Point of Care Molecular Diagnostics

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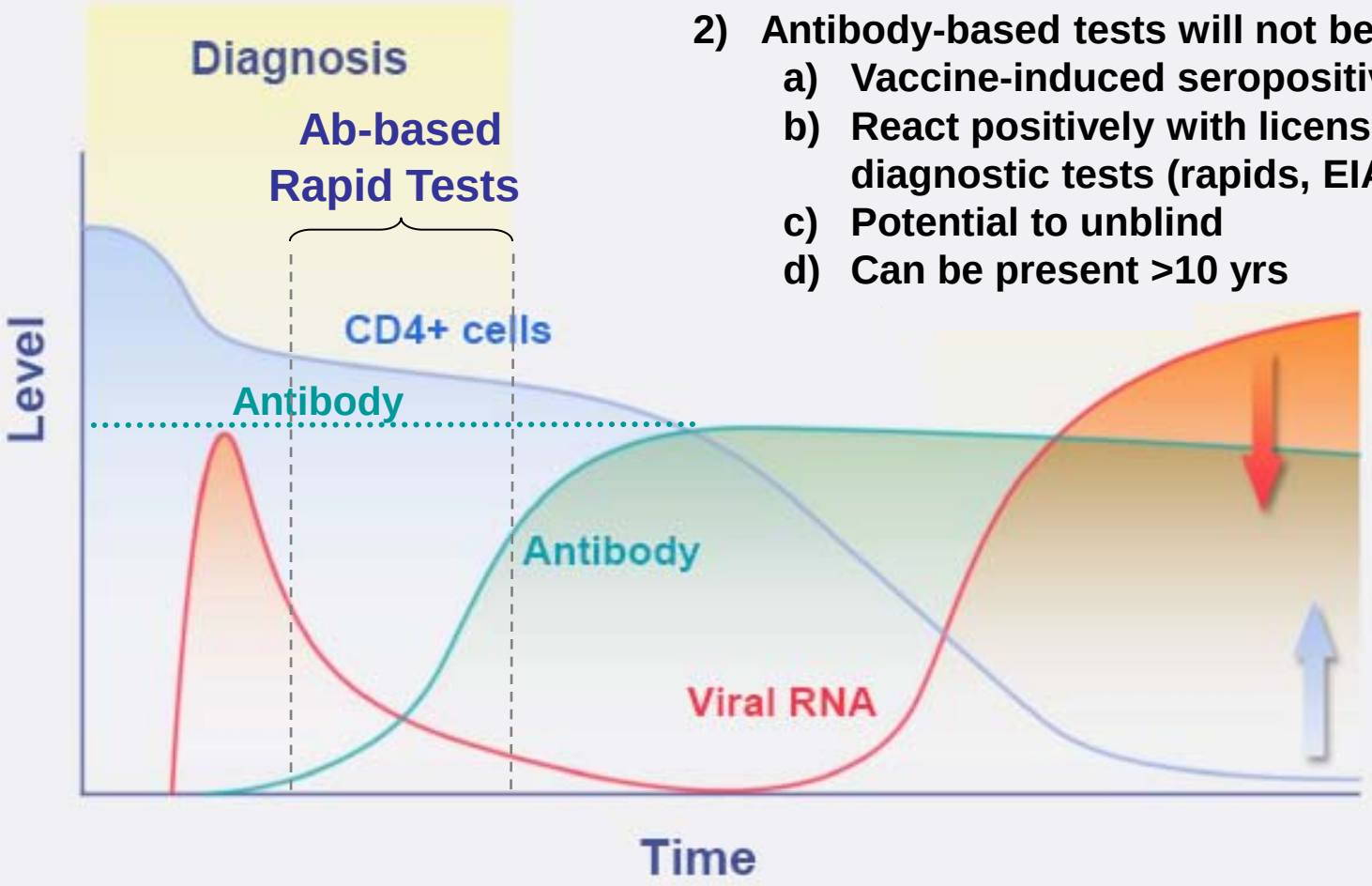
Henry Jackson Foundation

Vaccine Research Program, Division of AIDS, NIAID, NIH

Vaccine Specific Issue

1) Advantages of rapid tests
   a) Quick (loss to follow-up)
   b) Easy (minimal infrastructure)
   c) Cost ↓

2) Antibody-based tests will not be effective
   a) Vaccine-induced seropositivity (VISP)
   b) React positively with licensed diagnostic tests (rapids, EIA, WB)
   c) Potential to unblind
   d) Can be present >10 yrs



