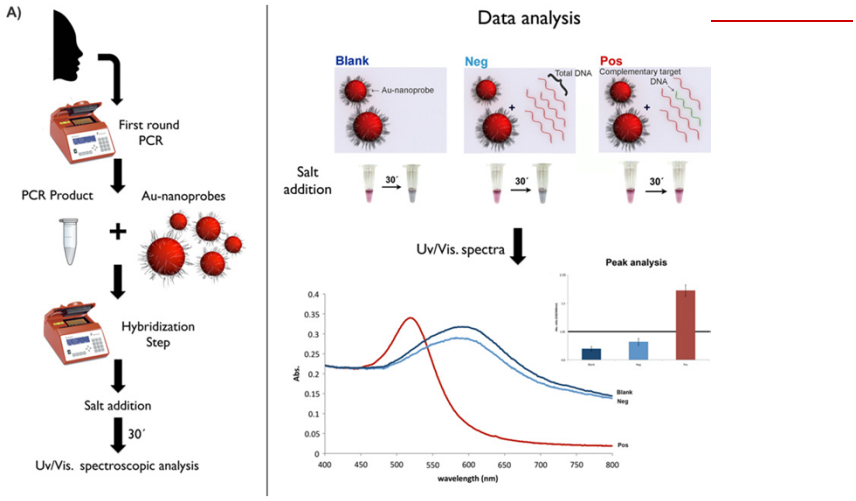
http://www.optigene.co.uk/applications.htm

EC detection platform for NAAT products



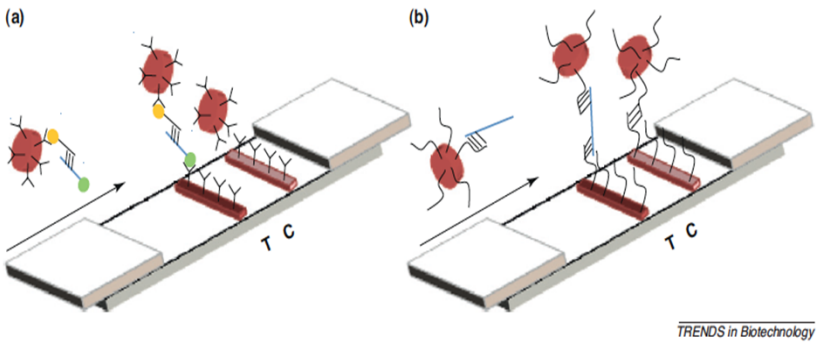
Gonzales- Diaz M, Biosens & Bioelec, 2005

Gold nanopartcles for detection of SNPs for rifampicin resistance using calorimetric readout

