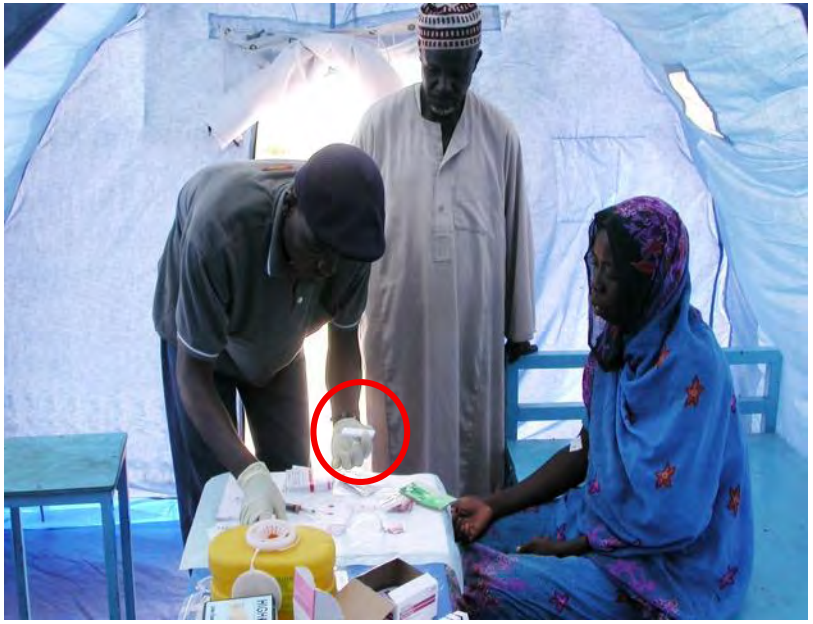
POC tests for disadvantaged settings – pushing the boundaries of lateral flow immunochromatography



A/Prof David Anderson

Deputy Director (Partnerships), and Co-Head – Global Health Diagnostics Lab

Burnet Institute, Melbourne

david.anderson@burnet.edu.au

Viruses in May, May 2018

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Pushing the boundaries of lateral flow

Rapid (point of care) diagnostics

- Why (mostly) lateral flow for resource-poor settings?
- Examples of POC tests in viral hepatitis, HIV (CD4, viral load), liver disease etc
- *Approaches to research translation, commercialisation and validation/implementation*

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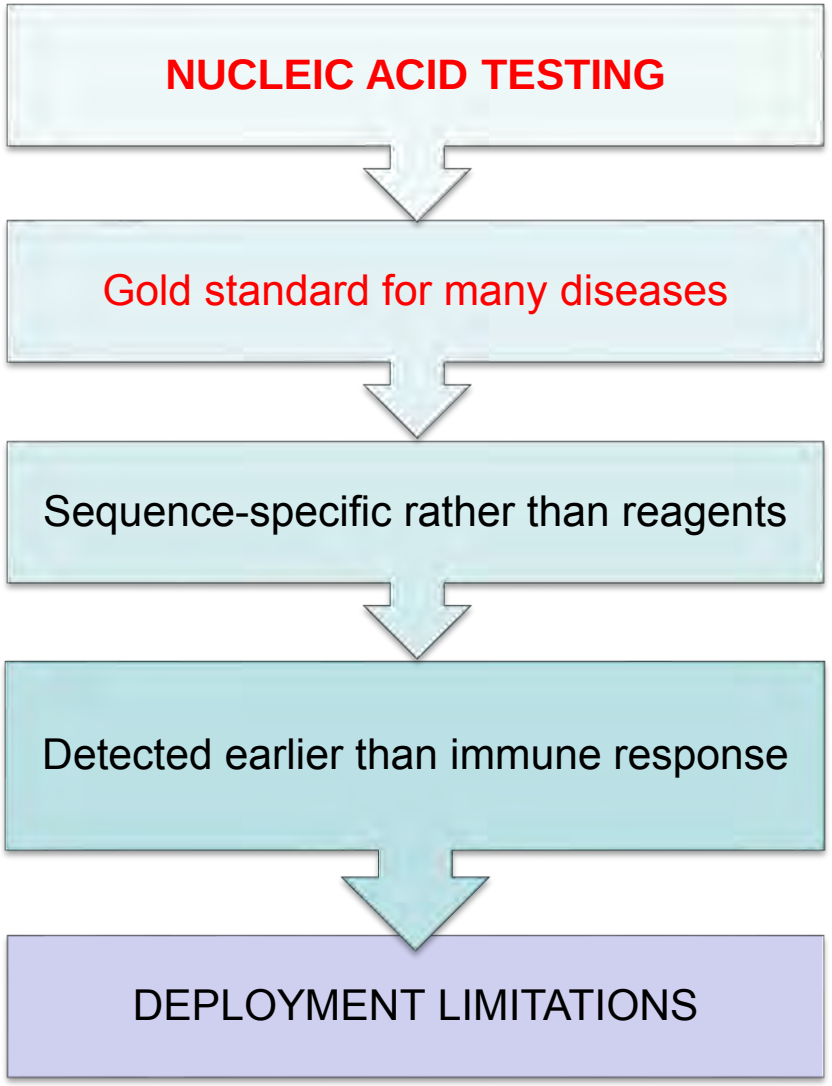
POC testing and lateral flow chromatography



cobas® Liat® System

Compact and portable, innovative **real-time PCR** platform designed for **on-demand STAT testing**, enabling **confidence** in rapid patient management, at the point of care or in the laboratory.

Patient sample t

1,500 units installed in US over past year
262% growth in test sales over past year

ARTG listed **cobas**® Liat® assays:

- Influenza A/B & RSV
- Influenza A/B
- Strep A
- Cdiff

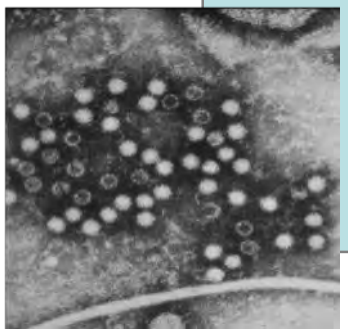
ANALYSER

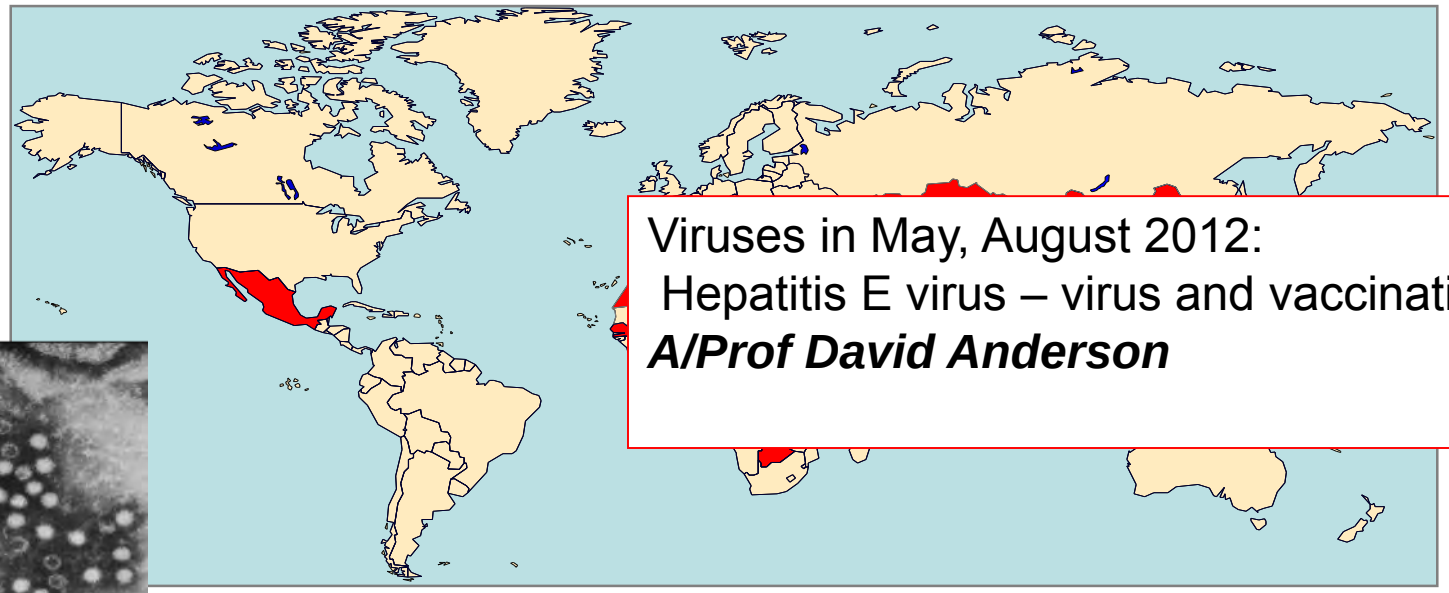
ASSAY TUBE

A pencil-sized, flexible single-use tube acts as the sample vessel and contains all assay reagents pre-packed in tube segments