Vaccine Diagnostic Needs

SELECTest

- Being developed by Westat® for blood donors (NHLBI)
  - Able to differentiate true HIV infection from VISP

- Identify conserved sequences in p6 and gp41
  - Recognized soon after infection (similar sensitivity to 3rd gen)
  - Do not contain protective epitopes
  - Are not part of most current HIV vaccines in development

Alternatively: directly detect virus

- Eliminate the chance of a future vaccine containing the same epitopes as the diagnostic assay
- Nucleic acid testing (NAT)
  - Efficacy trials are performed in resource-limited settings
  - NAT requires expensive equipment, reagents and highly skilled technicians with sufficient infrastructure to support testing
- Need for simple, affordable and robust molecular point-of-care diagnostic device

Beyond Diagnostic Needs of Vaccines

Additional applications for POC NAT

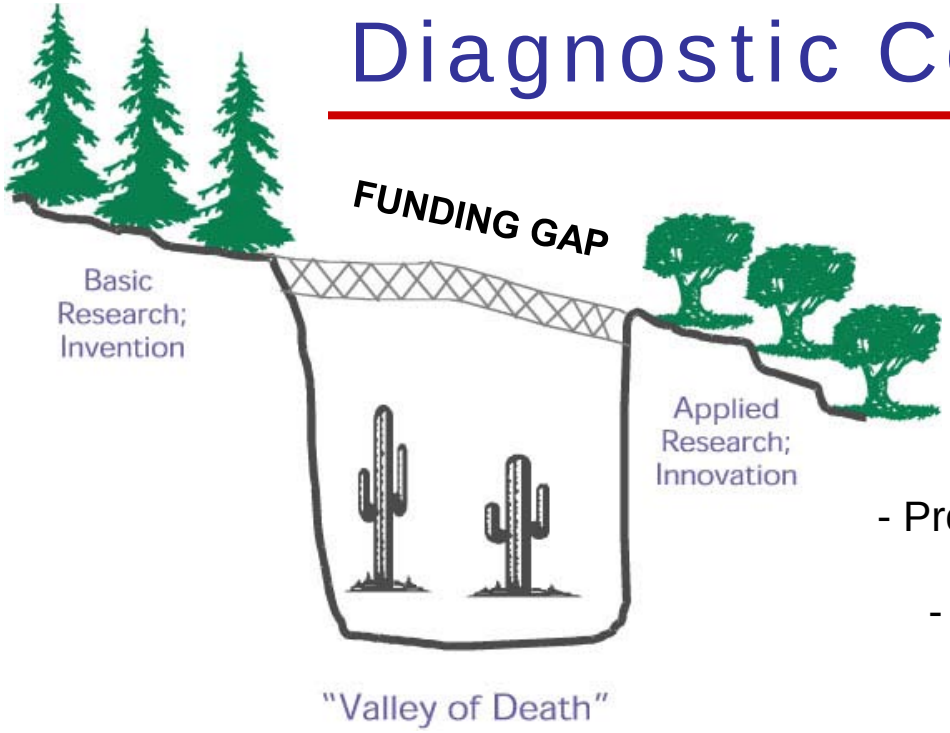
- Early infant diagnosis (maternal antibodies)
- Identify acutely infected (RNA+/Ab-) individuals (window phase highly viremic)
- Test and treat (limit transmission, reduce TB co-infections)
- Monitor for therapeutic efficacy (compliance and viral DR)
- Monitor for infection in PrEP (low dose treatment may accelerate DR)
- Disaster readiness (blood transfusions in earthquake)

