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

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New Diagnostics Working Group Annual Meeting 26th October 2011

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).

25th October 2011

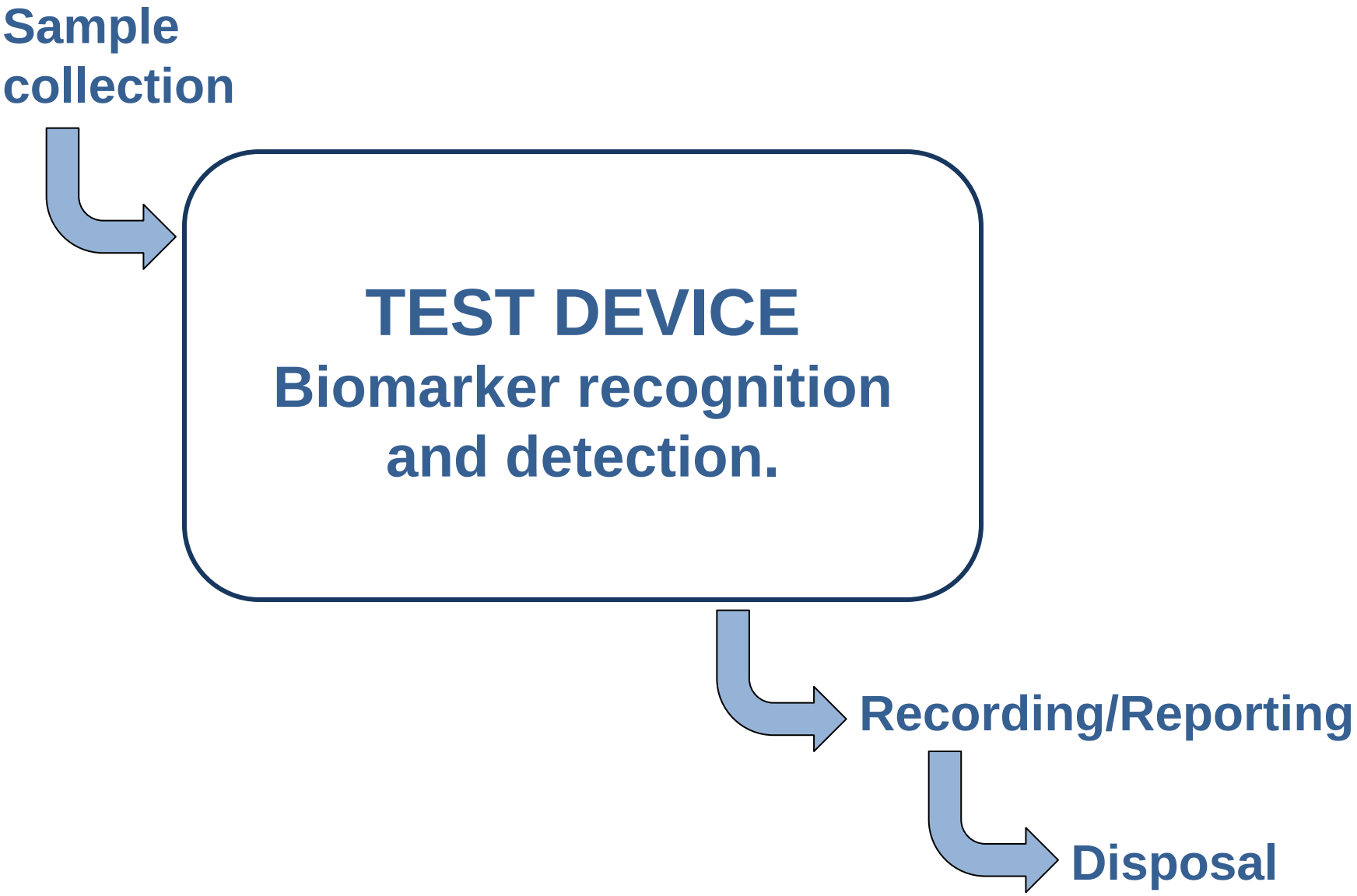
What is a POCT?

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



Test specifications

Sample?

- Breath
- Saliva
- Urine
- Blood
- Sputum

Technology?

