HIV Point Of Care Testing

Near to the patient but far from perfect?

Dr Mat Donati, PHE Bristol PHL
Overview

What are point of care tests?
HIV infection host response timeline
HIV test formats and history
What is the point, of point of care?
What’s in the box- the technology
Examples
Performance- on the box and in real life
POCT Definitions And Synonyms

- **Point of Care Testing** (POCT) can be defined as medical diagnostic testing performed outside the clinical laboratory in close proximity to where the patient is receiving care.

- POCT is typically performed by non-laboratory personnel and the results are used for clinical decision making.

- Also known as near patient tests (NPTs), rapid/quick tests.
Familiar POCTs

Based on

• Biochemical reactions
• Immunological/ serological reactions
Familiar POCTs

Based on

• Biochemical reactions
• Immunological/ serological reactions
• Increasingly molecular
Host Response To Infection

Standard sequence

• Infection

• Virus replication (detection: nucleic acid, antigen, culture)

• Host response (cellular, antibody, IgM then IgG)
HIV Diagnostics Evolution And Terminology

1985 first licensed HIV antibody test, blood supply testing begins

Different test principles/ formats = different ‘generations’

• **1st generation**: HIV antigens derived from cell culture whole virus lysate
  • Detects IgG only. Significant specimen dilution is required to overcome cross-reactivity with cellular protein contaminants. *[no longer used in UK]*

• **2nd generation**: Synthetic peptide or recombinant protein antigens
  • Improves sensitivity for HIV-1 group O and HIV-2
  • Eliminating cellular antigens that contaminate viral lysates improves specificity by eliminating cross-reactivity with cellular proteins *[some rapid tests]*
HIV Test Evolution (2)

- **3rd generation**: Synthetic peptide or recombinant protein in immunometric antigen sandwich format
  - Detection of IgM and IgG antibodies
  - Lower sample dilutions and the ability to detect IgM antibodies increase sensitivity during early seroconversion [*some commercial assays*]

- **4th generation**: Same antibody detection as 3rd generation, and monoclonal antibodies are also included to detect p24 antigen
  - Inclusion of p24 antigen capture allows detection of HIV-1 infection before seroconversion ("combo" assays") [*preferred screening test*]

- HIV1 focused- HIV2 diagnostics less developed; HIV2 equivalent of p24 antigen for HIV1 (p26/27) is not present in 4th gen tests [but cross reactivity may occur]
Relative Timelines

- Approximately 10 days after infection, HIV-1 RNA becomes detectable.
- HIV-1 p24 antigen rises to levels that can be detected by 4th generation immunoassays within 4 to 10 days after the initial detection of HIV-1 RNA.
- p24 antigen detection is transient because, as antibodies begin to develop, they bind to the p24 antigen and form immune complexes.
- IgM antibodies are expressed which can be detected by 3rd and 4th generation immunoassays 3 to 5 days after p24 antigen is first detectable (10 to 13 days after RNA).
- Finally, IgG antibodies emerge and persist, becoming reactive in the most sensitive assays 18 days after the initial detection of viral RNA.
Laboratory testing for the diagnosis of HIV infection, updated recommendations. CDC USA, June 27, 2014
The Point of POCT

• Immediate result, immediate care
  • Rapid decisions, entry to care pathway [A&E, GUM, ANC]
  • Rate of sexual transmission high during acute infection
  • 26x that of established infection; 10-50% all new HIV

• Inability to deliver standard laboratory test
  • Difficult to bleed, resource poor/ infrastructure

• Individual choice
  • Quick, home testing, testing outside traditional healthcare settings, outreach

• Expansion of those who can perform testing
Test Technology

Typical immunochromatographic lateral flow

Procedural Control
Or Human antibody addition control

Other formats exist
Examples

- Rapid vertical flow
  - Very fast
  - Second membrane prevents backflow of fluids

- Lateral flow

Immunochromatography

Immunofiltration

Sample pad  Test line  Control line

Rapid vertical flow
Very fast
Second membrane prevents backflow of fluids
HIV POCT Examples

Lots available from many different suppliers, some the same with different marketing names

- Variable in appearance, and performance
  - CE marked
  - FDA approved
  - WHO prequalified

Abon HIV 1,2,0 RTD
Insti HIV1/HIV2 Ab
MedMira Reveal G3 rapid HIV1 Ab
SD Bioline HIV 1/2 3.0
SD Bioline HIV Ag/Ab combo
Uni-gold HIV
Alere Determine HIV 1/2
Alere Determine HIV 1/2 Ag/Ab combo
Multispot HIV1/HIV2 Rapid Test
HIV 1/2 stat pak
Chembio DPP HIV 1/2
OraQuick ADVANCE Rapid HIV-1/2

http://www.who.int/diagnostics_laboratory/evaluations/PQ_list/en/
http://www.cdc.gov/hiv/pdf/testingListofModCompHIVRTforlabs6514.pdf
Formats And Performance

