



Prospects for a point-of-care test (POCT).

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Stop TB Partnership

I shall mention several technologies and tests, this will not be an exhaustive list because there are other tests in development that I don't know about or where information about their development is confidential.

I shall begin by describing tests that are close to the market and then move backwards through the pipeline to end with some brief examples of novel technologies in development.

And then a little advocacy . . .

Conflict of interest statement:

I have no financial interest in the sale of any diagnostic test.

I work on the development of new TB diagnostic tests, including a 'point of care' molecular test, detection of volatile compounds, detection of cell wall components and methods to assist sample collection. Should any of these projects be successful they may be commercialised.

In the past 12 months I have collaborated with TwistDx Ltd and Applied Nanodetectors Ltd (public funding).

In past 5 years I have received financial support from Technology Strategy Board (UK), the Wellcome Trust, the European Union, WHO, B&M Gates Foundation and Department for International Development (UK).

I am a trustee of TB Alert (unpaid).

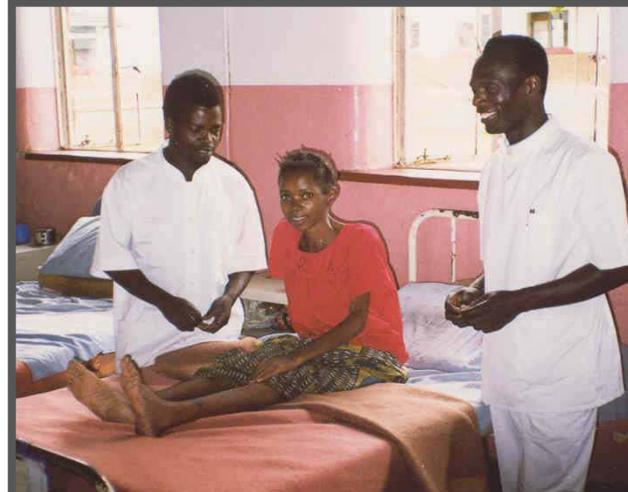
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What is a POCT?

A test which can be performed at the site at which care is provided with immediate results, without referral to a specialist laboratory.

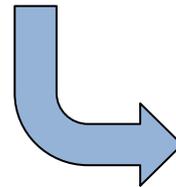
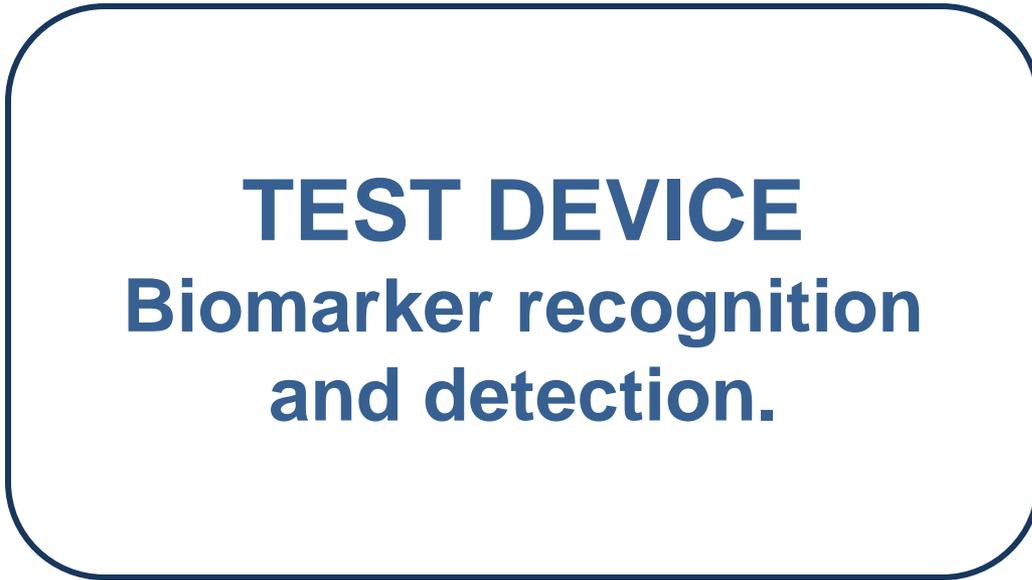
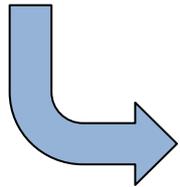
A test which can be performed at the site of specimen collection.

For TB this may be within a community or home setting, a clinic or at the hospital bedside.