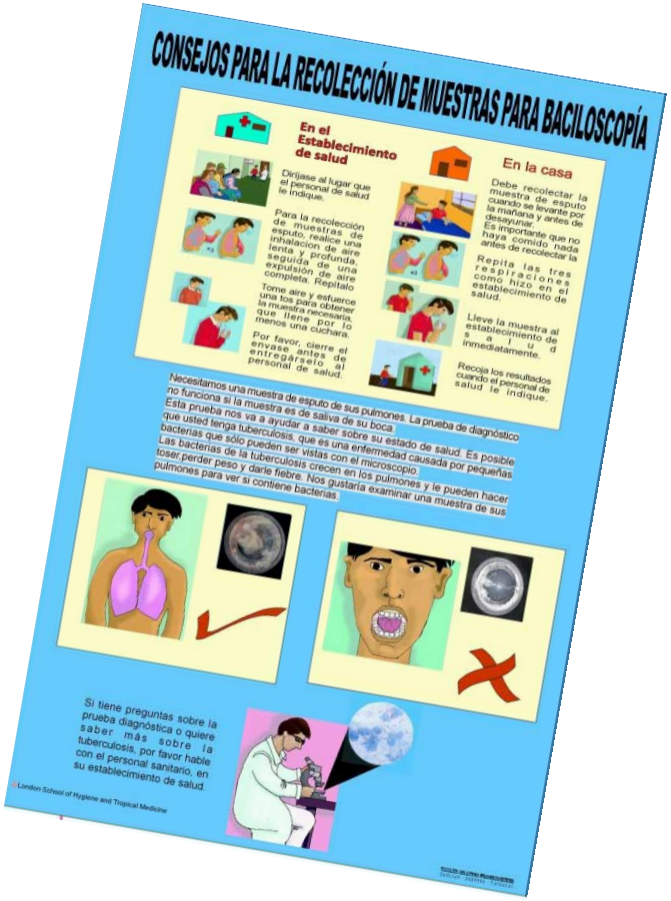
- 5 min instrument free
- 5 min bedside gadget
- ½ hr ‘kitchen’ gadget
- 2-3 hr ‘kitchen’ test

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.

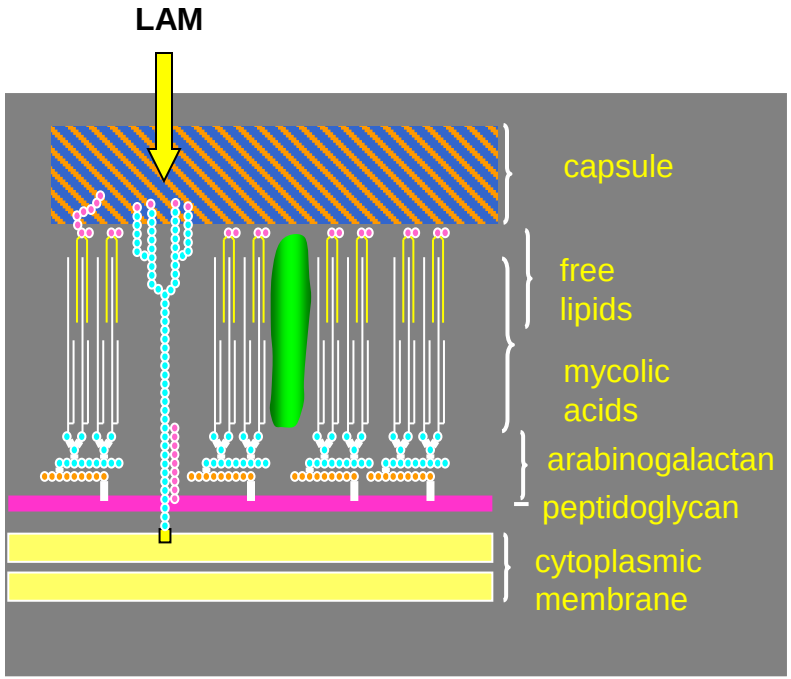


www.youtube.com/watch?v=HhW85reE0Ug

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 Vogt, 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 µL

51·7% at <100 cells per µL

39·0% at <200 cells per µL

Specificity was greater than 98% for all strata.