Diagnostic Companies

- Genuine desire to do good

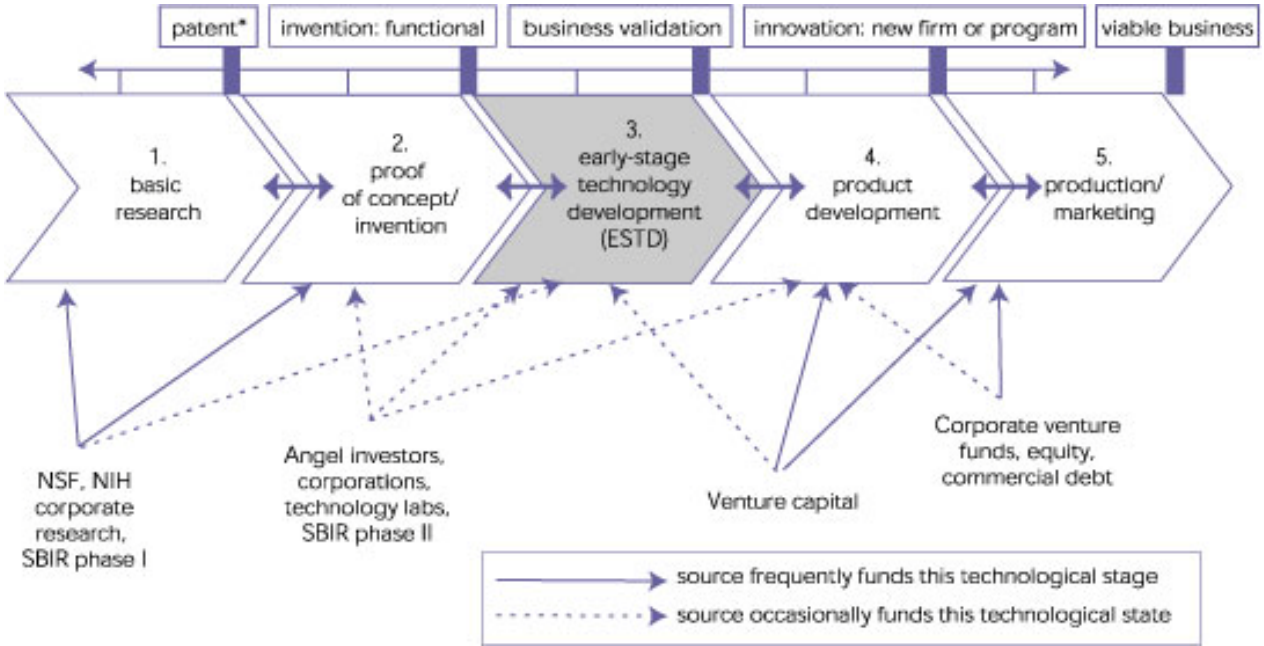
- Identified medical need

- Matched technology



- Product launch

- Access to markets



Venture Capital

Difficult to convince investing in a product with the intended use in disadvantaged and vulnerable populations in the poorest settings

BAA: Contract mechanism

BAA: Broad Agency Announcement (peer reviewed)

- Government identifies research area and specifications
- Offeror responds with the statement of work (SOW)

Three contracts were awarded September 2009

- Advanced Liquid Logic (5.2M)
- Diagnostics for the Real World (4.7M)
- Wave 80 Biosciences (7.5M)

Scope of work to be funded: All phases of technology development, product development, validation *except:*

(1) basic research on core POC platform

(2) Phase I, II, or III clinical trials

Anticipated timeline: 3-5 year program

- Technology/product development
- Analytical/pre-clinical studies
- Clinical studies

BAA: Performance & Operational Characteristics

sample	type volume preparation	whole blood (plasma…) 100-200 uL 1-3 steps
assay	LOD sensitivity specificity subtypes	200-1000 copies/mL 90-95% 99.5-99.9% M,N,O
diagnostic	time-to-result shelf life at 37 C humidity transportation stress	90-120 minutes 12-24 months 70 % 50 C for 48-72 hours
controls	negative positive	full process negative internal positive
biosafety	containment	closed, self-contained system no biosafety cabinet required unprocessed sample transfer only, no open handling of material
instrument	handheld power requirements	portable battery powered
reporting	interface	LED readout, electronic data transfer flexible database architecture: capture, store, integrate
training	community health worker high school diploma	< 1 hr < 8 hrs
cost	per test result	$12 - $20 USD

Balancing the Needs

Sensitivity
Limit of detection

Robust
Fast
Low sample vol.
Whole blood
Low Cost

WHO guidelines (2006)

- Do not recommend the routine use of VL testing for diagnosing treatment failure due to the high cost and feasibility (currently being revised) in resource-limited settings
- Affordable molecular diagnostics which reduce loss to follow up will allow health care workers to monitor patient compliance and viral drug resistance

Diagnostics for the Real World: SAMBA Device and Point of Care Machine

Front view

Rear view

Simple technology

- Sample preparation module in development
- Cartridge with breakable seals
- Isothermal NA amplification ~1 hr
- Dipstick-based visual detection

SAMBA (Simple AMplification BAsed nucleic acid test) machine

Lee et al. J. Infect. Dis., April supplement 2010

Performance of SAMBA detection of HIV-1 in clinical samples (Barts hospital, London)