- Many rapid devices detect antibody only and are second generation (IgG)
- Some newer kits can detect p24 antigen in addition
- Third generation formats exist but performance often similar to 2\textsuperscript{nd} gen
  - Important to consider performance according to stage of infection
Performance - Key Features

Sensitivity

• Percentage of results that will be correctly positive when the person is HIV infected
• Low sensitivity = more false negative results

Specificity

• Percentage of results that will be correctly negative when the person is not HIV infected
• Low specificity = more false positive results

[Caution - gold standard, stage of infection]
<table>
<thead>
<tr>
<th>Sample types</th>
<th>Determine Combo (March 2012)</th>
<th>SD Bioline Combo (Feb 2013)</th>
<th>Insti HIV1 /2 Ab (Aug 2013)</th>
<th>Uni-Gold Ab (Dec 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum/plasma/ capillary (whole blood)</td>
<td>Serum/plasma/capillary</td>
<td>Serum/plasma/ capillary</td>
<td>Serum/plasma/capillary</td>
</tr>
<tr>
<td>Performance Clinical specimens (~1080)</td>
<td>Sens 100% (99.1-100) Spec 98.78 (97.6-99.5)</td>
<td>Sens 100% (99.1-100) Spec 98.6 (97.4-99.4)</td>
<td>Sens 100% (99.1-100) Spec 99.7% (98.9-100)</td>
<td>Sens 99.76% (98.7-100) Spec 99.85 (99.2-100)</td>
</tr>
<tr>
<td>Mixed titre panel</td>
<td>All correct but one</td>
<td>All correct</td>
<td>Missed two</td>
<td></td>
</tr>
<tr>
<td>Subtype panel</td>
<td>Detected HIV1A,B,C, CRF01_AE, O, HIV2</td>
<td>Detected 1A,B,C, CRF01_AE, O, HIV2</td>
<td>Detected 1A,B, CRF01_AE, HIV2; NOT HIV1C or O</td>
<td>Detected 1A,B,C, CRF01_AE, HIV2; NOT subtype O</td>
</tr>
<tr>
<td>Culture supernatant</td>
<td>Detected HIV1, NOT HIV2</td>
<td>Detected HIV1, NOT HIV2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variability</td>
<td>No indeterminate or invalid, inter-reader variation 0.7%</td>
<td>No indeterminate, invalid one, variation 0.8% (0.3 Ab;0.5Ag)</td>
<td>No indeterminate or invalid, no variation</td>
<td>No indeterminate, invalid 0.09%, variation 0.09%</td>
</tr>
</tbody>
</table>

Notes: Established infection/ Manufacturer data usually superior/ Initial sens/spec quoted/ Comparator assays/ Technician use

http://www.who.int/diagnostics_laboratory/evaluations/hiv/en/
Predictive Values Of Results

\[
PPV = \frac{(prevalence)(sensitivity)}{(prevalence)(sensitivity) + (1-prevalence)(1-specificity)}
\]

\[
NPV = \frac{(1-prevalence)(specificity)}{(1-prevalence)(specificity) + (prevalence)(1-sensitivity)}
\]

Probability of the result being correct relates to population prevalence as well as assay performance.
# Range of PPV and NPV

<table>
<thead>
<tr>
<th>Prevalence HIV %</th>
<th>Poor 99% sens 98% spec</th>
<th>Best likely 99.9% sens 99.8% spec</th>
<th>PPV</th>
<th>NPV</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>~0</td>
<td>~100%*</td>
<td>5%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>4%</td>
<td>~100%</td>
<td>33%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33%</td>
<td>~100%</td>
<td>83%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>76%</td>
<td>~100%</td>
<td>97%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>90%</td>
<td>99.8%</td>
<td>99%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*99.999
Range of PPV and NPV

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</tr>
<tr>
<td>1</td>
<td>33%</td>
<td>~100%</td>
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</table>

- High NPV critical to not miss a true infection

- High PPV helps to prevent unnecessary distress/ cost/ loss of belief in testing due to false positives

- Laboratory testing algorithms employ confirmatory tests to ensure the correct result and distinguish HIV 1 from 2, and acute infection from established infection
# Seroconversion Performance

Interval in days between HIV-1 RNA detection and first reactivity in test classes