When combined with smear sensitivity was

72·2% at CD4 counts less than 50 cells per µL

65·5% at less than 100 cells per µL

52·5% at less than 200 cells per µL

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)

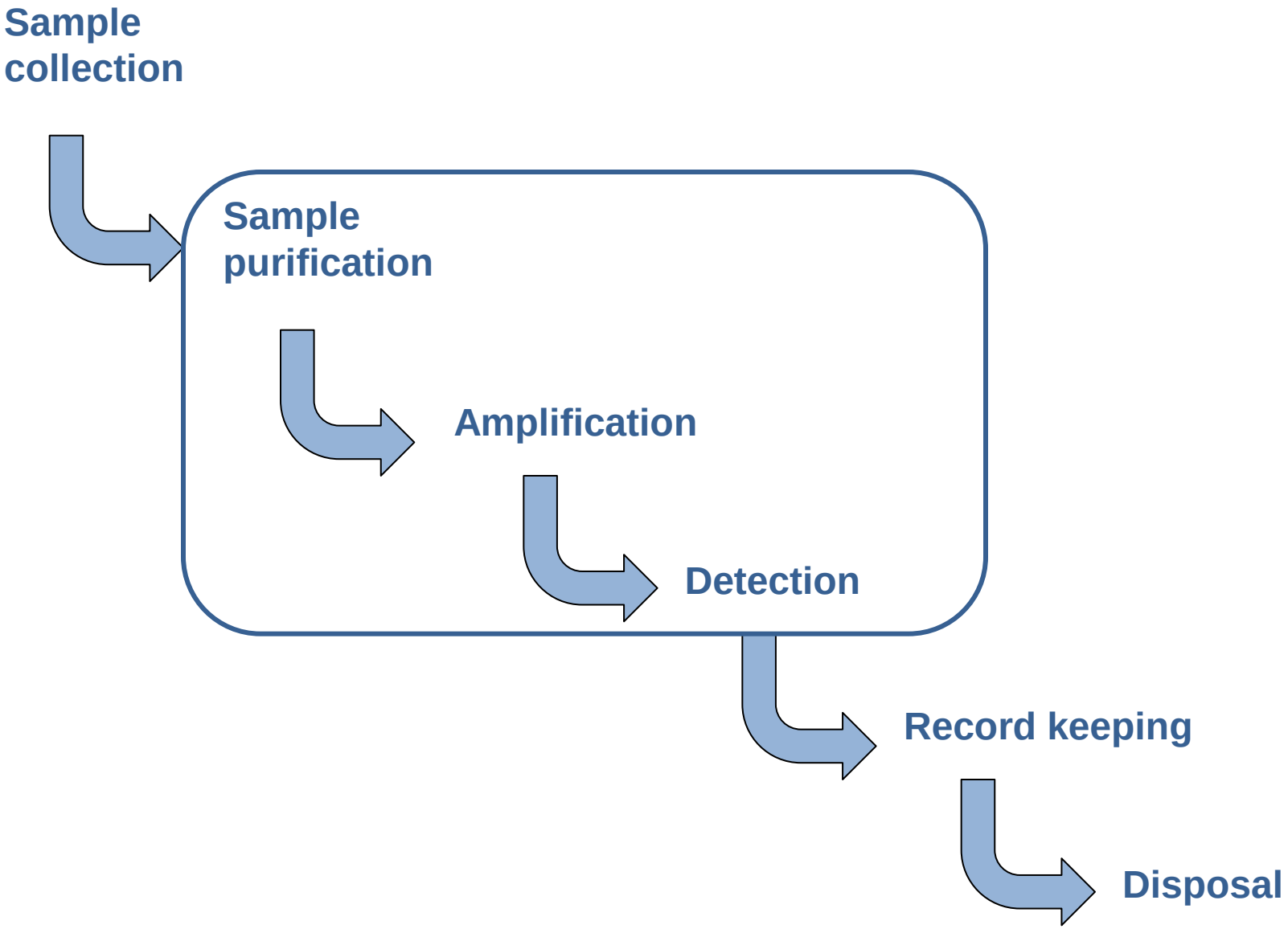
Pros

- 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

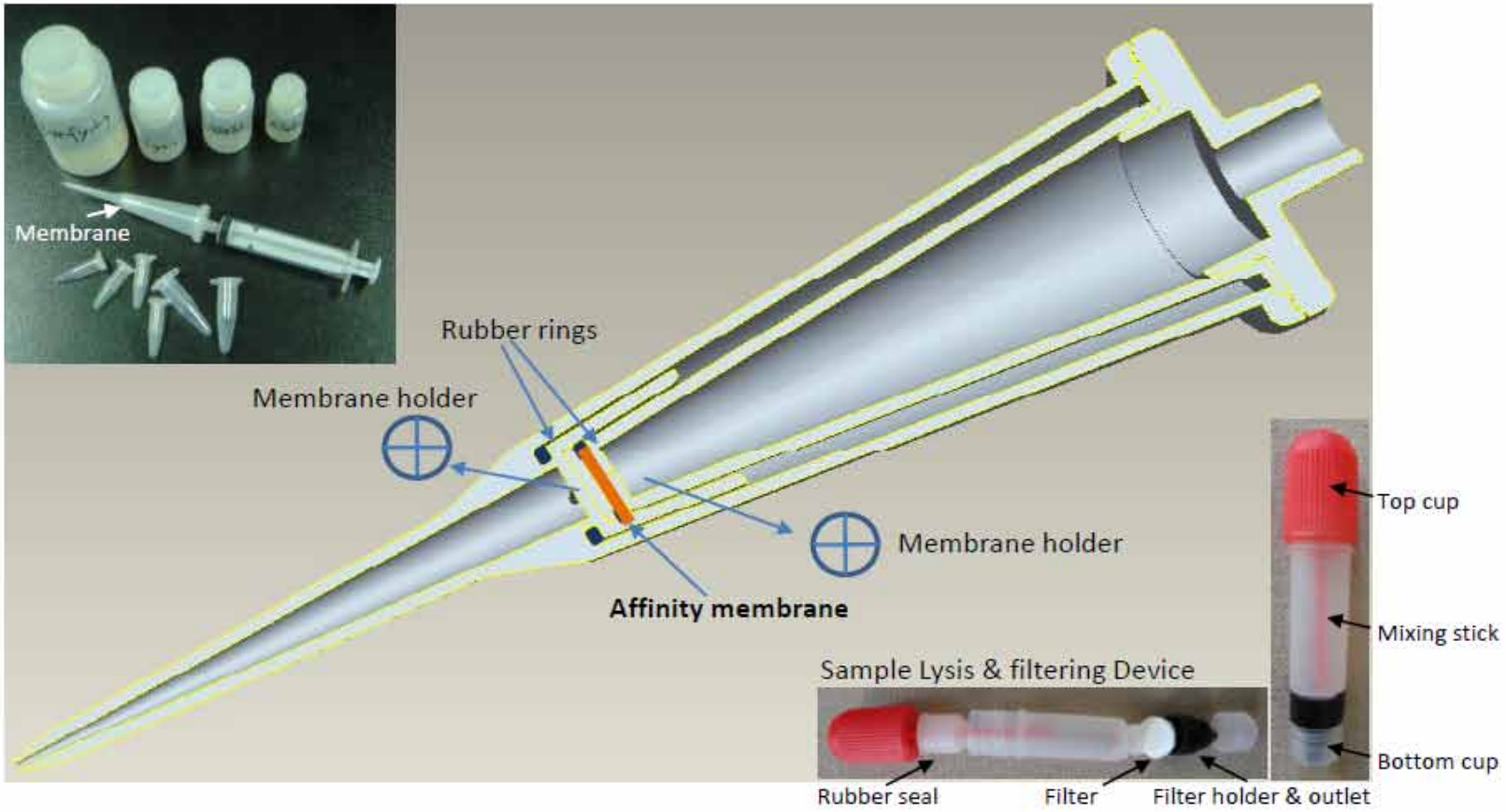


Sample purification at POC (and concentration?)

- **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.