*Turnaround times may vary by assay

Diagnosis of Hepatitis E virus infection





Viruses in May, August 2012:
Hepatitis E virus – virus and vaccination
A/Prof David Anderson

- Enterically transmitted: 30 million cases/yr
- Large epidemics (contaminated water)
- 30% mortality during 3rd trimester
- No vaccine - major public health problem
  (vaccine licensed in China 2012 but not used)

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First reported outbreak of locally acquired hepatitis E virus infection in Australia

Chaturangi M Yapa, Catriona Furlong, Alexander Rosewell, Kate A Ward, Sheena Adamson, Craig Shadbolt, Jen Kok, Samantha L Tracy, Scott Bowden, Elizabeth J Smedley, Mark J Ferson, Vicky Sheppeard and Jeremy M McAnulty

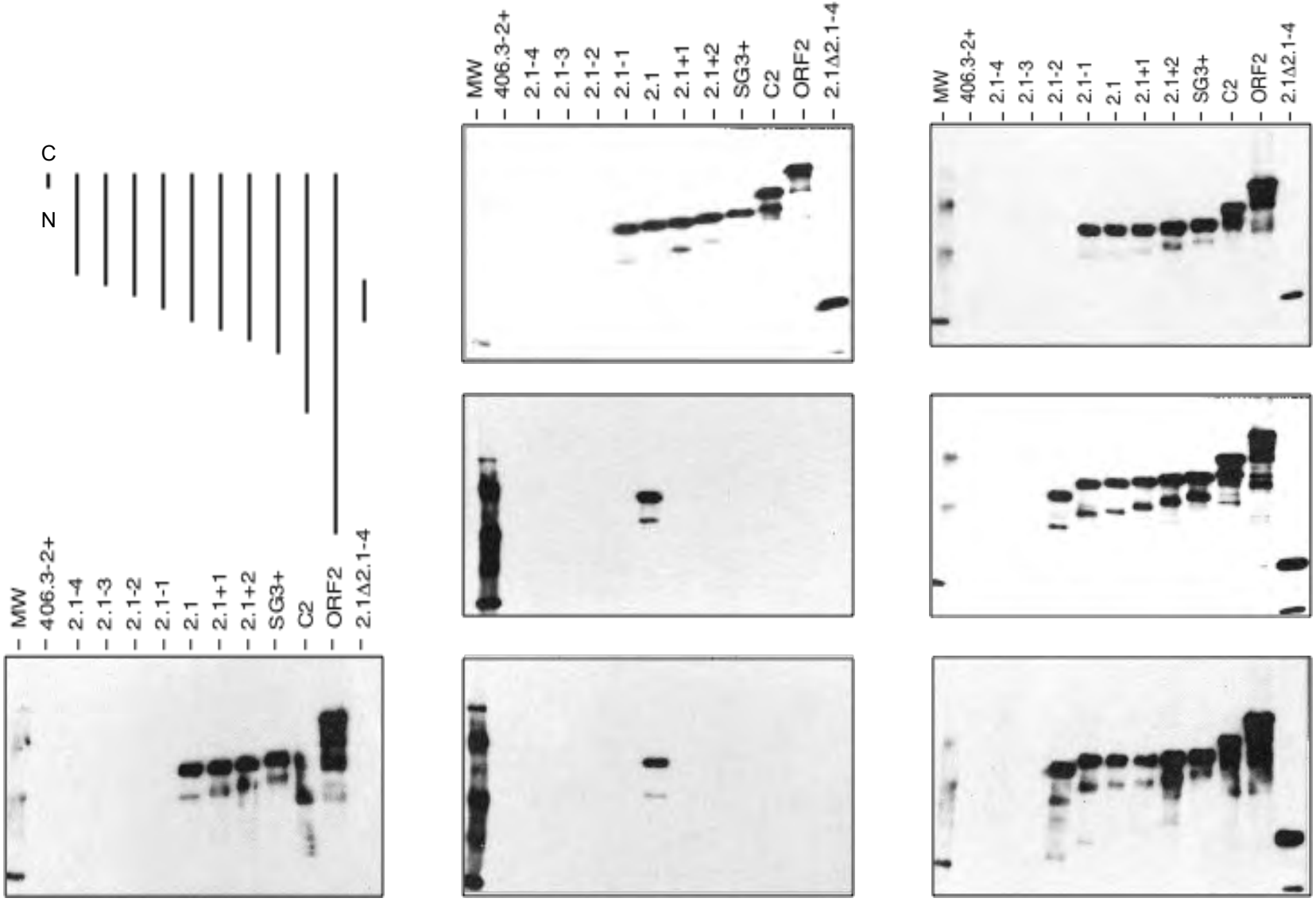
Med J Aust 2016; 204 (7): 274. || doi: 10.5694/mja15.00955

Published online: 18 April 2016

(cases beginning October 2013)

"Results: In 55 serologically confirmed cases of HEV infection, 24 people had not travelled overseas during their incubation periods. Of the 24, 17 reported having eaten at restaurant X, 15 of whom could be interviewed. All reported consuming pork liver pâté, compared with only four of seven uninfected co-diners ($P < 0.05$). The other seven people with locally acquired infections each reported consuming a pork product during their incubation periods. HEV RNA was detected in 16 of the 24 cases; all were of genotype 3. Sequencing indicated greater than 99% homology among restaurant X isolates. HEV RNA was isolated from pork sausages from a batch implicated in one of the locally acquired infections not linked with restaurant X".

Western blot of MAbs against the deletion series



HEV IgM Rapid Test: Reverse Flow Technology

See red colour on Test Line

Always see red colour on Control Line

GOLD

Anti-HEV Mab HEVAg

GOLD

Mab HEVAg

conjugate buffer

flow

Anti-Hu IgM

Anti-mu

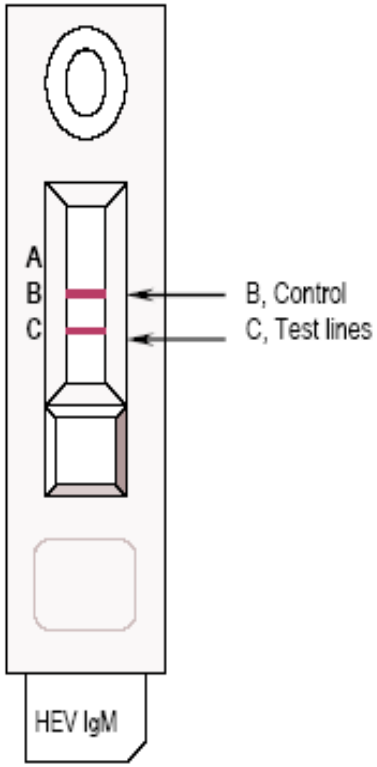
CONJUGATE PAD:

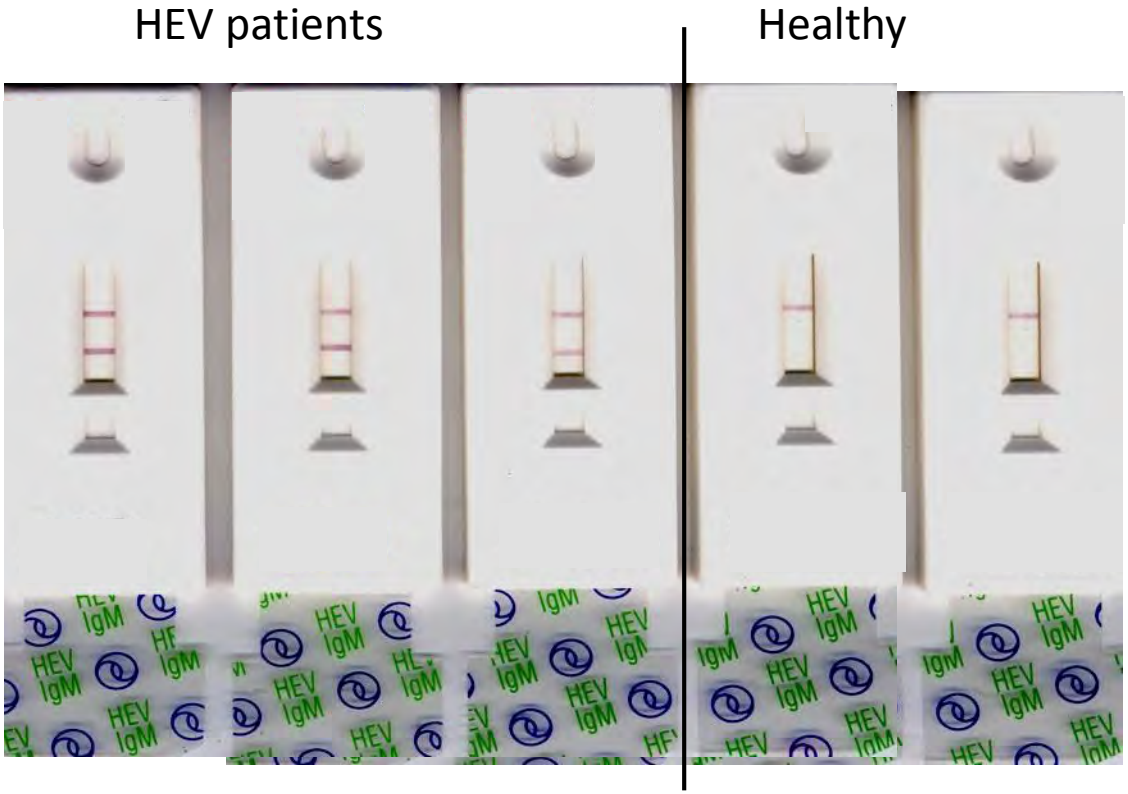
- Gold conj. to anti-HEV MAb, bound to HEV antigen
- reverses flow

----------NITROCELLULOSE MEMBRANE----------

Hepatitis E IgM rapid test

Burnet Institute: MP Biomedical HEV IgM Assure™





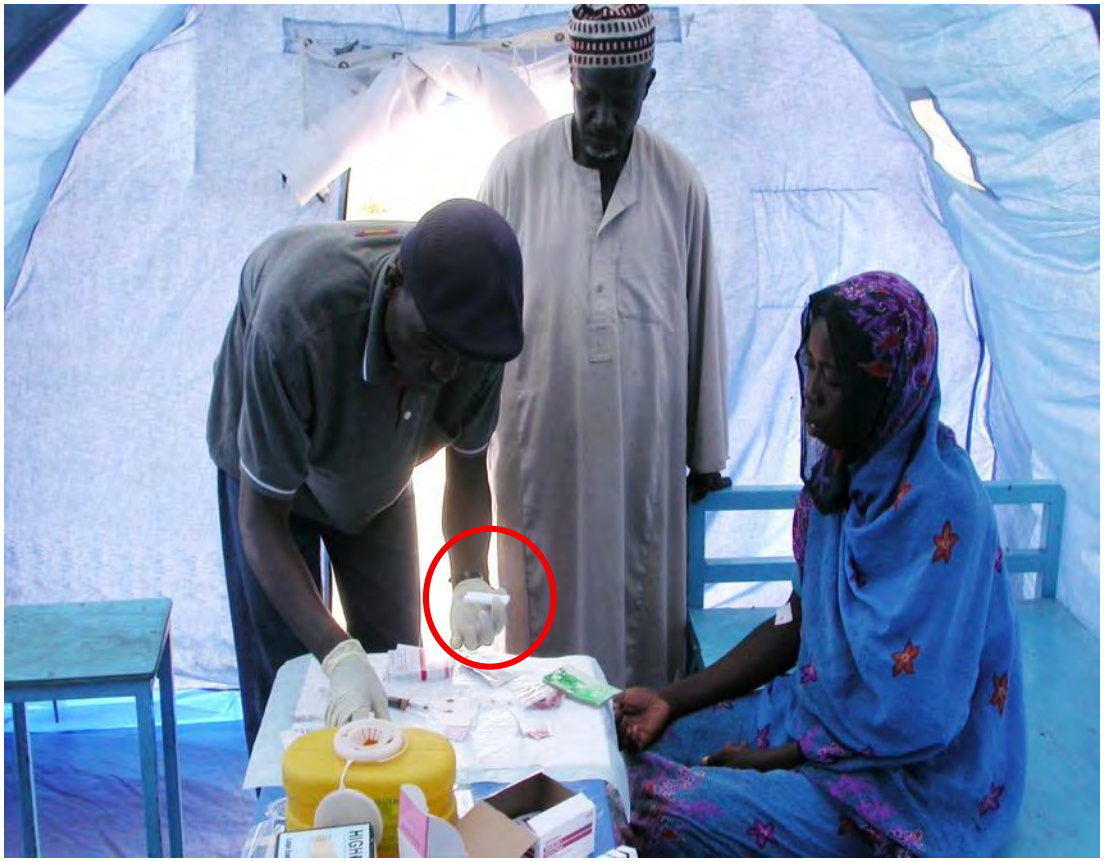
Plus 3 different lab-based ELISA products based on same licensed technology

Priority Diagnostics Roadmap

UNMET MEDICAL NEEDS

•Access to treatment, control of disease spread, targeting of vaccines or drugs

PATIENT BENEFITS



Burnet Hepatitis E test in field use.