<table>
<thead>
<tr>
<th>Class Interval (all tests)</th>
<th>Value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th-Generation Laboratory Tests</td>
<td>6.8 (3.7, 9.7)</td>
</tr>
<tr>
<td>3rd-Generation Laboratory Tests</td>
<td>11.4 (9.7, 13.4)</td>
</tr>
<tr>
<td>2nd-Generation Rapid Tests</td>
<td>18.5 (16.0, 21.6)</td>
</tr>
<tr>
<td>Western Blot Laboratory Test</td>
<td>24.3 (18.8, 31.0)</td>
</tr>
</tbody>
</table>

Figure 12. Relative seroconversion sensitivity compared to an antigen/antibody combined detection enzyme immunoassay (Vironostika Ag/Ab Combo [bioMérieux])

**DETECTION BEFORE REFERENCE ASSAY (Vironostika Ag/Ab Combo)**

**DETECTION AFTER REFERENCE ASSAY**

- 95% confidence limits
- mean relative seroconversion sensitivity index

Antibody only assays
<table>
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</tr>
<tr>
<td><strong>Performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical specimens</strong></td>
<td></td>
<td></td>
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<tr>
<td>(~1080)</td>
<td>Sens 100% (99.1-100)</td>
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<tr>
<td></td>
<td>Spec 98.78 (97.6-99.5)</td>
<td>Spec 98.6 (97.4-99.4)</td>
<td>Spec 97.87 (96.4-98.8)</td>
<td>Spec 99.85 (99.2-100)</td>
</tr>
<tr>
<td><strong>Seroconversion</strong></td>
<td>Earlier 0.75 (3G reference)</td>
<td>Earlier 0.75 (3G) and 0.125</td>
<td>Later 0.25 (3G) average</td>
<td>Later 0.125 (3G) average</td>
</tr>
<tr>
<td><strong>panels (number of</strong></td>
<td>and 0.125 (4G reference)</td>
<td>(4G) average</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>samples detected</strong></td>
<td>average</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>later or earlier</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>than reference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>assay)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P24 Ag panel</strong></td>
<td>All correct</td>
<td>Missed one</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Detected 3.125 iu (ref 12.5iu)</td>
<td>Detected 3.125 iu (ref 12.5iu)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

http://www.who.int/diagnostics_laboratory/evaluations/hiv/en
Field Use Data - Combo POCT

Determine HIV Ab/Ag

Manufacturer quotes sensitivity 92% in seroconversion panels; may be less

Sensitivity for acute infection on basis of antigen line alone ranges from 0-90% 1-5

Many studies laboratory based or not whole blood in field use

True Field Use

Sydney Sexual Health Clinic, clinician/nurse testing fingerprick bloods

Compared to standard laboratory algorithm (4G plus confirmatory)

- 3,190 evaluation specimens, 39 were confirmed as HIV-positive (12 with early infection) and 3,133 were HIV negative
- Determine HIV Combo sensitivity was **87.2%** overall
- **94.4%** for the antibody and **0%** for antigen (zero of nine)
- Sensitivity in early infection was **66.7%** (all DHC antibody reactive)
  - Specificity overall was 99.4% with the antigen component contributing to 33% of false positives
- Antigen component did not enhance performance during point of care HIV testing in a high risk clinic-based population

2014 Annual Influenza European Meeting, Vienna
Sample Types- Blood/ Saliva/ Urine

- Antibody levels in urine (1mg/mL) are much lower than in oral fluid (15mg/mL IgG) which are lower than in blood
  - Oral fluid specimens consist mostly of saliva, which predominantly contains IgA class antibody, and oral mucosal transudates, which mostly contain IgG
- Very sensitive techniques are needed to achieve good performance in urine samples
- Assay performance may be suboptimal with saliva samples
  - Especially if IgG detection only
Oral Fluid

OraQuick test saliva vs blood systematic review and meta analysis\(^1\)
- Pooled sensitivity lower for saliva vs blood; specificity similar
- Saliva sens \textbf{98.3\%} (95.85-99.08); spec \textbf{99.74}
- Blood sens \textbf{99.68\%} (97.31-99.96); spec \textbf{99.91}

Oral fluid sample testing (Avioq microelisa) detected less early antibody responses in a longitudinal Nigerian cohort (9 of 14 positive first in plasma)
- Mean delay between plasma and OF reactivity was 29 days
- RNA detection to OF positivity median 69.5 days\(^2\)

Also see Stekler JD et al. J Clin Viro 2013;58S:e119
OraQuick- Home Testing in USA