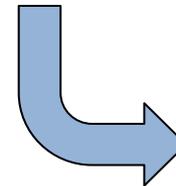


Multiple challenges at POC

**Sample
collection**



Recording/Reporting



Disposal

Test specifications

Sample?

Breath

Saliva

Urine

Blood

Sputum

Technology?

5 min instrument free

5 min bedside gadget

½ hr 'kitchen' gadget

2-3 hr 'kitchen' test

Infrastructure?

No cold chain

No electricity

Intermittent electricity

Specialist training

Cold chain

Constant electricity

Specialist supervision
or maintenance

Affordable

Low cost tools can improve specimen quality and increase case detection.

Advice for you to produce a sample for testing

At the clinic



Move away from other patients to the place where the doctor or nurse tells you.



To help you produce a sample from your lungs slowly take a deep breath in and then blow out fully. Do this three times.



3x

Take a breath and quickly cough up sputum from deep down in your chest, into the pot. Enough to fill a spoon is sufficient. Do not cough up food from your stomach.



Please put the lid on the pot before giving it to the nurse.

At home



You should cough up the sputum when you first wake in the morning.



Repeat the breathing like you did in the clinic. Take a deep breath in and out three times.



3x

Take a breath and cough sputum from your lungs into the pot.



Bring your sample to the clinic straight away. Make sure you know when to collect your results.



CONSEJOS PARA LA RECOLECCIÓN DE MUESTRAS PARA BACILOSCOPIA

En el Establecimiento de salud



Diríjase al lugar que el personal de salud le indique.



Para la recolección de muestras de esputo, realice una inhalación de aire lenta y profunda, seguida de una expiración de aire completa. Repítalo.



Tome aire y esfuerce una vez para obtener la muestra necesaria. que tiene por lo menos una cucharita.



Por favor, cierre el envase antes de entregarlo al personal de salud.

En la casa



Debe recolectar la muestra de esputo cuando se levante por la mañana y antes de desayunar.



Es importante que no haya comido nada antes de recolectar la muestra.



Repita las tres respiraciones como hizo en el establecimiento de salud.



Lleve la muestra al establecimiento de salud a la hora indicada.



Reciba los resultados cuando el personal de salud le indique.

Necesitamos una muestra de esputo de sus pulmones. La prueba de diagnóstico no funciona si la muestra es de saliva de su boca. Esta prueba nos va a ayudar a saber sobre su estado de salud. Es posible que usted tenga tuberculosis, que es una enfermedad causada por pequeñas bacterias que sólo pueden ser vistas con el microscopio. Las bacterias de la tuberculosis crecen en los pulmones y le pueden hacer toser, perder peso y darle fiebre. Nos gustaría examinar una muestra de sus pulmones para ver si contiene bacterias.



Si tiene preguntas sobre la prueba diagnóstica o quiere saber más sobre la tuberculosis, por favor hable con el personal sanitario, en su establecimiento de salud.

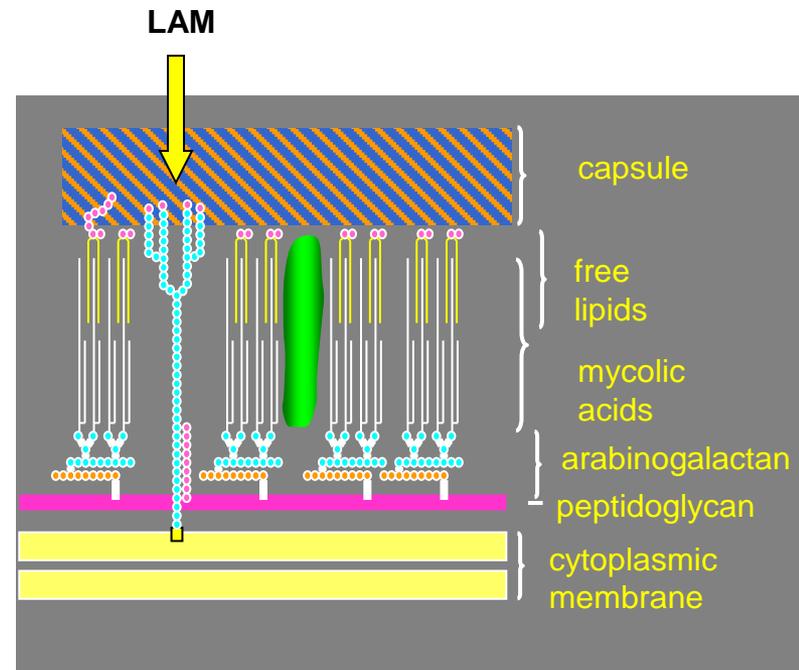
London School of Hygiene and Tropical Medicine

SAÚDE PÚBLICA - PORTUGAL

Determine TB-LAM (Alere)

Test for lipoarabinomannan (LAM) antigen in urine.