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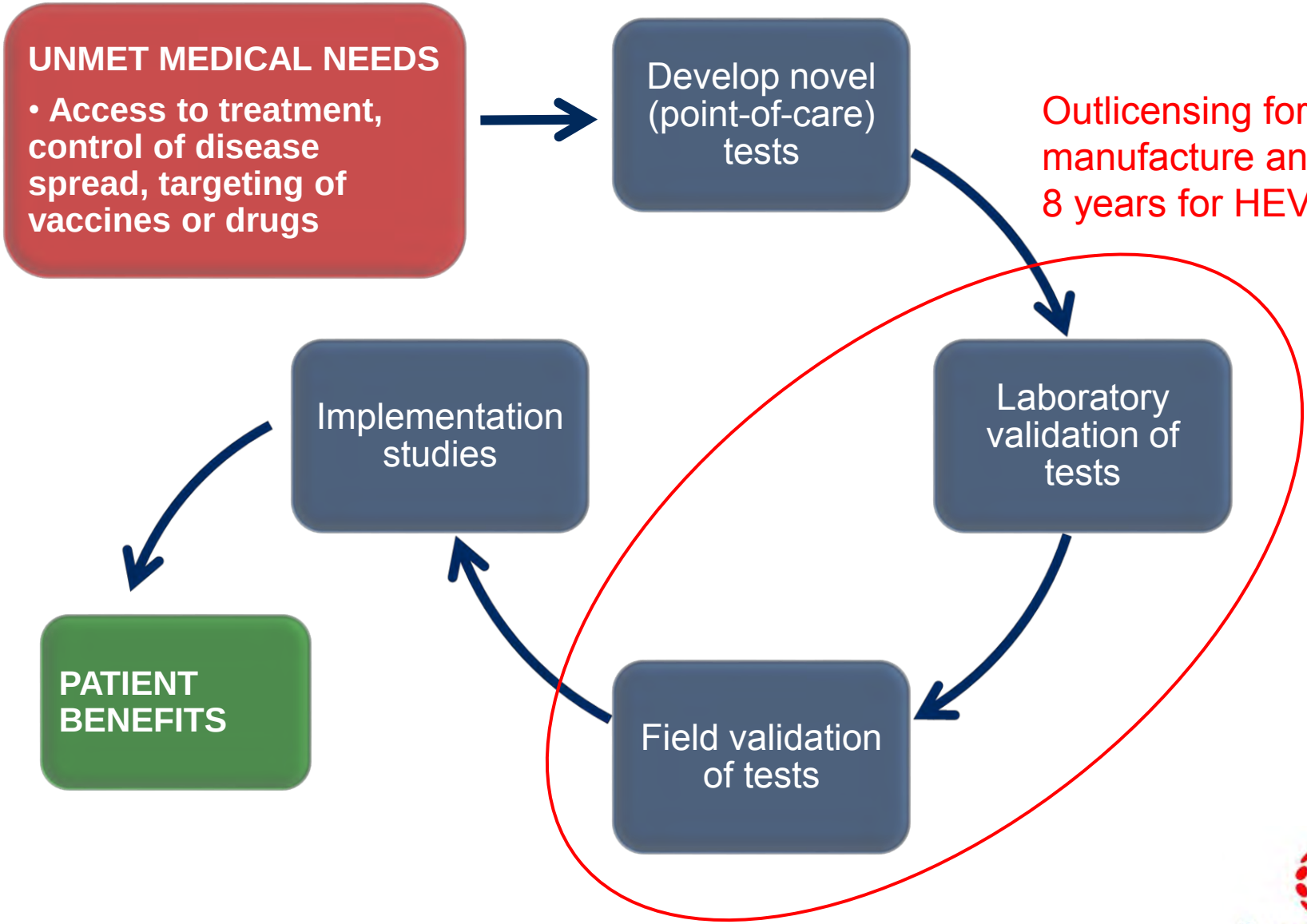
POC Diagnostics Roadmap



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Pushing the boundaries of lateral flow

- An alternative to Flow Cytometry?
  - CD4 T-cells in HIV (Visitect CD4); neutrophil CD64 in sepsis
  - Visitect CD4 achieved CE Mark in November 2017
- An alternative to enzymatic tests?
  - Alanine aminotransferase (ALT) in liver disease
- An alternative to centrifugation
  - Plasma separation for HIV viral load, OTHER BIOMARKERS
- Improved markers of acute viral infections
  - Dimeric IgA (dIgA) as a more specific marker than IgM
- Novel biomarkers
  - CD64 for sepsis
  - Treponemal IgA for confirmation of active syphilis (Omega)
  - G6PD/Hb for malaria

CD4 T-cells in HIV/AIDS

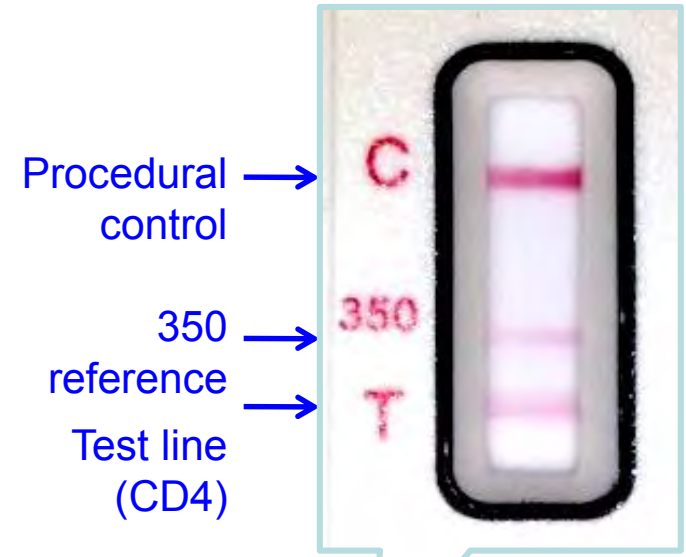
- WHO now recommends treatment regardless of CD4 T-cell count, but prioritised for those with counts <350 / µl

- Many patients diagnosed at much lower counts, and needing additional interventions (cryptococcal Ag, etc) when they have very low CD4 count (<100-200 / µl)

- Continuing need for CD4 testing but poor access for most of the HIV patients in resource-poor settings

- Need for simple, robust and cheaper CD4 tests that can be delivered at decentralised health facilities

A


Procedural control
350 reference
Test line (CD4)
C
350
T


C
350
T
A
B
VISITECT CD4

B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Test commercialisation

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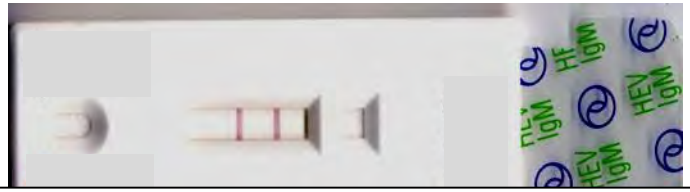
Hepatitis E IgM



MP

Visitect CD4 (HIV)

SAVING LIVES AT BIRTH:

omega DIAGNOSTICS

Visitect Syphilis IgA

SAVING LIVES AT BIRTH:
A GRAND CHALLENGE FOR DEVELOPMENT

omega DIAGNOSTICS GROUP PLC

Novel biomarkers

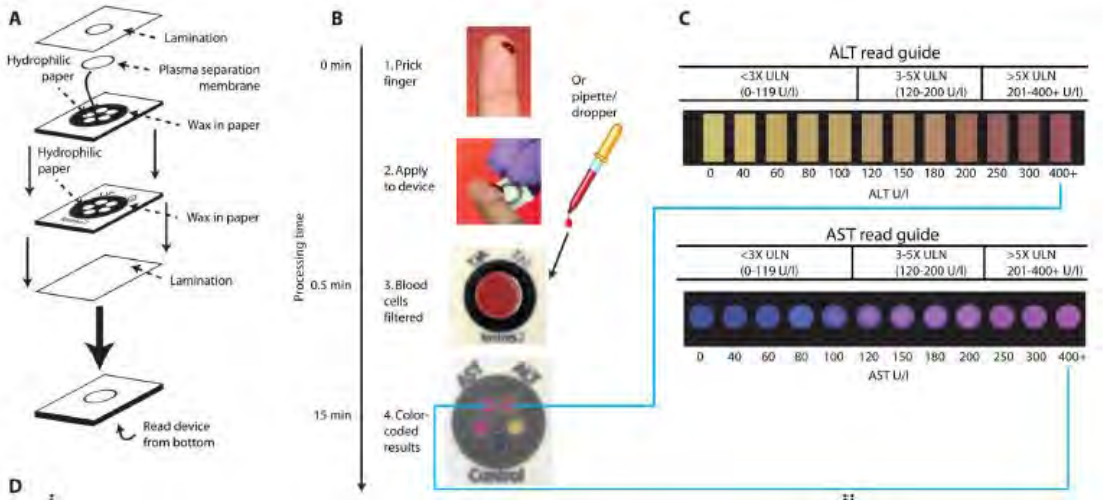
- ALT (alanine aminotransferase) as a POC test for liver disease

- Plasma separation (and drying) for centralised lab testing (HIV viral load, serology)

- Dimeric IgA (dIgA) as an improved marker of acute infection compared to IgM (higher specificity)

POC test for ALT – Why?