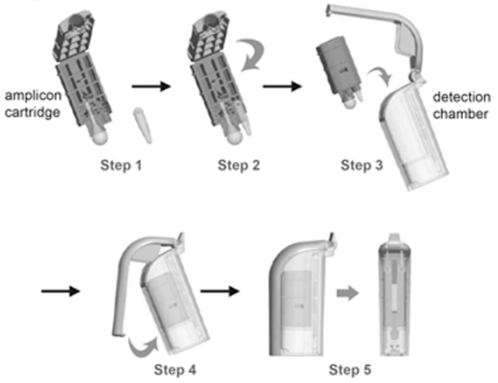


Veigas B, Nanotech, 2010

Lateral flow readout for NAAT products



Best Cassette- detection platform

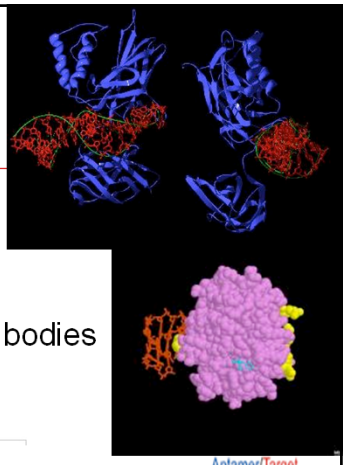


http://www.biohelix.com/products/BESt_Cassettes.asp

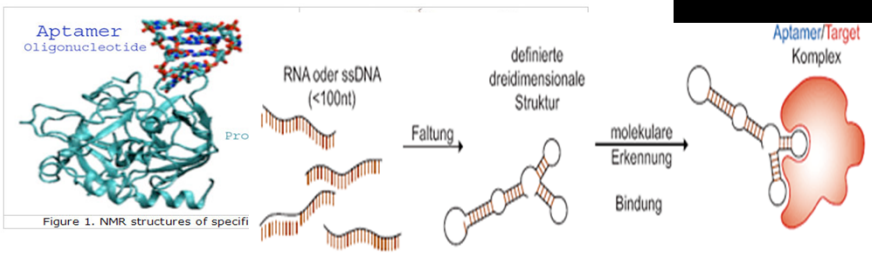
Antigen or antibody immunoassays

- My use antibodies in multiplex or array format
- Generated through peptide arrays (Antigen Discovery Inc) or from exposing fractionated antigen mixtures to antibody libraries

Alternative detection technologies: Aptamers

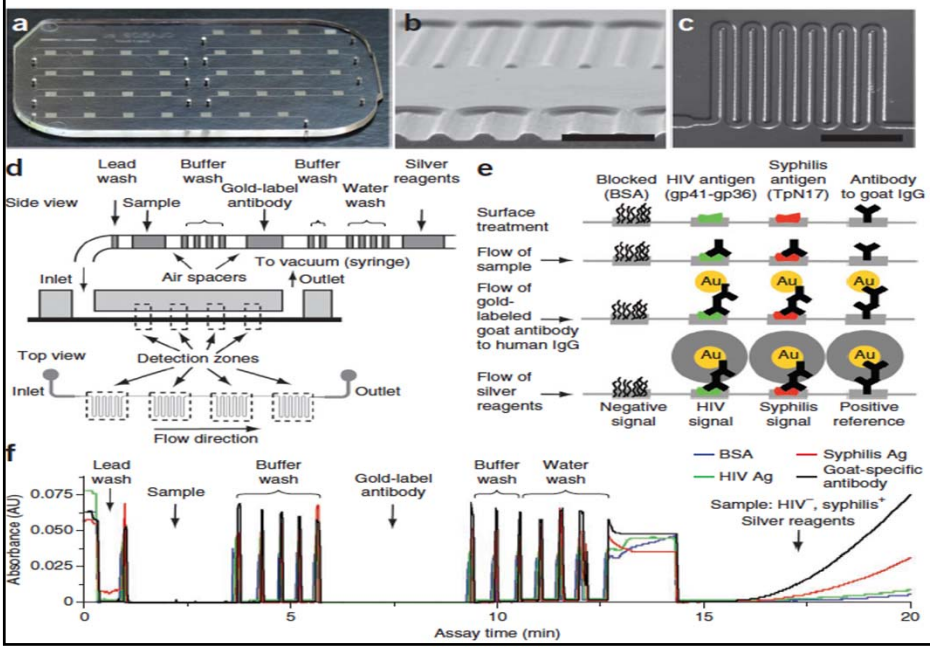


- Aptamers, in contrast to protein-based antibodies, are simply 'chemical' or NA antibodies



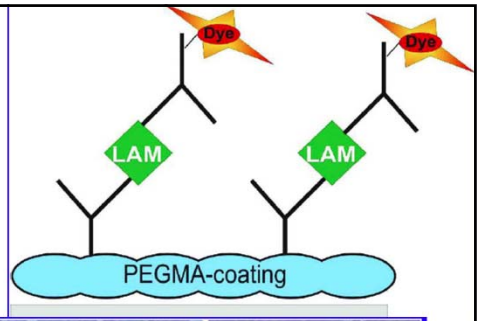
Microfluidics M-Chip platform using optical density detector