To be commercialised 1st quarter 2012



M. tuberculosis cell wall

Multi-centre evaluation studies in South Africa. Results from one study published last week.

Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study

Stephen D Lawn, Andrew D Kerkhoff, Monica Voqt, Robin Wood

Funded by Wellcome Trust

www.thelancet.com/infection Published online October 18, 2011 DOI:10.1016/S1473-3099(11)70251-1

Determine TB-LAM had highest sensitivity at low CD4 cell counts

66.7% at <50 cells per μ L

51.7% at <100 cells per μ L

39.0% at <200 cells per μ L

Specificity was greater than 98% for all strata.

When combined with smear sensitivity was

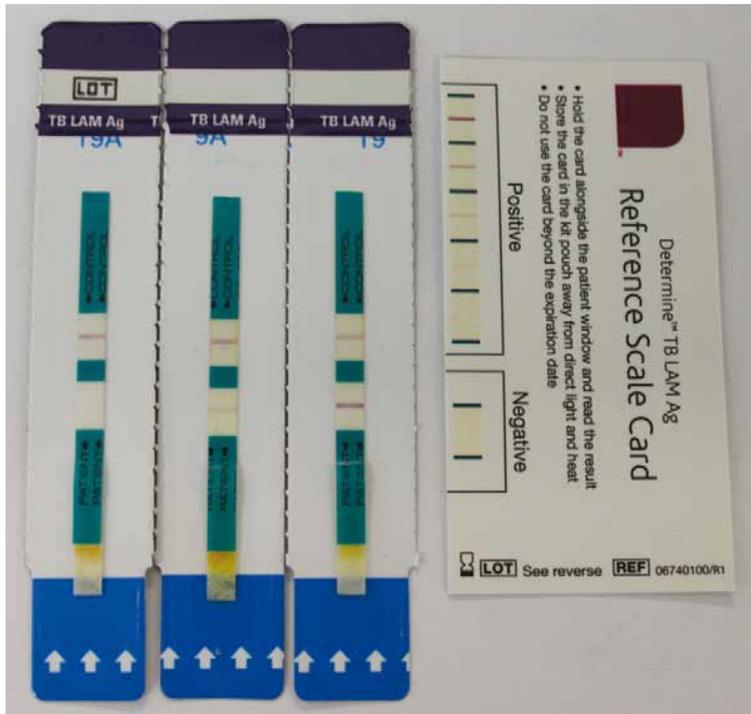
72.2% at CD4 counts less than 50 cells per μ L

65.5% at less than 100 cells per μ L

52.5% at less than 200 cells per μ L

Did not differ statistically from the sensitivities obtained when testing a single sputum sample with the Xpert MTB/RIF assay.

Determine TB LAM Ag (Alere)



Test takes approx 30 min
Price \$3-3.50 per test

A 'rule in' test
(not a 'rule out' test)

Lawn *et al*: Determine TB-LAM is a simple, low-cost, alternative to existing diagnostic assays for tuberculosis screening in HIV-infected patients with very low CD4 cell counts and provides important incremental yield when combined with sputum smear microscopy.

Nucleic acid amplification (NAAT)

Pros

- Can be highly specific
- Can detect drug resistance
- Does not require live organisms