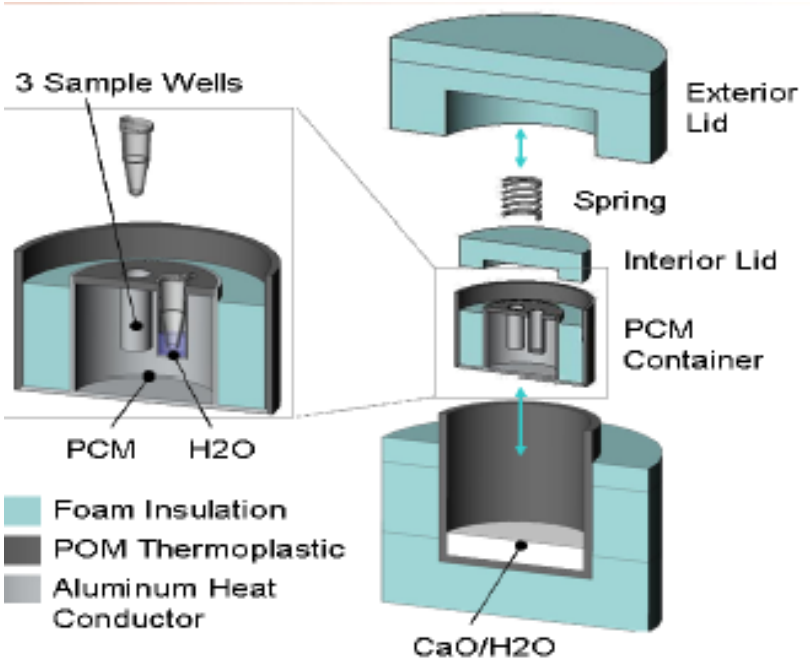
Many to choose from!

- PCR is slow and requires thermocycling
  (i.e. programmable instrument and power supply).

- Isothermal methods are faster.
  but most work at elevated temperatures (~65°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.



http://www.path.org/

Amplification at reduced temperatures

e.g. RPA, TwistDx Ltd

Recombinase Polymerase Amplification

The reaction takes place at temperatures between 22^{o}C to 45^{o}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)

www.**finddiagnostics**.org

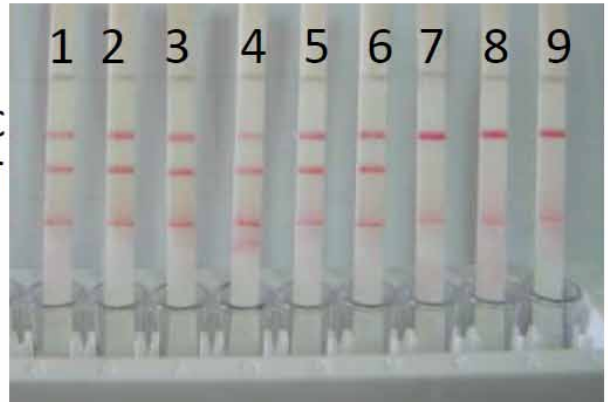
Magnesium pyrophosphate is a by-product of the amplification reaction - white turbidity can be seen in the tubes

Multiplexing not possible (internal controls not possible).

Visual detection technologies

Lateral flow devices (cassettes).

Multiplexing possible, but limited.



Nucleic acid strips:

1-3:10^4copies/ml

4-6:10^3copies/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.

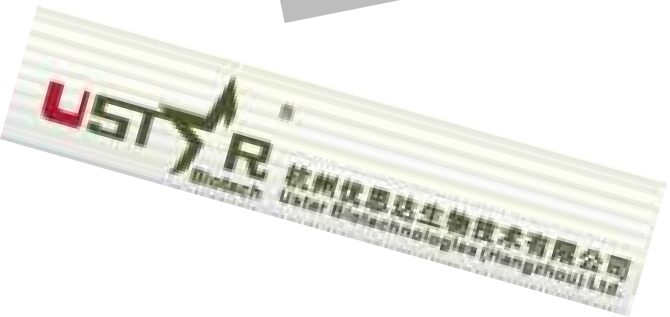
TrueLab instrument launch price approx $6000

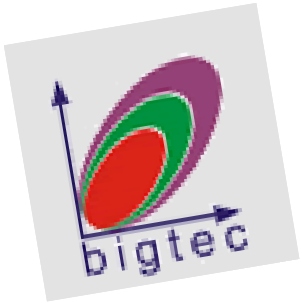
Cost per TrueNat chip approx $10

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

NAAT Summary

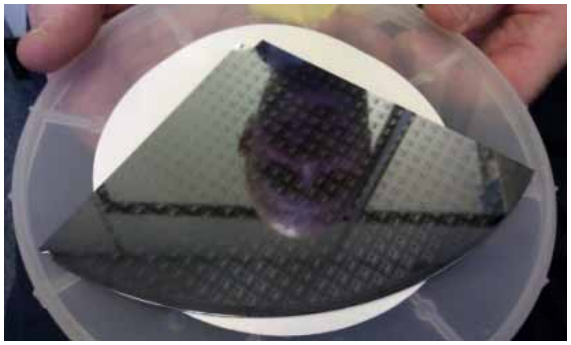
Several competing NAAT technologies to choose from. Only GeneXpert has moved into clinical practice in developing countries so far but cheaper technologies are in development, with some early phase evaluation studies ongoing.