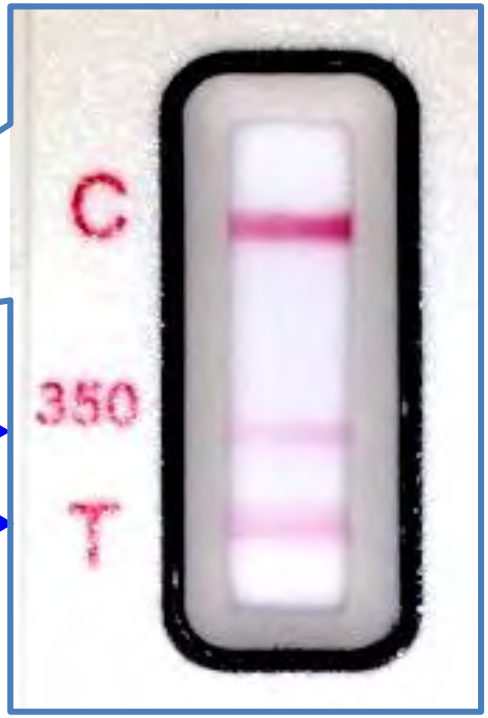
- ALT (Alanine aminotransferase) is a commonly used marker of liver damage (acute and chronic)
- EASL guidelines suggest 40 U/L as upper limit of normal
- ALT enzymatic reaction requires expensive instruments



POC test for ALT – How?

A

B



PROTEIN

C

INTERPRETATION:

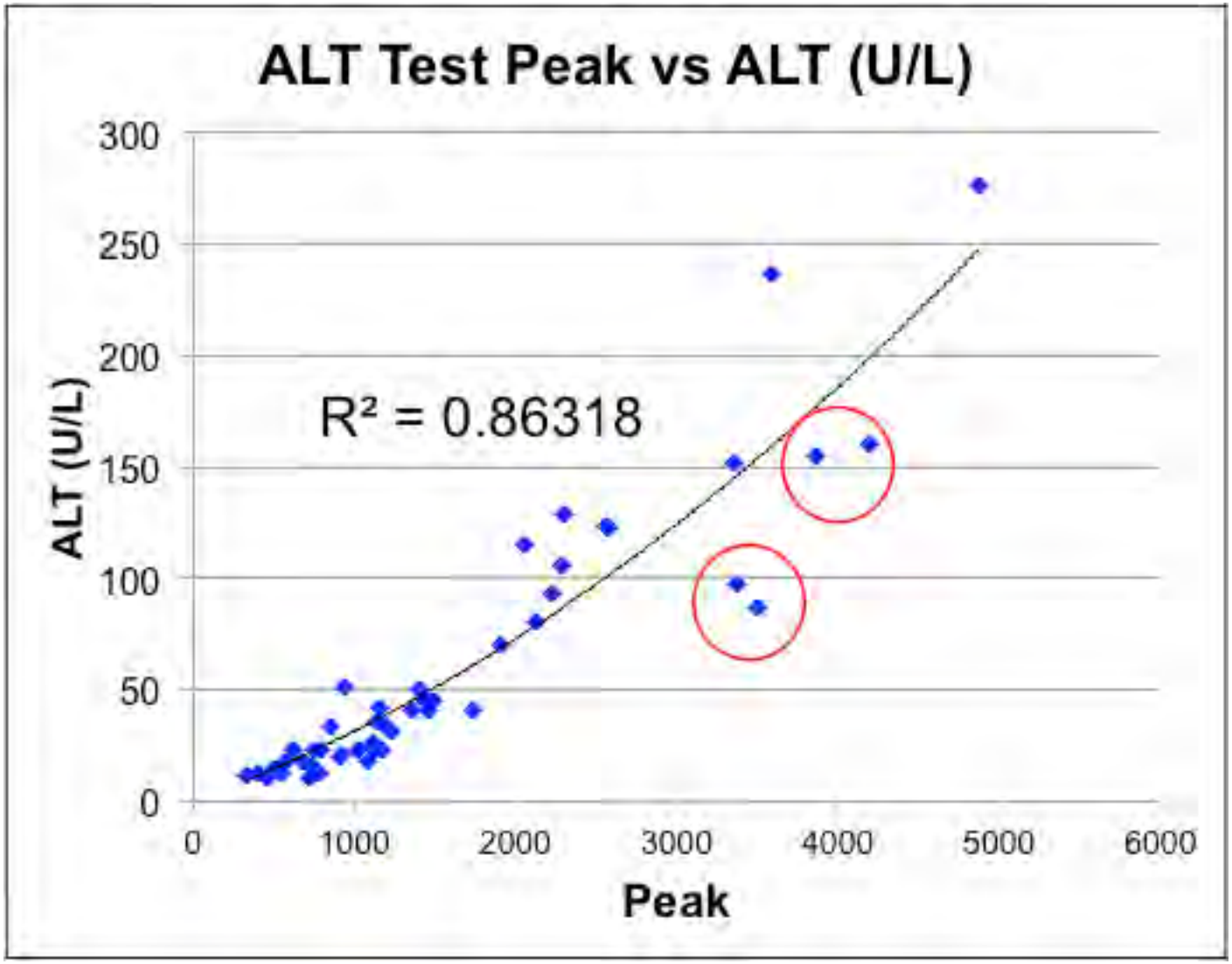
Test line ≥ reference = CD4 T-cells > 350

Test line < reference = CD4 T-cells <350

Example results for lateral flow POC ALT

C T

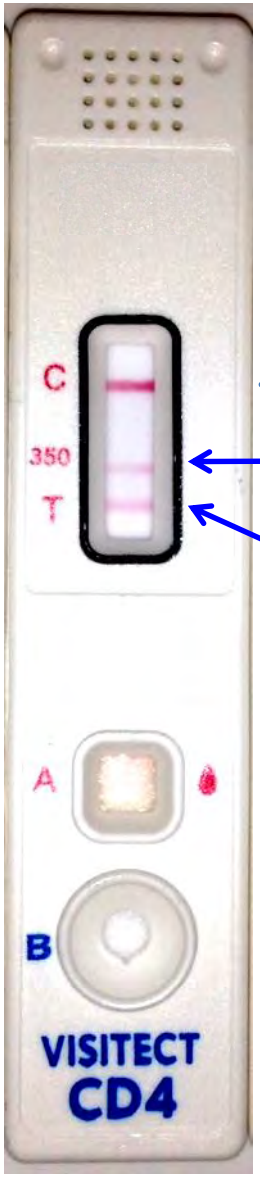
Correlation with standard (enzymatic) ALT



Melbourne, n=48

Proposed final POC test for ALT

A

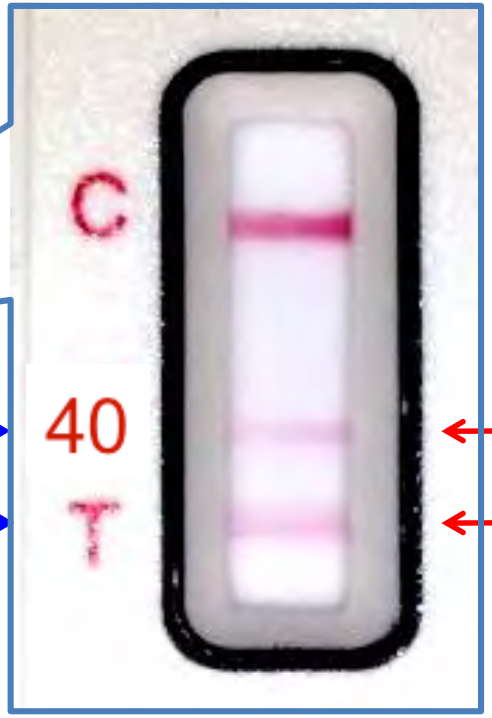


B

Procedural control

350 reference

CD4 Test line



Alanine aminotransferase (ALT) test

Reference (40 U/L)

ALT test line

C

INTERPRETATION:

Test line ≥ reference = liver disease

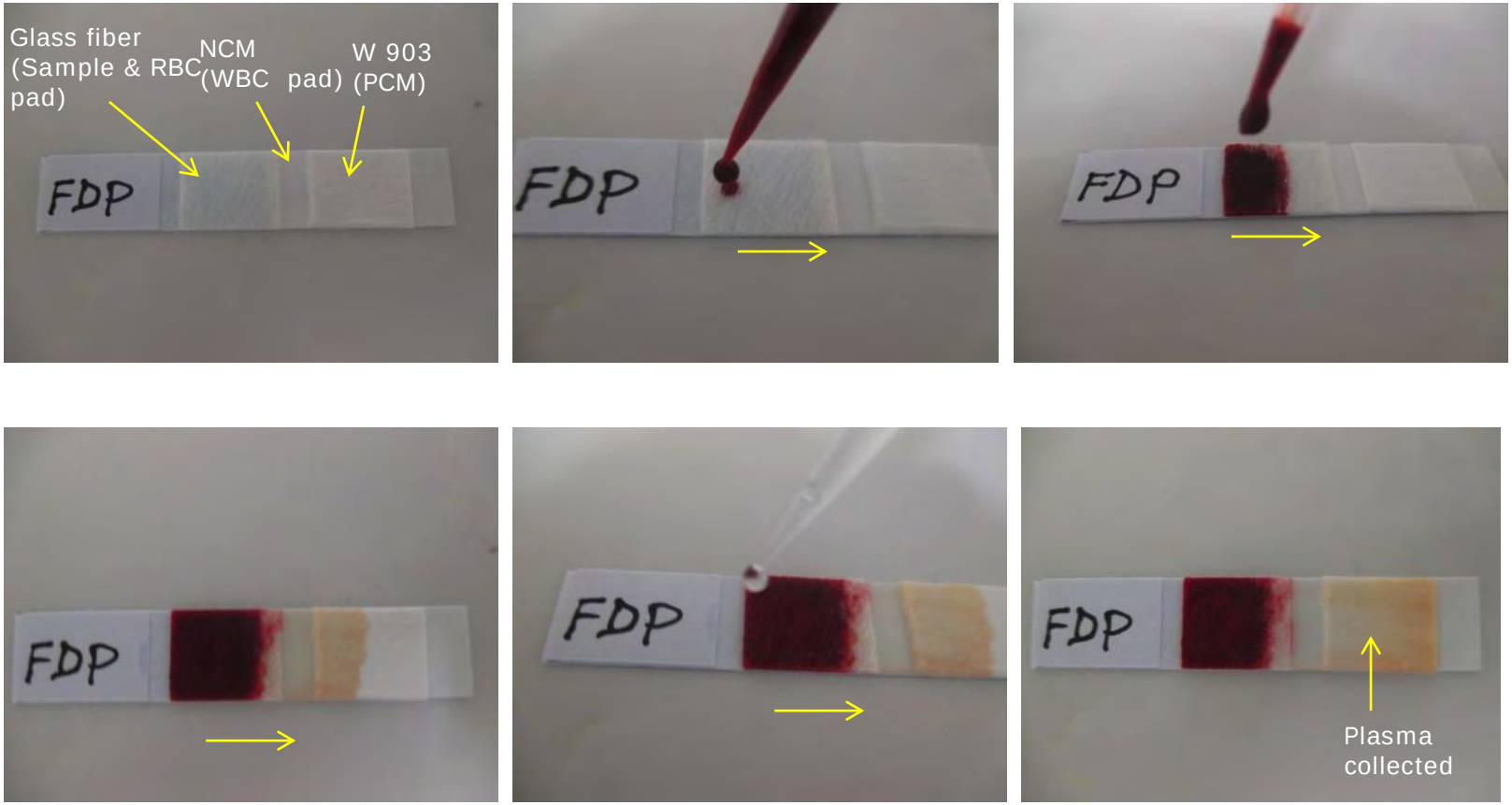
Test line < reference = not liver disease

Plasma Separation Device

- Preparation of cell-free plasma from whole blood at point of care for HIV viral load, avoiding false positive results from white blood cells in dried blood spots
  - oVL-Plasma or FDP (filtered dried plasma)

- Lateral flow design, adapted from Burnet's CD4 test
- Berhan Ayele Haile, PhD project
- Cartridge design in collaboration with Axxin Ltd, Melbourne
- Patent pending for device and cartridge
- Licensed to Nanjing BioPoint for manufacture and sale
- Now pursuing use for serological testing as well as HIV viral load

Plasma separation



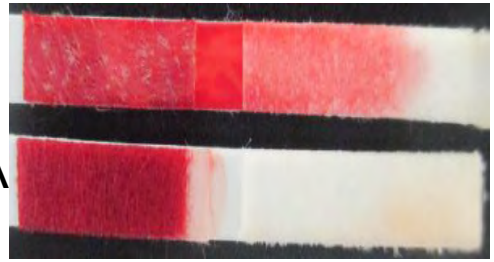
Efficient retention of RBCs and WBCs

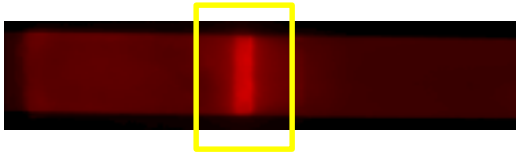
RBCs

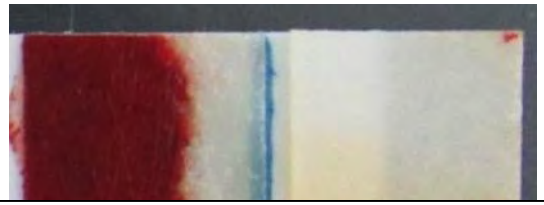
WBCs

Non treated GF

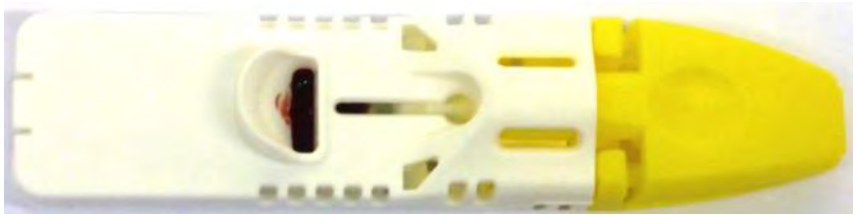
Anti-Glycophorin A Treated GF







BioPoint VL-Plasma



南京澳百生物技术有限公司
Nanjing BioPoint Diagnostics

Efficiency to diagnose ART failure at 1000 copies/ml cut off (n=200)

Sample type	Sensitivity (CI)	Specificity (CI)	Positive predictive value (CI)	Negative predictive value(CI)
DBS	100 (78- 100) %	46 (35-57)%	27(14- 37)%	100(91 -100)%
FDP	100(85 -100)%	100 (98 -100)%	100(85 – 100)%	100 (98 -100)%
FVE	87(66 -97)%	100 (98 -100)%	100 (83 -100)%	98 (95 -99%)

Manufacturing of complete device (Q1 2017)
200 patient trial underway in Malaysia (Burnet Institute, University Malaya) with 100% accuracy (n=106 at January 2018)