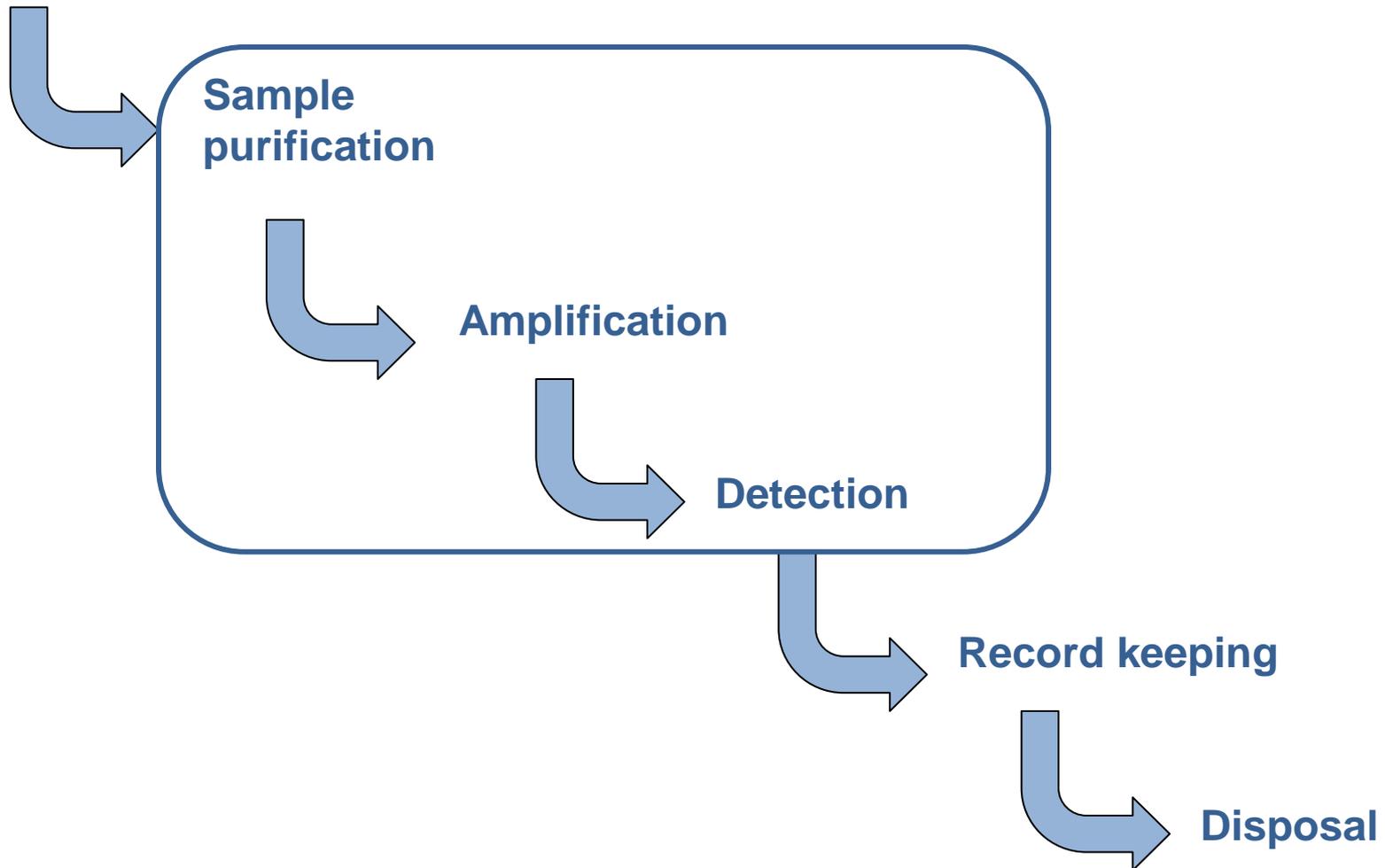
Cons

- Needs pathogen DNA/RNA
- Needs sample processing
- Does not differentiate viable/dead bugs