- SAMBA detected 189 of 191 HIV positive samples

Subtype	No. samples (%)	Viral load	No. SAMBA +
A	31 (16.3)	214 - $5x10^5$	31/31
B	25 (13.2)	78 - $6x10^6$	24/25
C	30 (15.8)	278 - $6x10^5$	30/30
D	9 (4.8)	$7x10^3$ - $1.8x10^5$	9/9
F	7 (3.8)	268 - $6x10^4$	7/7
G	5 (2.7)	937 - $5.7x10^4$	5/5
H	1 (0.6)	526	1/1
J	4 (2.2)	$7x10^3$ - $2x10^5$	4/4
K	5 (2.7)	$1x10^3$ - $2x10^4$	5/5
Recombinants	74 (38.8)	54 - $4x10^7$	73/74
Negatives	225	0/225	

Sensitivity = 98.95% (189/191)

Specificity 100% (0/225)

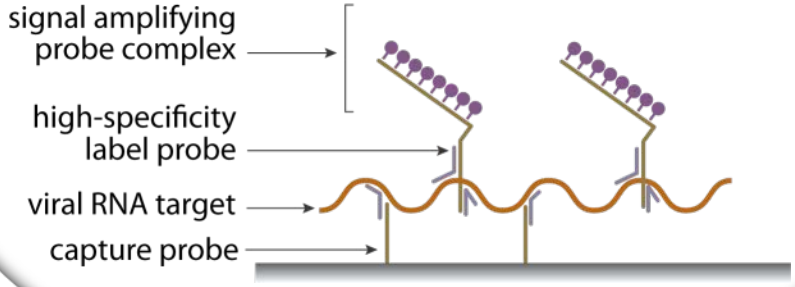
WAVE 80 Biosciences

L. Mazzola: Poster #40

- Continuous-flow microfluidics
- Onboard lyophilized reagents
- No fluid exchange
- Disposable
- Finger-stick sampling
- Licensed microchip and assay technology
- Wave 80 proprietary IP

Branched DNA-like

Nucleic Acid Signal Amplification



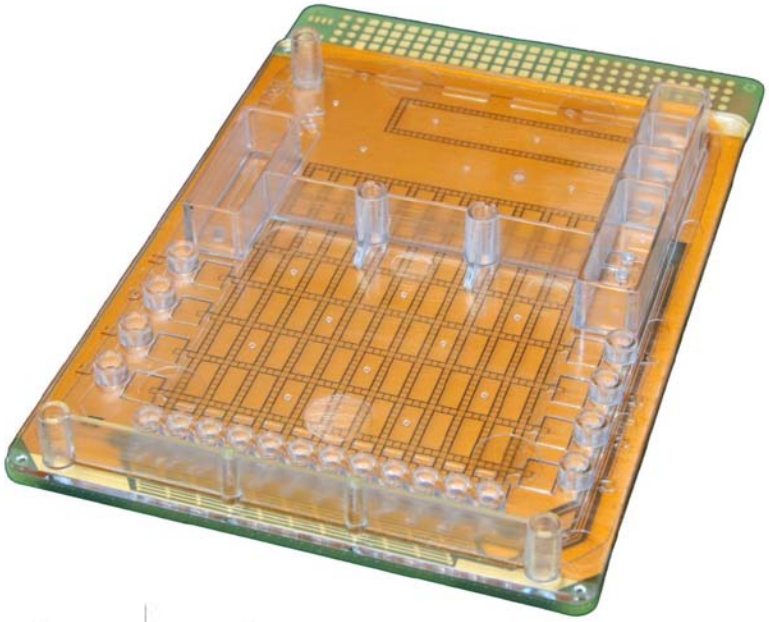
- Highly sensitive signal amplification
- No risk of amplifying non-targeted RNA
- No temperature or stability issues