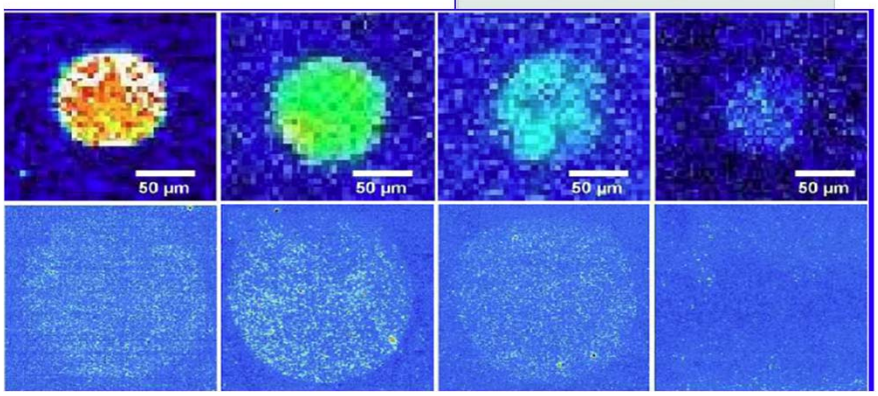
Chin CD, Nature Med, 2011



FLISA- detection of fluorescent signal at high magnification- 3 orders of magnitude more sensitive than ELISA

Schmidt R, J of Proteome R, 2011

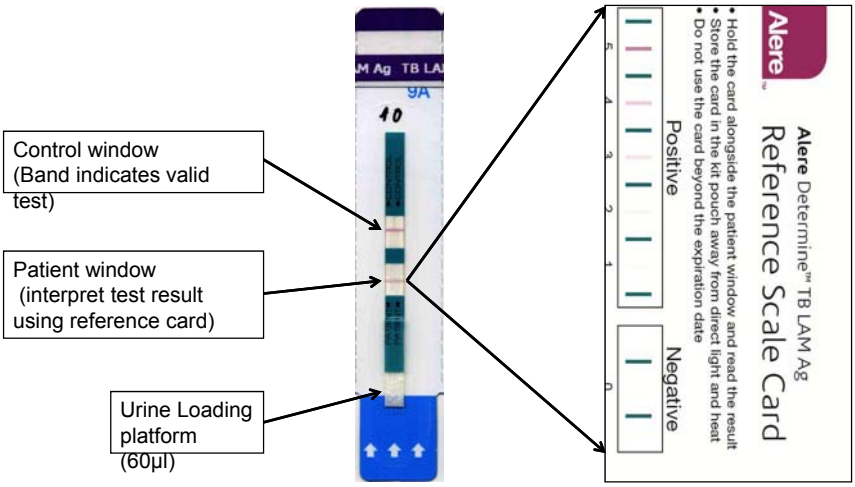




Development of readouts limited by POC detection technologies

- NAAT adapted to a lateral flow format
- LAM antigen detection test
- SERS
- Biosensors- (electro-chemical detection, piezoelectric quartz crystal biosensors, magnetoeleastic biosensors)

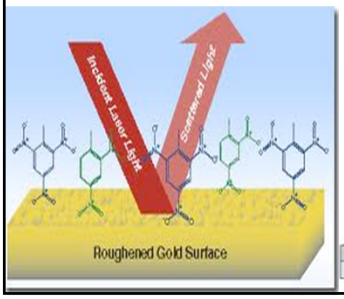
Alere Determine™ TB LAM Ag rapid test and reference card for used for grading and interpretation

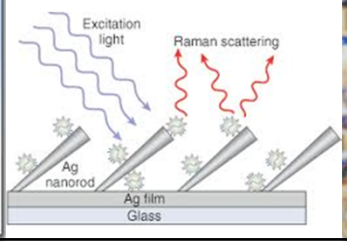


Strip test (15 to 20min; under $5)

Aptamers and a SERS detection platform

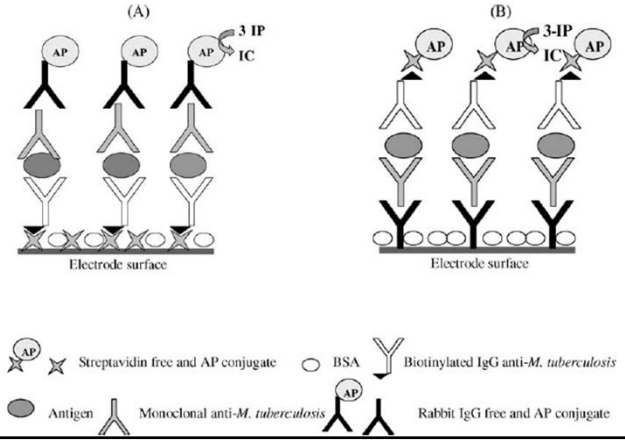
- We have generated aptamers to TB-specific antigens (CSIR-Shooz Kathi)
- Grand Challenges Canada (J Blackburn) to develop a platform using antigen-specific aptamers and a SERS detection platform
- Surface enhanced resonance spectroscopy