Many challenges at POC

Sample collection

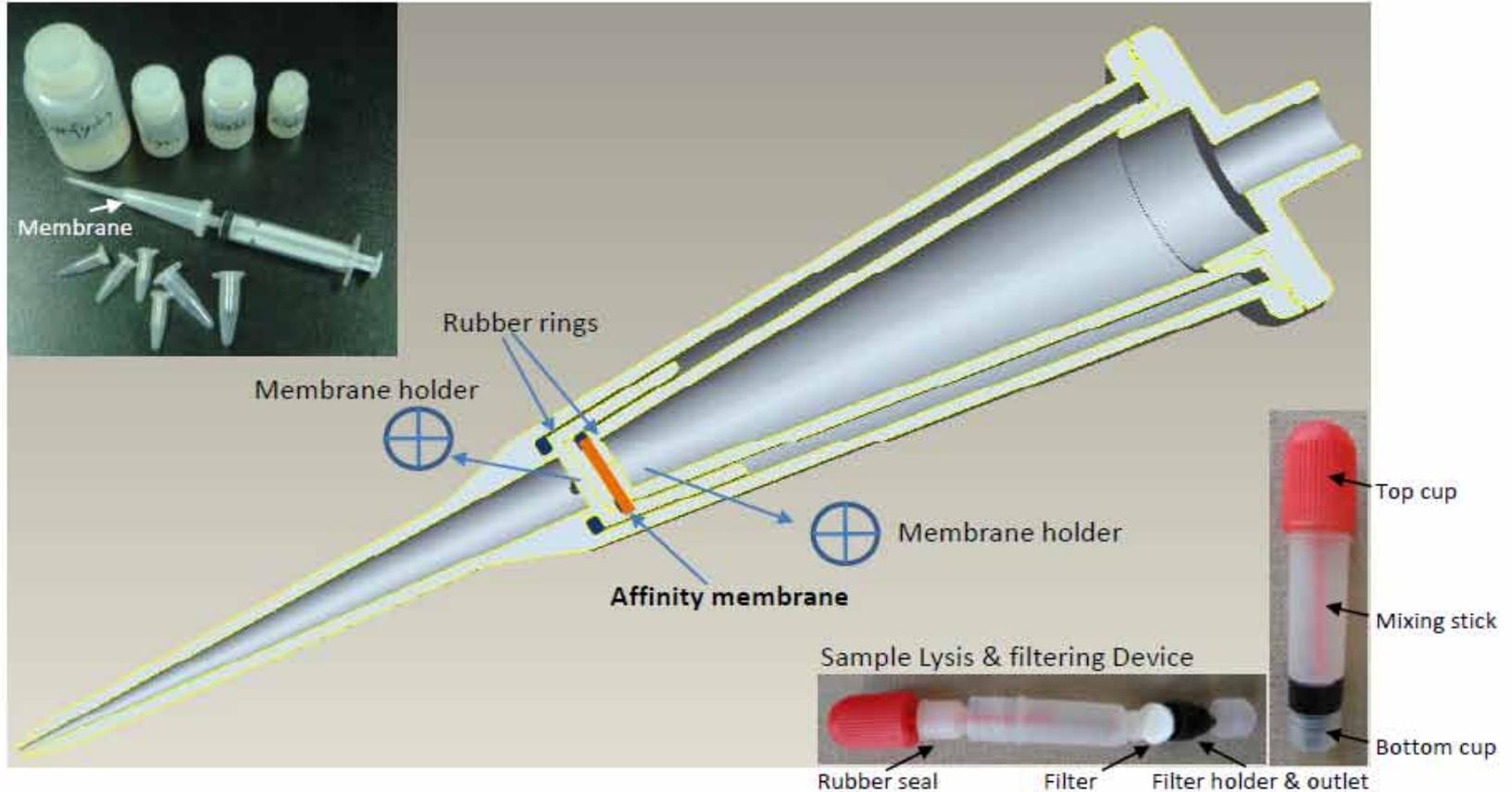


Sample purification at POC (and concentration?)

- **Use robust amplification technology.**
Some isothermal reactions are *believed* to be more tolerant of dirty samples than PCR. (e.g. LAMP, RPA).
- **Use mechanical separation e.g. Ustar**
- **Use automated sample prep e.g. GeneXpert**
- **Use magnetic beads**
- **Use magnetised nanoparticles**

Instrument-free sample preparation

A rapid, simple method of purifying/concentrating DNA/RNA from clinical samples, such as blood, sputum and vaginal swabs, using a disposable syringe and membrane unit and proprietary reagents. No instrument is needed. The protocol takes only a few minutes.