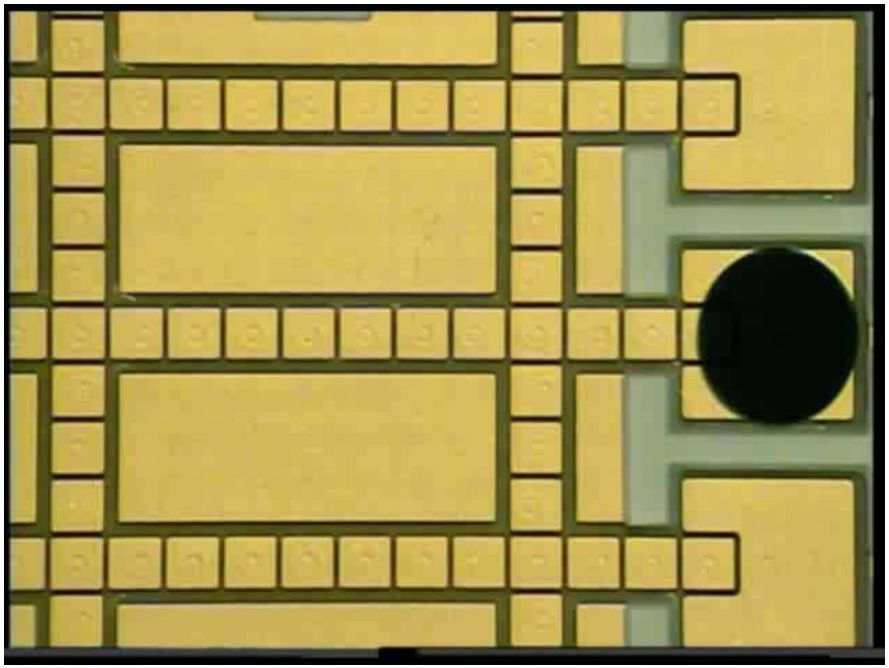
- Flexible instrument design
- Luminescent readout
- Robust operation
- Low maintenance

Advanced Liquid Logic

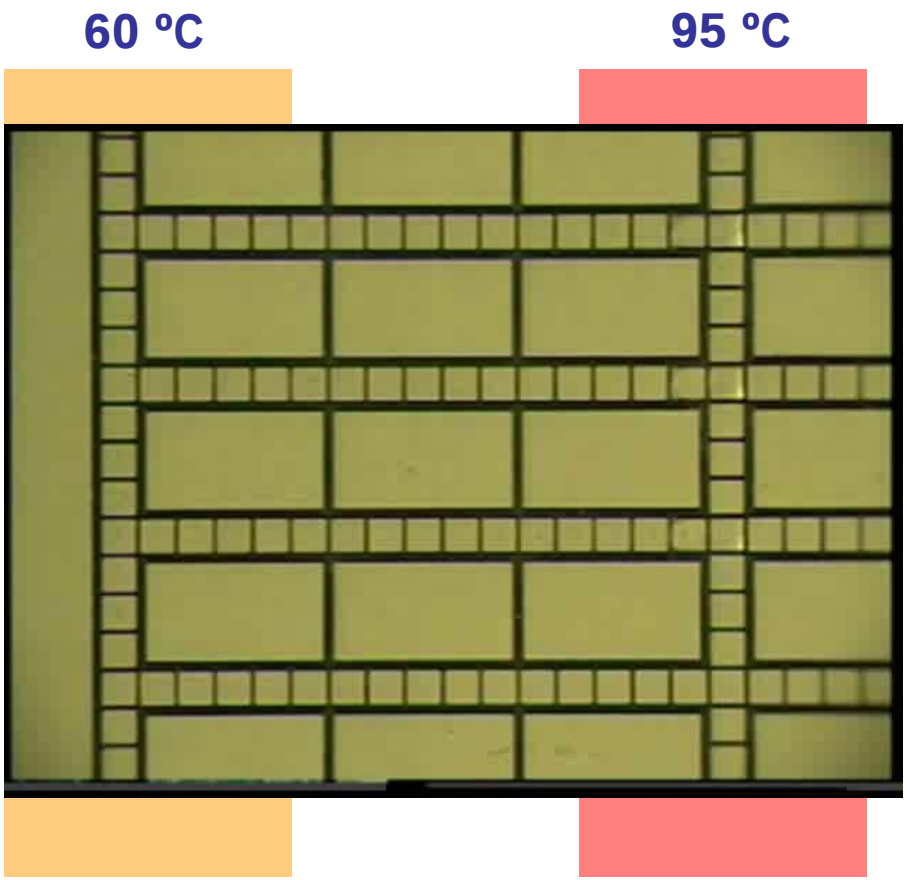
Digital microfluidics

- Cartridge is fabricated using low-cost printed-circuit-board technology
- No pipes, pumps or valves
- Discrete droplets are manipulated electrically (electrowetting) within an oil-filled cartridge
- Use whole blood with a magnetic bead capture protocol