Novel technologies in development

Several projects utilising nanotechnology



Antigen detection in breath

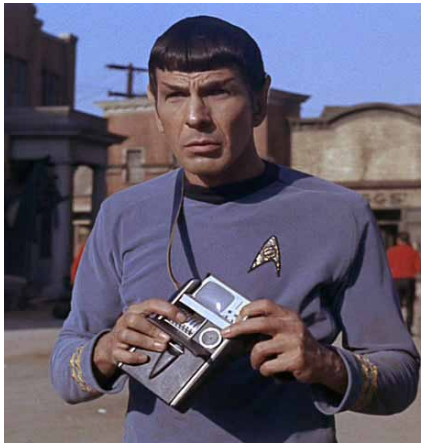
Rapid Biosensor Systems

VOC analysis

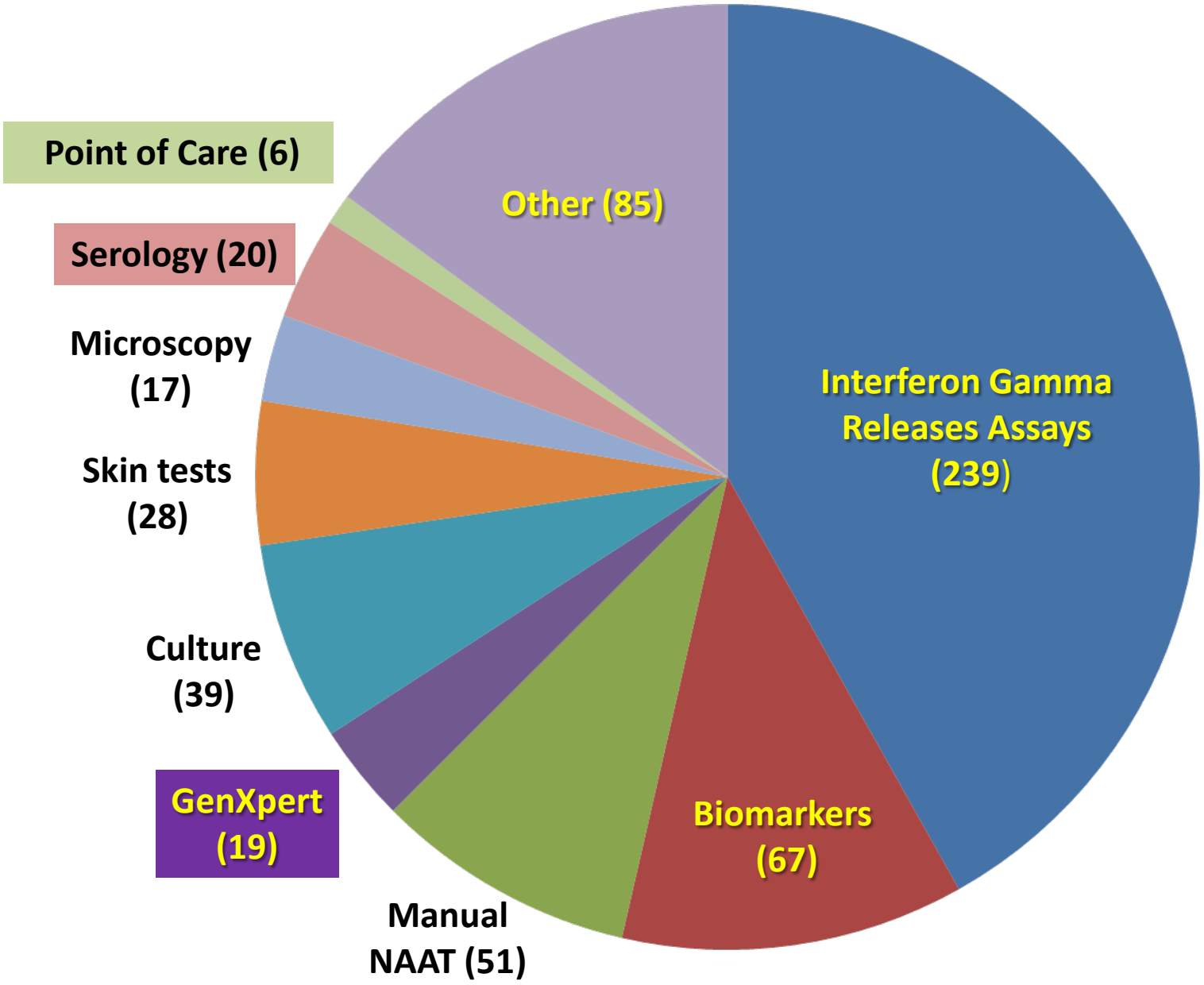
Raman spectroscopy

www.smithsdetection.com

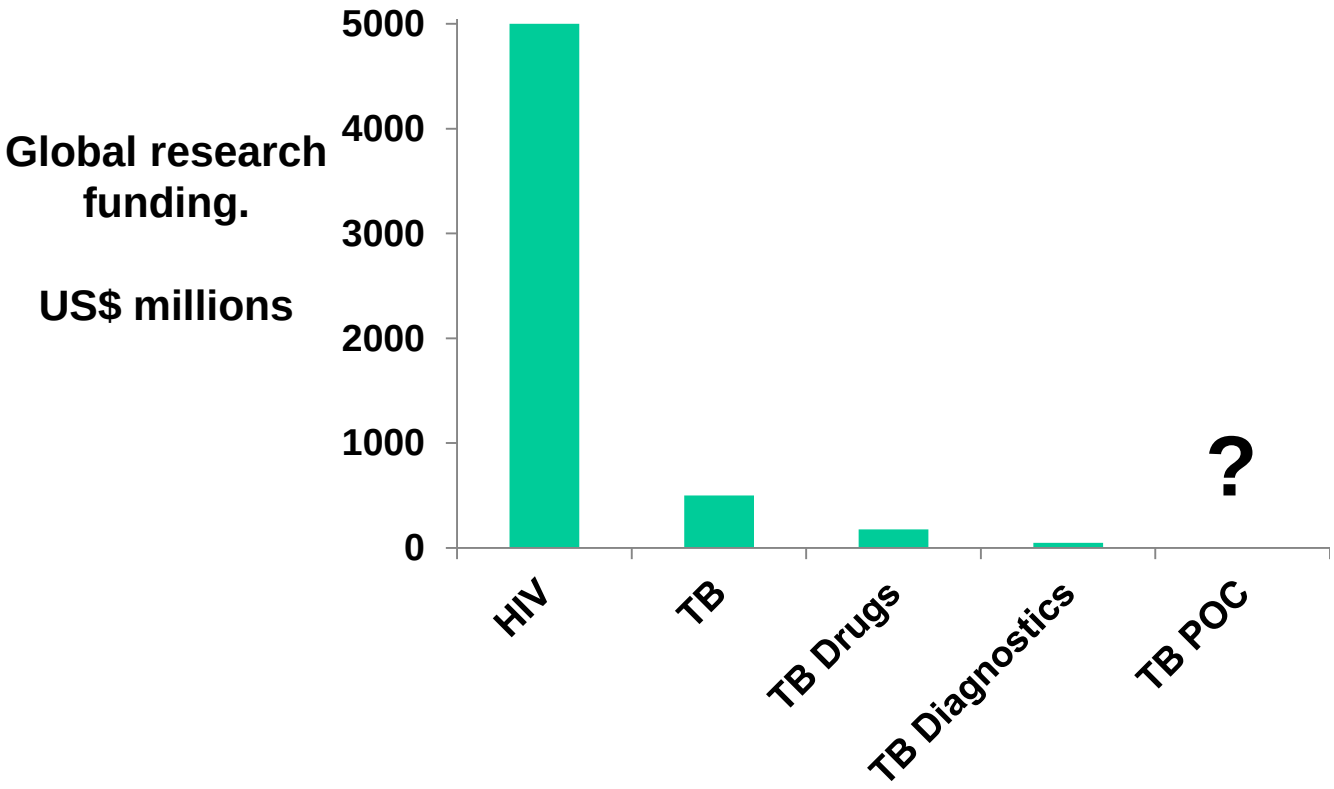
Coming soon . . .



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