Ustar Biotechnologies

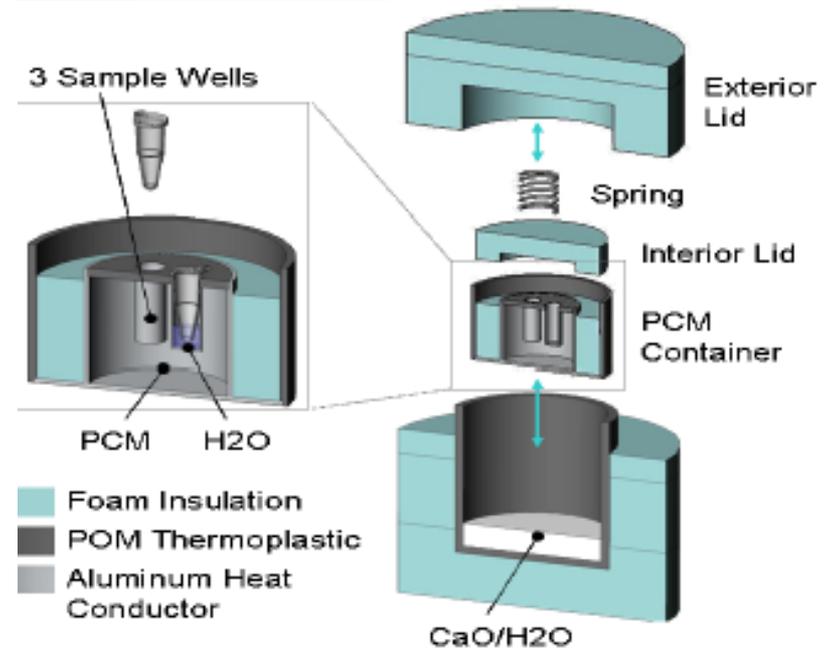
<http://www.bioustar.com/en/index.aspx>

Amplification technologies.

Many to choose from!

- PCR is slow and requires thermocycling
(i.e. programmable instrument and power supply).
- Isothermal methods are faster.
but most work at elevated temperatures ($\sim 65^{\circ}\text{C}$)
and require a cold chain for the reagents.
- Not all technologies can multiplex or have
an internal control.

Isothermal amplification using instrument free heat source developed by PATH. Is being tested with LAMP.



Amplification at reduced temperatures

e.g. RPA, TwistDx Ltd

Recombinase Polymerase Amplification

The reaction takes place at temperatures between 22°C to 45°C.

No heating and cooling cycles.

Low energy requirement.

Detection technologies

Fluorescence, provides real time detection.
Requires instrument (can be simple/portable)
Signal not stable.



Visual detection technologies

LAMP Loop-mediated Isothermal Amplification

(Eiken Chemical Co., Ltd. & FIND)

Magnesium pyrophosphate is a by-product of the amplification reaction - white turbidity can be seen in the tubes

Multiplexing not possible (internal controls not possible).

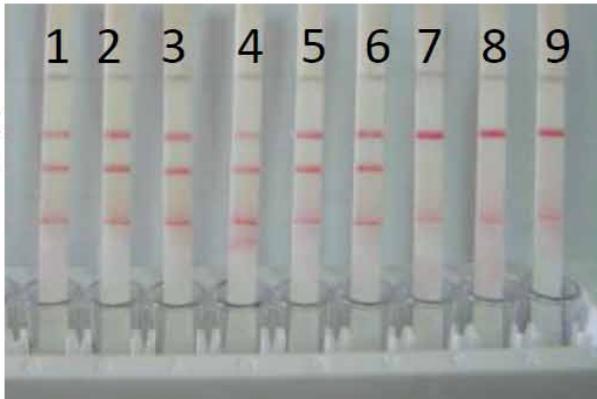


— +
www.finddiagnostics.org

Visual detection technologies

Lateral flow devices
(cassettes).

Multiplexing possible,
but limited.



Nucleic acid strips:

1-3: 10^4 copies/ml

4-6: 10^3 copies/ml

7-9: Negative Control

Ustar

<http://bioustar.com/newSite/>

Visual detection technologies

Aggregation of functionalised gold nanoparticles.

Nanoprobes are tiny particles labelled with specific markers. Surface plasmon resonance results in the nanoprobe suspension exhibiting a red colour. At high salt concentrations, nanoprobe aggregation turns the solution purple. If target DNA is present (i.e. amplicons) specific probe hybridization to the complementary sequence prevents aggregation of the nanoparticles, and the solution remains red.

TrueLab

A real time PCR platform from The Tulip Group, developed by Bigtec Labs.

A battery powered handheld device used in conjunction with a TruePrep sample prep platform. and TrueNat chips.

Tests for other pathogens available very soon.

TB test in development and being evaluated.

**Time to result less than one hour
(incl sample prep).**

**Prototype expected to move to manufacture
towards the end of 2011.**

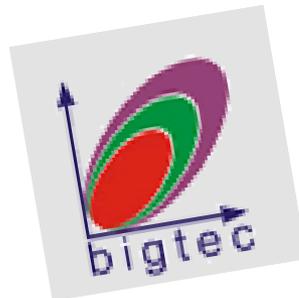
TrueLab instrument launch price approx \$6000

Cost per TrueNat chip approx \$10

Watch this space . . . **(nsriram@tulipgroup.com)**

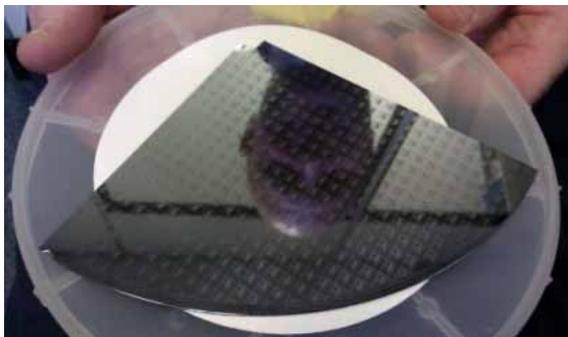
NAAT Summary

Several competing NAAT technologies to choose from. Only GeneXpert has moved into clinical practice in developing countries so far but cheaper technologies are in development, with some early phase evaluation studies ongoing.