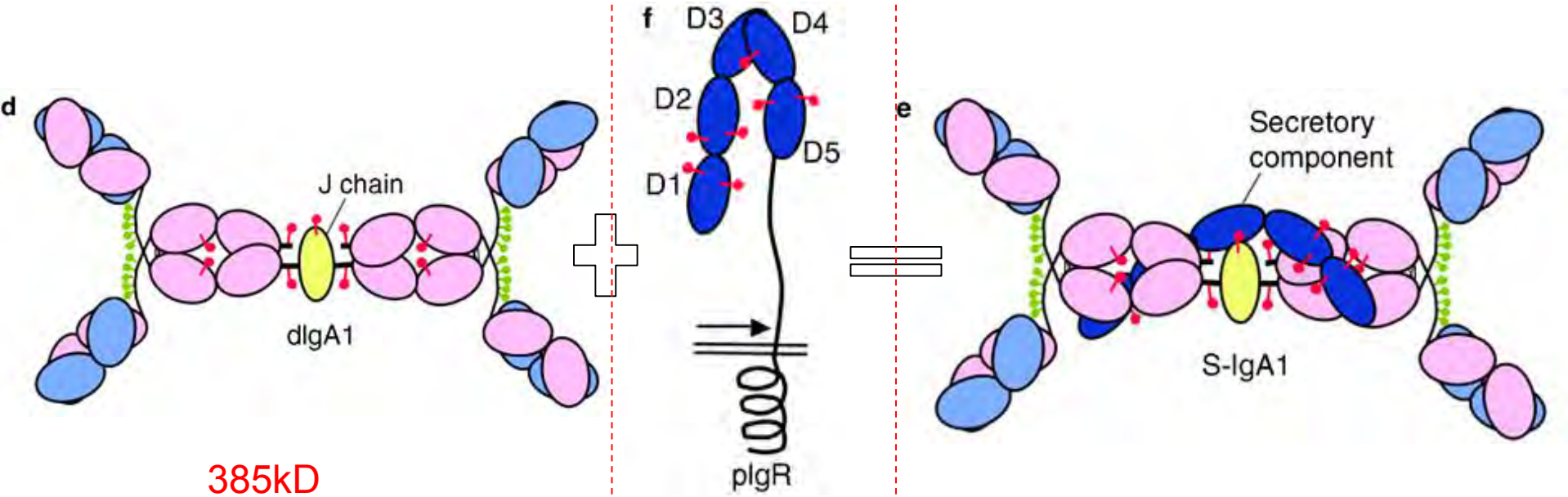
Dimeric IgA (dIgA) as a serological marker

- IgG persists for life; IgM is usually a marker of acute infection but commonly cross-reactive (often 3-5% false positive even in lab-based assays)

- IgA has shown some promise but fails in larger studies
  - ≈ 90% of serum IgA (1.8 mg/ml) is monomeric and unrelated to mucosal antigen exposure

- Dimeric IgA (dIgA) is produced by B-cells in the lamina propria, directly exposed to antigens at the mucosa
  - ≈ 10% of serum IgA (0.2 mg/ml) is dIgA, with a short half-life due to rapid export (excretion as secretory IgA)

Sub-epithelium (Plasma) Epithelium (Basal) Mucosal surface or lumen (Apical)



385kD

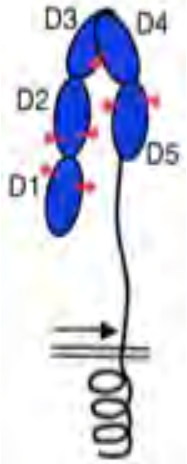
83kD

Detect the fraction of dIgA that is NOT secreted as SIgA but instead found in blood

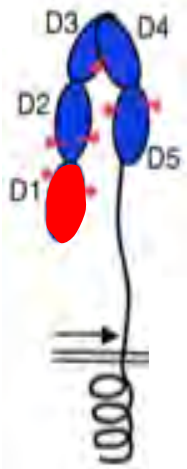
Woof JM and Russell MW. *Mucosal Immunology* **4**, 590-597 (November 2011)

Experimental approach

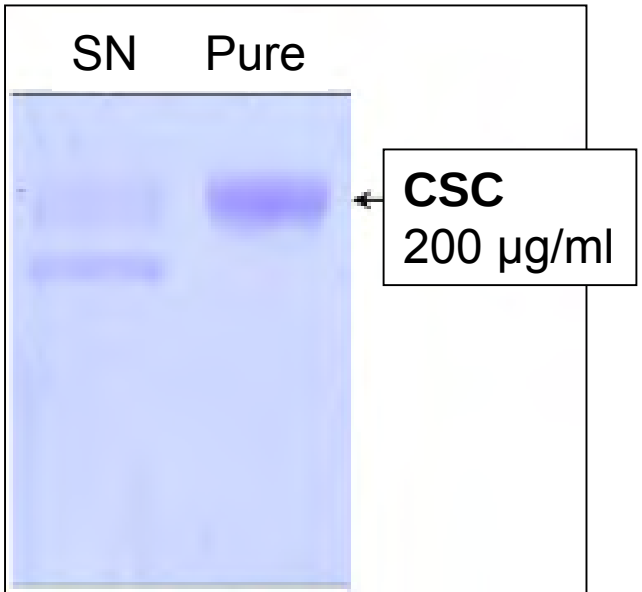
- IgM, IgG detected using anti-IgM, anti-IgG
- dIgA detected using recombinant pIgR expressed in mammalian cells, and anti-SC
  - Chimera of rabbit and human pIgR (R/HpIgR, or CSC)
    - reduced binding of IgM compared to human pIgR
      - Roe et al. 1999. J Immunol 162:6046-52.



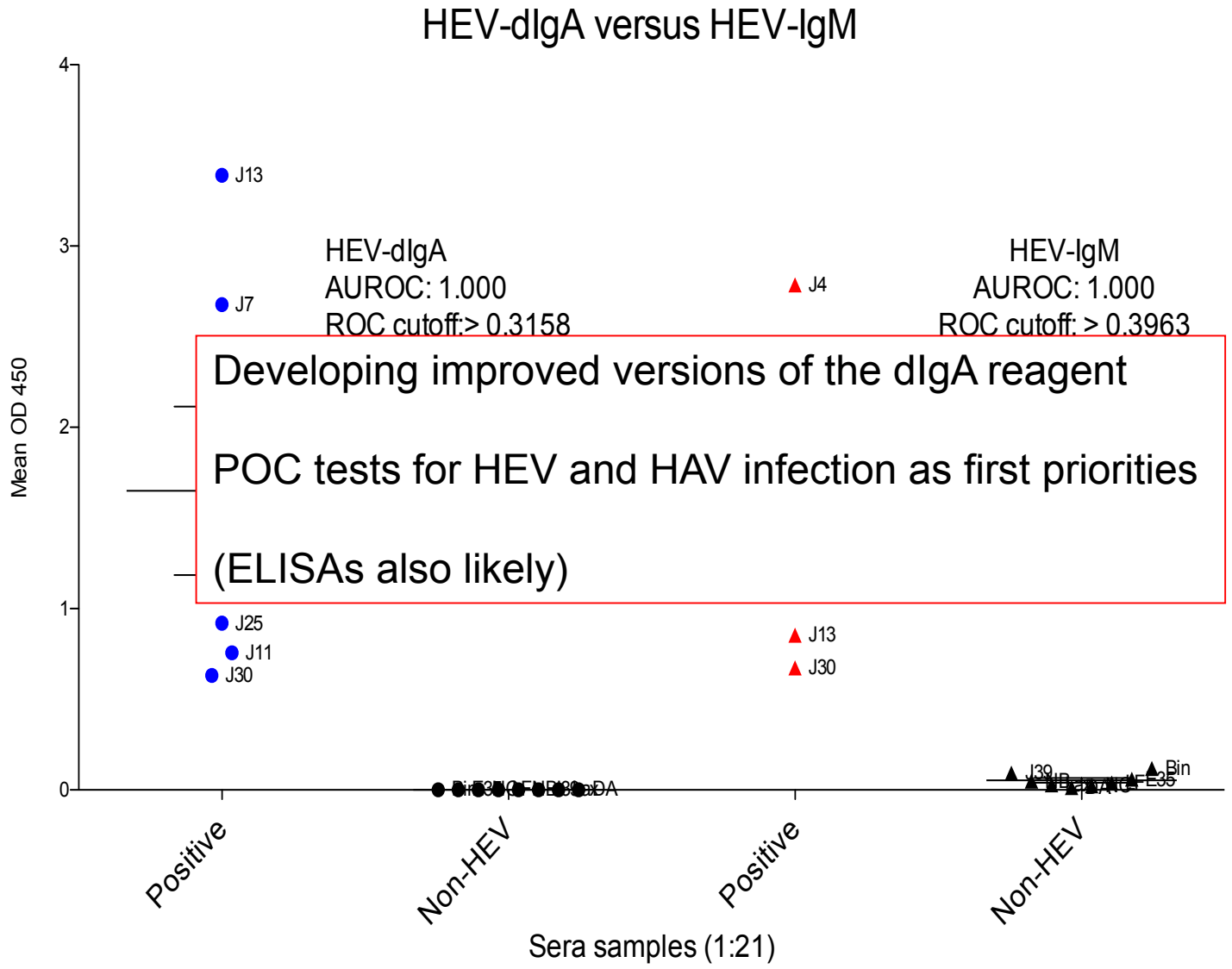
HpIgR



R/HpIgR (**CSC**)



Proof of concept – hepatitis E



Burnet Diagnostics pipeline

- Focus on strengths – innovation using conventional lateral flow platform
- Current projects at Burnet:
  - CD4, and syphilis IgA (outlicensed)
  - ALT (Liver disease and progression)
  - Plasma separator (HIV viral load)
  - dIgA (HAV, HEV, other infections in future)
  - Neonatal and adult sepsis (Longitude Prize, philanthropic)
  - G6PD for malaria therapy (primaquine sensitivity - NHMRC)
  - Improved malaria (vivax) tests (FIND)

- Approaches to translation/commercialisation?