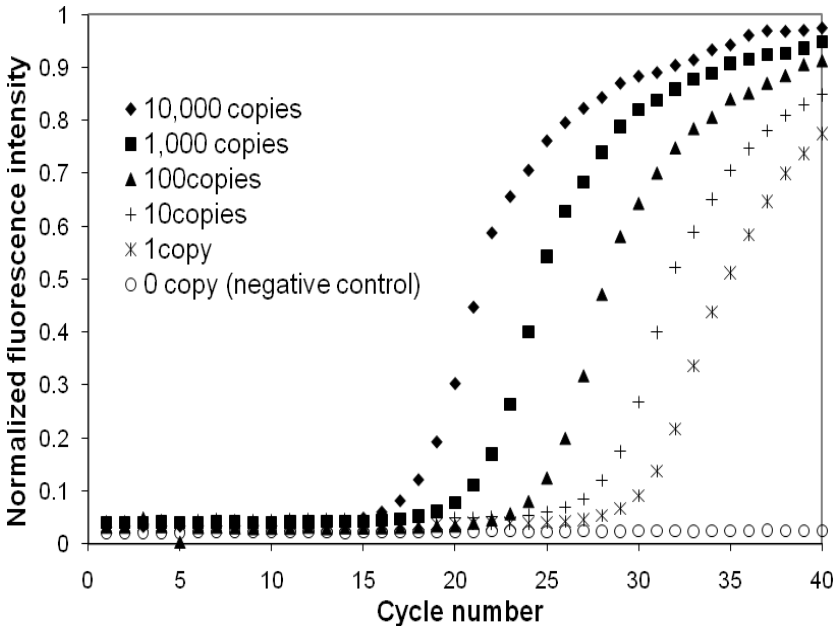




Flow-Through Real-Time PCR



MRSA Titration



Hua et al., Analytical Chemistry, 2010

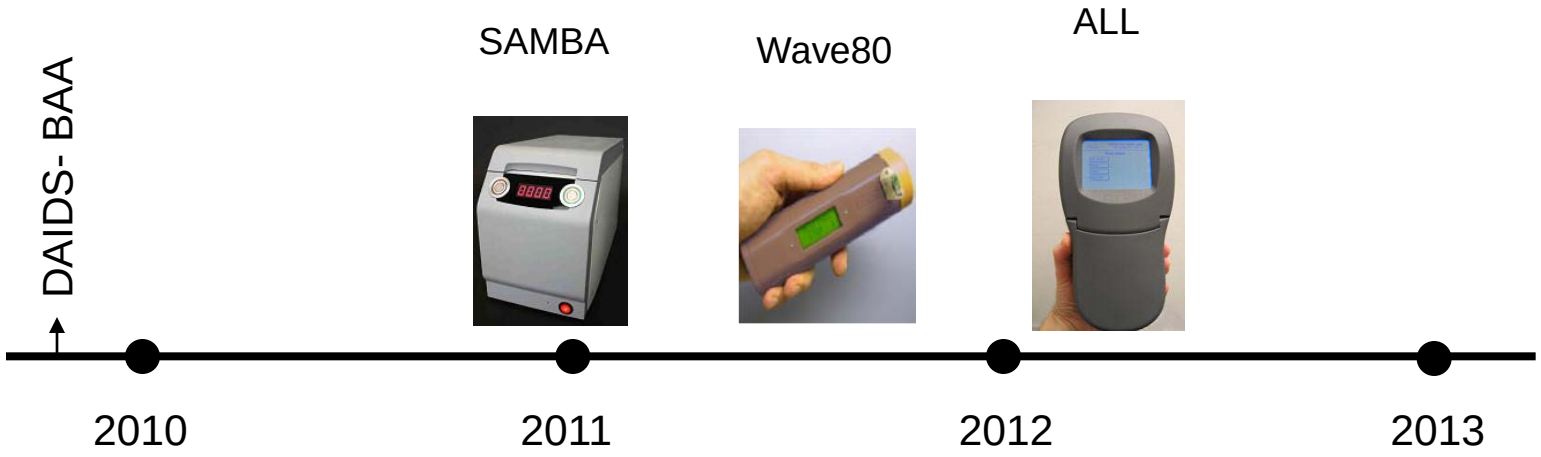
POC Technology Pipeline

Near POC

- Tabletop assays using finger-stick blood will be evaluated in clinical trials over the next year (DAIDS can assist in evaluation)

Next generation POC

- Handheld microfluidic-based battery powered assays require an additional year of development before clinical trials



Other Potential Products

Acknowledgements

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