Novel technologies in development

Several projects utilising nanotechnology



Antigen detection in breath



Rapid Biosensor Systems

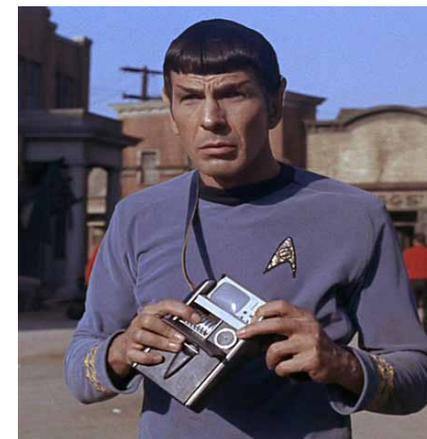
Raman spectroscopy

VOC analysis

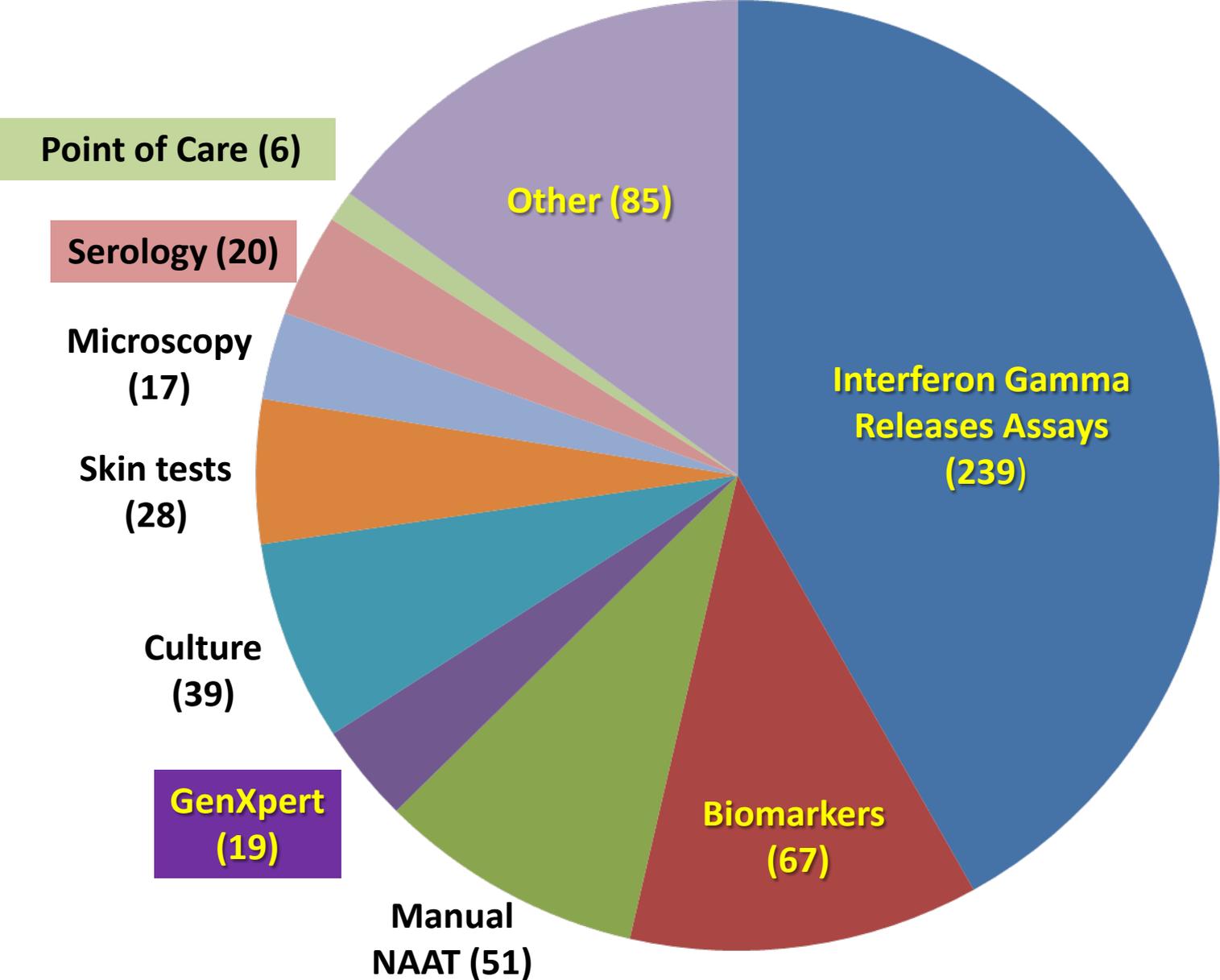


www.smithsdetection.com

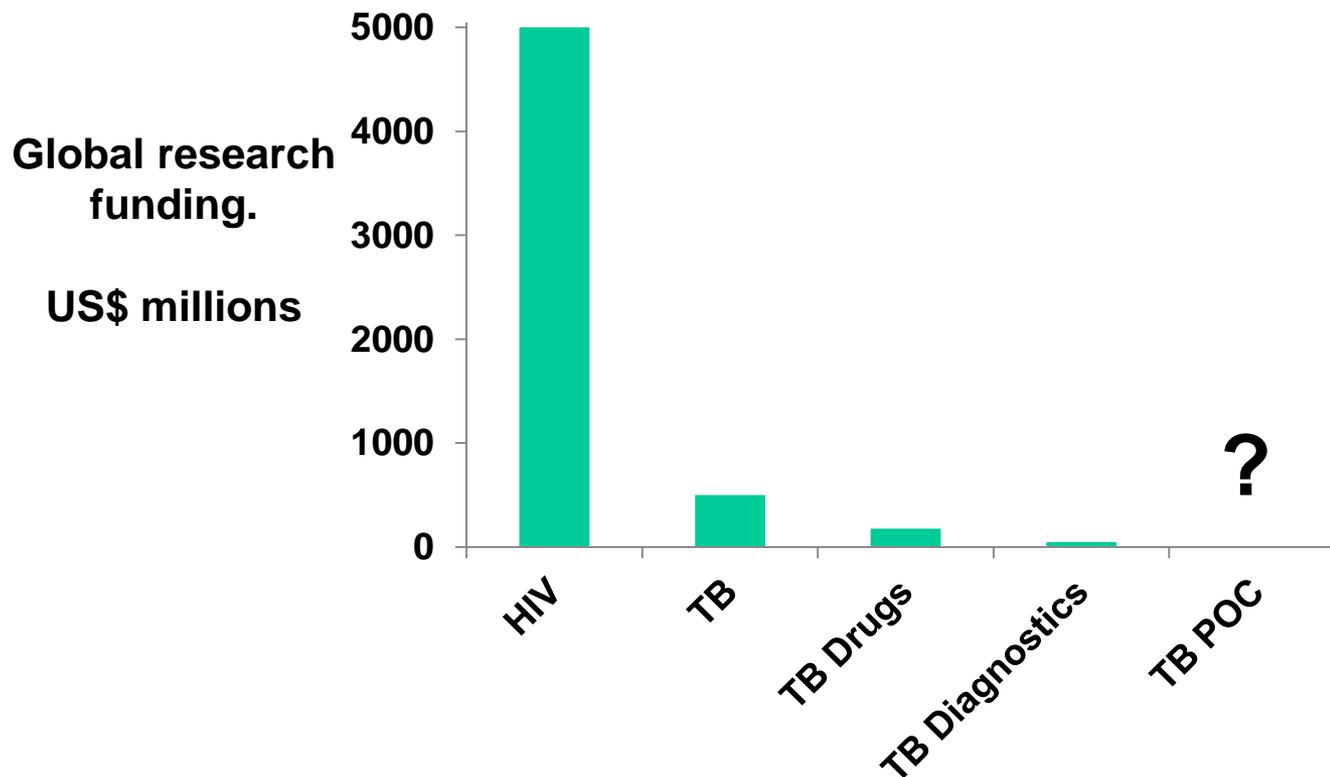
Coming soon . . .



PubMed survey: 1st January 2008 to 7th August 2011



Inadequate investment in R&D has delayed new test development. Now we have a better lab tests efforts **MUST focus on the search for simple affordable devices that will change the way we control TB.**



Merci de votre attention