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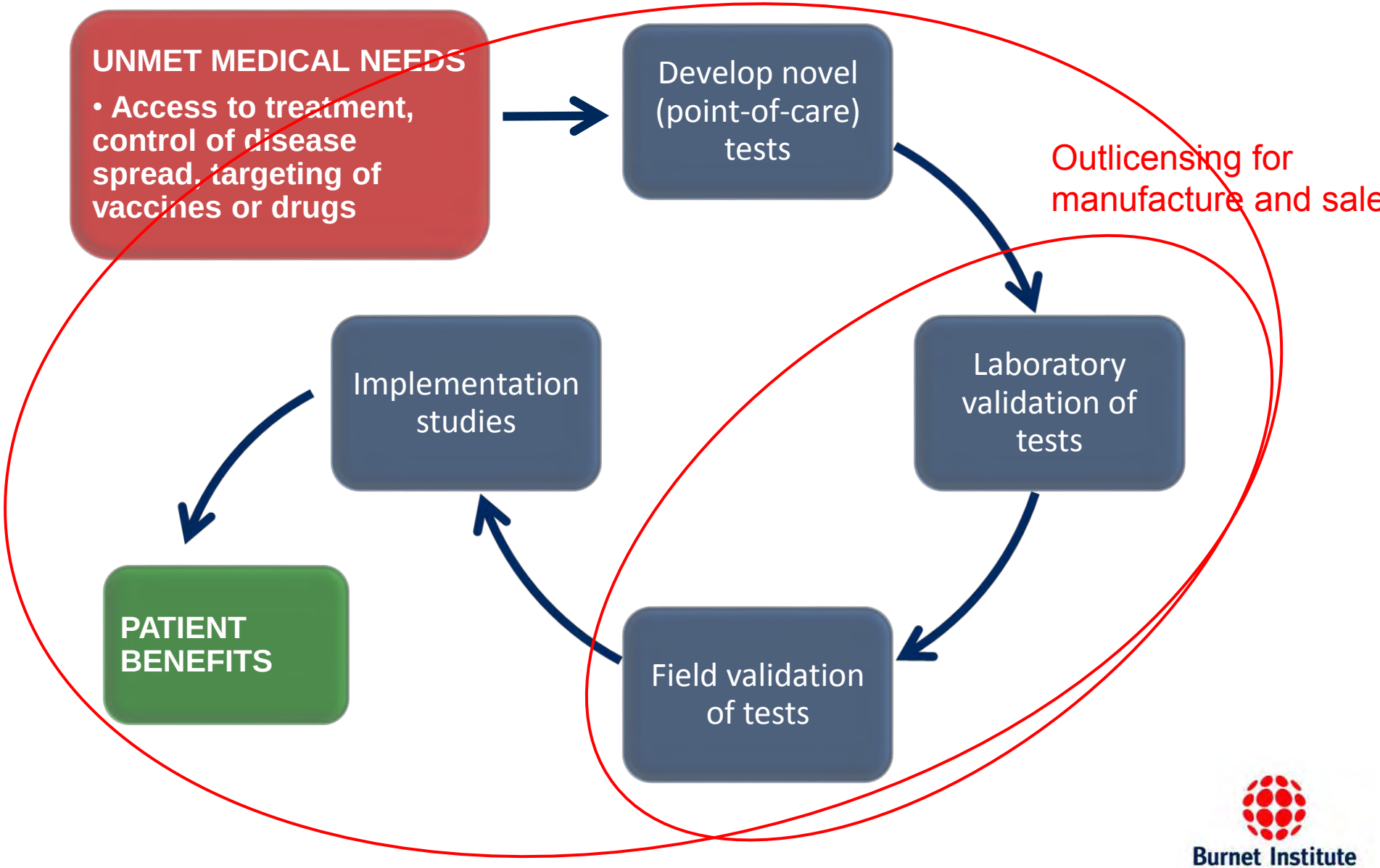
POC Diagnostics Roadmap



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Commercialisation (2)

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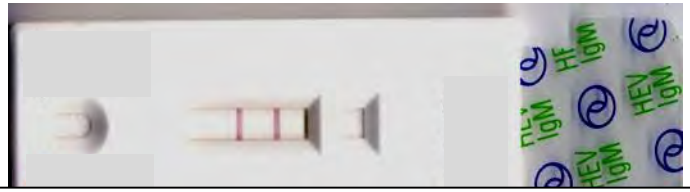
Hepatitis E IgM



MP

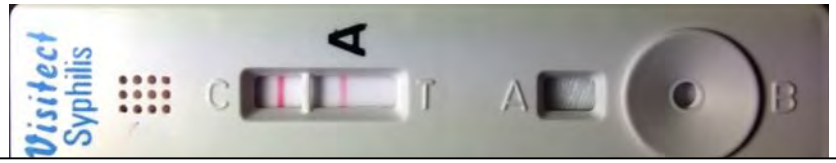
Visitect CD4 (HIV)

SAVING LIVES AT BIRTH:

omega DIAGNOSTICS

Visitect Syphilis IgA

SAVING LIVES AT BIRTH:

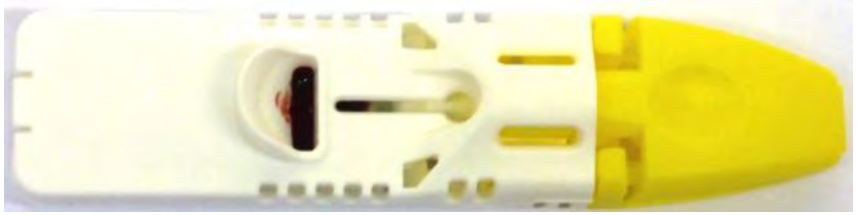


omega DIAGNOSTICS GROUP PLC

BioPoint Liver

南京澳百生物技术有限公司

BioPoint VL-Plasma



南京澳百生物技术有限公司
Nanjing BioPoint Diagnostics

Burnet in China – Building on 20 years....

- **Nanjing BioPoint Diagnostic Technology Co. Ltd.**

- Wholly foreign-owned entity (WFOE), established in 2013 with 321 grant in Jiangsu Life Science Technology and Innovation Park
  - ≈$240,000 from Nanjing Government, plus ancillary support
  - 12.5 million RMB (≈2.5M $A) Chinese investment 2014

- Development and commercialisation of point-of-care diagnostic tests for China and global markets in areas of **unmet medical need, starting with ALT test (liver disease) and VL-Plasma (plasma separator)**

南京澳百生物技术有限公司
Nanjing BioPoint Diagnostics

Burnet Institute
Medical Research. Practical Action.

Jiangsu Life Science Technology and Innovation Park, Nanjing

Nanjing BioPoint – GMP facility

Burnet Institute and BioPoint Hong Kong

BioPoint Hong Kong

100% ownership

南京澳百生物技术有限公司
Nanjing BioPoint Diagnostics

Late-stage R&D
Manufacturing and reg.
Commercial operations

78% ownership

Burnet Institute
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Mid to late-stage R&D
(Pipeline)
Clinical validation studies
Implementation studies