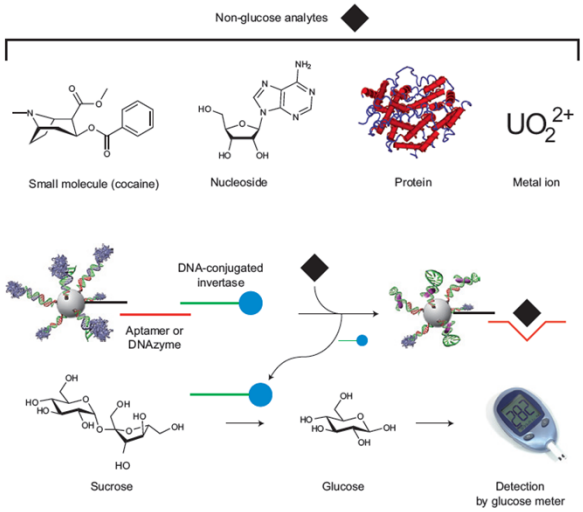
Electrochemical detection

- Nanoparticle filament/ wire which changes resistance when binding antigen



Gonzales- Diaz M, Biosens & Bioelec, 2005

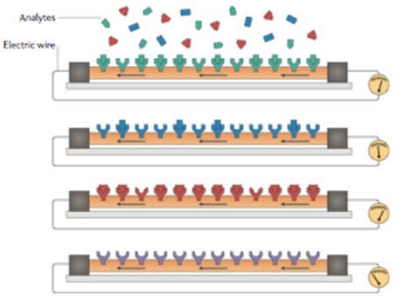
Electrochemical detection using aptamers



Xiang Y, Nature Chem , 2011

Other: Electrochemical detection

- Nanoparticle filament/ wire which changes resistance when binding antigen
- Nanoparticles which change colour when aggregate (quantum dots)

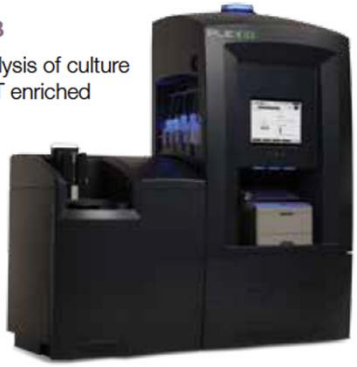


McNurney R, Nat Rev Micro, 2011

Combined approaches: PCR and mass spec (alternative to Hain and Xpert for isolate ID and DST

PLEX-ID MDR TB

- Suitable for analysis of culture isolates or MGIT enriched culture broth



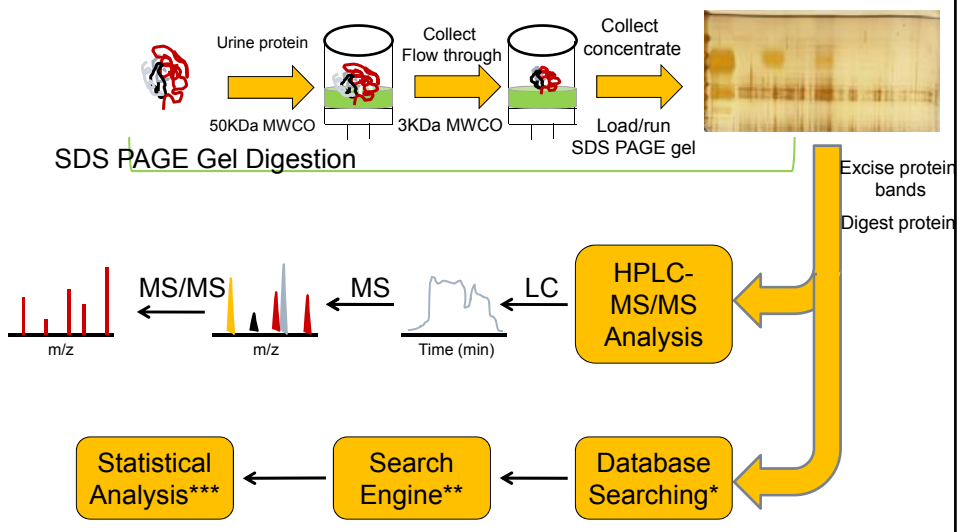
Rapidly identify known and unknown organisms with PCR Assay and Electrospray Ionization Mass Spectrometry

