Experiences and opportunities in the use of dried blood spot specimens in resource limited settings

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Presentation

• Why Dried Blood Spots?
• Screening newborns / childbearing women (EID)
• Needle Exchange Surveillance – ANSPS
• Future opportunities

Dried blood spot (DBS)

• Widely used in early infant HIV diagnosis
• Applicable to ‘hard-to-reach’ and remote settings
• Dried samples are stable at room temperature
• Simple transport via post possible
• Self-collection possible
• Conventional lab testing possible
• Seroprevalence surveys for HIV/HCV - ANSPS

Comparison of plasma and DBS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Plasma</th>
<th>DBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requires venipuncture</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Requires centrifugation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Stable at “room temperature”</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>Biohazard for shipping purposes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dry ice required for shipping</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>HIV DNA PCR (EID)</td>
<td>No</td>
<td>Yes*</td>
</tr>
<tr>
<td>HIV RNA virus load</td>
<td>Yes</td>
<td>Not routine</td>
</tr>
<tr>
<td>HIV drug resistance (GART)</td>
<td>Yes</td>
<td>Yes*</td>
</tr>
<tr>
<td>Volume range</td>
<td>1-5 ml</td>
<td>0.25-0.5 ml</td>
</tr>
</tbody>
</table>

* if kept dry, for at least 2 weeks

Challenges in resource limited settings

Transport and Logistics

Laboratory Capacity

Health System Capacity
Common failures in regional laboratories

- Lack of Infrastructure – testing environment
- Lack of sustainable transport referral pathways
- Lack of or inadequate training
- Lack of proficiency testing
- Inconsistent availability of supplies, poor quality, inappropriate test kits
- Failures in management support

The testing cycle

Development of a DBS collection SOP

Early infant diagnosis – HIV nucleic testing
Typical EIA Assay Procedure for Dried Blood Spots

Elution Plate
- Punch 3 or 6 mm disks into microwell plate
- Use 1:30 dilution

Elution Plate
- Add Kit Diluent (150 μL, 1:30)
- Cover plate, incubate overnight at 4°C
- Shake plate gently to mix
- Add Diluent to assay plate (125 μL)
- Transfer DBS eluate (25 μL) to assay plate (1:150 final serum dilution)

Assay Plate
- Cover plate, incubate 90 min at 37°C
- Wash plate 4x
- Add IgG-Enzyme Conjugate
- Cover plate, incubate 30 min at 37°C
- Add Substrate (150 μL)
- Incubate 10 min at 25°C
- Add Stop Solution (150 μL)
- Read plate at 405 nm

HIV infection reasonably excluded in non-breast fed infant if negative in 2 or more (21 month and 24 months)

>2 Negative HIV Ab tests (<1 month apart)

Infant still Ab Pos at 12 mo – retest 15-18mo

HIV DNA testing in the infant blood

- Anti-HIV Positive >18m = HIV infection
- Positive 14d (likely intrauterine infection - late)
- Consider stop prophylaxis?
- Months post partum
- >15-18m
- >6-12mo
- <1-2mo
- 3-6mo
- 6-12mo
- Positive 48h (likely intrauterine infection - early)
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CDC

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1. Cover plate, incubate plate 90 min at 37°C
2. Wash plate 4x
3. Add IgG-Enzyme Conjugate
4. Cover plate, incubate 30 min at 37°C
5. Add Substrate (150 μL)
6. Incubate 10 min at 25°C
7. Add Stop Solution (150 μL)
8. Read plate at 405 nm
DBS and surveillance - ANSPS

- ANSPS conducted annually since 1995 and NSP attendees have participated on ~40,000 occasions.
- Majority of primary NSP services in Australia participate in the ANSPS.
- Serological surveillance using DBS (HIV and HCV) serology
- 2013 – HCV RNA PCR pilot
- Monitor blood borne viral infections and associated risk behaviour in PWID

Opportunities

- Conventional HIV antibody laboratory tests possible
- Full confirmation by western blot possible
- HIV DNA/RNA detection possible
- ‘Window period’ same as lab testing
- Access to ‘hard-to-reach’ or remote groups
- Personalises the sample collection – ‘no immediate test result’
- May appeal to people not wanting to engage with health provider/community testing settings
- May appeal to other priority populations

Potential considerations

- Regulatory – few approved tests make DBS sample claims
- How are DBS collection kits distributed?
- Non-return rates may be costly (wasted kits)?
- DBS not a familiar venous specimen
- Labs not familiar or set up for DBS processing and testing
- Turn around time for results (batched)?
- Separates HIV testing from other STI tests – e.g. bacterial STI and syphilis?
- Loss to follow up?

Emerging technologies for DBS

WHO HIVResNet – global resistance

DBS and HIV viral load assays

A Loftie, R Kshatriya, K McCall-Culbreath, S Fiscus and J Nelson. IAS 2009

Expected

Observed

NHP
BMK
URN
CSF
DBS
CXS
NHP2
SEM2

Day/Month/Year
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Day/Month/Year
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**Costs**

- DBS collection kit ~ $5.00
- Australia Post pre-paid envelope for DBS return ~ $5.46
- Processing and testing by conventional lab test - $12.00
- Supplemental HIV testing for DBS:
  - HIV western blot $70
  - HIV DNA/RNA PCR = $50

**Active projects**

- HIV DBS included in clinical trials
- NSW HIV Strategy - new means to improve testing uptake
- PNG and Western Pacific EID and confirmatory testing
- Indonesia – HIV DR country threshold survey for HIV drug resistance and test and treat implementation research
- HCV simplified monitoring pre and post Rx