Report 2012

Approx 5000 subjects, mimic real life use
  Sensitivity 93%
  Specificity 99.98%
  Test failure rate 1.08%

- Failed pre-set criteria but accepted due to perceived public health benefit
Operator Effect

Training
Experience
Education/ language
Ease of use, clarity of instructions

- Shown to impact on ability to correctly perform the test and interpret results
## Simple Testing Procedure

**Oral Fluid (CLIA-waived)**  
Patient-preferred: ideal for outreach programs and paraprofessionals.  
*Please note the test must be used in conjunction with CDC guidelines for HIV testing and the communication of reactive test results. Please see step-by-step instructions or package insert for complete direction.*

### Step 1 - Collect sample.

- Swab between the teeth and upper and lower gum line.

### Step 2 - Insert the device into the buffer.

- Non-Reactive Line in the C-Zone

### Step 3 - Read between 20 and 40 minutes.

- Preliminary Positive Line in the C and T Zones

### Instructions:

1. Collect 50ul of fingerstick blood, venous whole blood, serum or plasma and add bottle number 1, Sample Diluent, re-cap and invert 3-4 times.
2. Add the entire contents of bottle number 2, Colour Developer, into the center of the Membrane Unit well to generate a blue control spot and a second spot if HIV-1/HIV-2 antibodies are present.
3. Add the entire contents of bottle number 3, Clarifying Solution into center of the Membrane Unit well to reduce background colour and produce more distinct test and control spots. The control spot will appear only if human blood or blood component is present.
Untrained User Study

Uni-Gold™ Recombigen® HIV-1/2 (Package insert)

3 sites with 100 participants in total
No professional medical laboratory training, or prior experience
Tested blinded panel of 6 samples without prior training, using package insert only
Samples consisted of a negative, a positive and a low positive sample (a weak positive close to the visual detection limit of the test)

<table>
<thead>
<tr>
<th>Negative</th>
<th>Low Positive</th>
<th>High Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.0% (198/200)</td>
<td>94.0% (188/200)</td>
<td>100% (200/200)</td>
<td>97.7% (586/600)</td>
</tr>
<tr>
<td>95%CI (96.4-99.9)</td>
<td>95%CI (89.8-96.9)</td>
<td>95%CI (98.2-100)</td>
<td>95%CI (96.1-98.7)</td>
</tr>
</tbody>
</table>

http://www.trinitybiotech.com/Product%20Documents/1206506-29%20EN.pdf
User Effect Variable

Study\(^1\) using the Insti HIV1 POCT

Good concordance between untrained operators and laboratory workers

<table>
<thead>
<tr>
<th>Measure of agreement</th>
<th>Point estimate</th>
<th>95% one sided CI(^a)</th>
<th>95% 2 sided CI(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent positive agreement</td>
<td>517/517 (100%)</td>
<td>99.48–100</td>
<td>99.26–100</td>
</tr>
<tr>
<td>Percent negative agreement</td>
<td>869/871 (99.77%)</td>
<td>99.31–99.92</td>
<td>99.17–99.94</td>
</tr>
</tbody>
</table>

\(^a\) Score method.

Multiple operators over 3 sites
Community service organization/ outreach/ Dr Office
All had experience of rapid tests (not Insti)

Technology Can Help
## Ideal POCT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Potential</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allows quick clinical decision</td>
<td>✓</td>
<td>Advice and referral</td>
</tr>
<tr>
<td>At point of care</td>
<td>✓</td>
<td>Clinic or outreach</td>
</tr>
<tr>
<td>Safe</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rapid (in clinic)</td>
<td>✓</td>
<td>&lt;5min to &lt;30 mins</td>
</tr>
<tr>
<td>Performance</td>
<td>✓</td>
<td>Established infection only</td>
</tr>
<tr>
<td>Sensitivity &gt; 99%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity &gt; 98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost effective</td>
<td>✓</td>
<td>Situation dependent</td>
</tr>
<tr>
<td>Ease of use</td>
<td>✓</td>
<td>Variable according to user experience</td>
</tr>
</tbody>
</table>
Conclusions

- HIV POCT assays with adequate performance are available
  - 99% sensitivity, 98% specificity recommended
- Standards can be set to achieve correct balance of performance
- Clear instructions and advice regarding result interpretation are key/ control line essential
- Users must understand specific limitations of the test they are using
  - Performance and predictive values
  - Established versus acute/ recent infection
  - HIV2
- Benefits can outweigh potential disadvantages
- They are here to stay, but in what format…….
"TRI-function reCORDER", referring to the device's primary functions; Sensing, Computing and Recording.