- PCR for gene-specific mutations followed by R, I, E and FQ resistance; already commercially available

Why many of these platforms might not work for TB diagnosis?

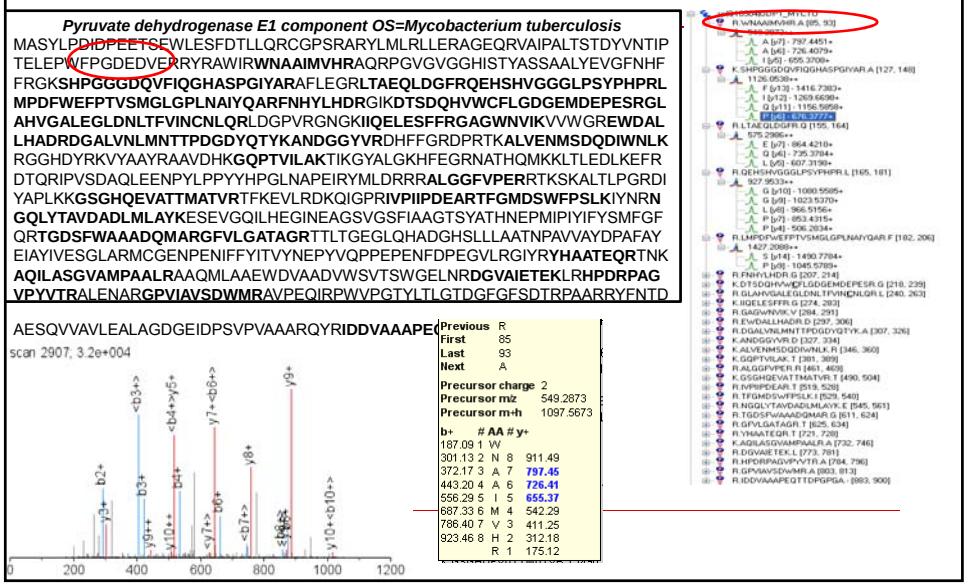
- Antibody profiles overlap in those with LTBI, previous TB or NTM exposure
- Antigens are differentially expressed in different body compartments
- LTBI/ HIV-infected/ NTM colonisation/ PTB vs EPTB
- Sensitivity is a problem with lateral flow assay formats
- Sample like sputum may not be available
- One of the biggest hurdles is lack of suitable or limited antigenic targets

Bottleneck- lack of suitable diagnostic targets



*A combined human and TB (H37Rv) database, **Crux and X!Tandem, ***Trans-Proteomic Pipeline (TPP) and Barista

Peptide spectral matching (PSM) using database searches



Technological innovation is not enough

- Lack of private investment because of perceived lack of return (changing rapidly)
- Need better regulatory standards for approval, and these need international harmonisation
- Variable quality of diagnostic services, need to improve quality control
- More innovation in developing countries (ANDI, Gates etc) including involvement from EDCTP, Wellcome etc
- New ways to deal with IP and patent fees
- Translation from research to policy- need streamlining of approval process and guidance for high burden settings
- Robust health care systems with good supply chain management
- Better representation in medical and nursing curricula

Summary

- Revolution in the development of new and POC diagnostic platforms
- Major bottlenecks are antigenic targets and detection technologies suitable for POC
- Major challenge is to include DR-TB readouts
- Need to move to platforms that will give multiplex readouts – TB, HIV, pneumonia, other OI, malaria etc
- Several other factors including knowledge translation are critical to bring new products to the market
- Then comes the challenge of how to incorporate these into clinical algorithms and niche areas taking into account clinical context

Funding Agencies:

EUFP7

Discovery

NIH Fogerty

South African National Research Foundation

EDCTP

South African MRC