Diagnosis of HIV Infection in children

by

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WHO Testing Protocol
Experiences with dry blood spot technique
Entry points for testing
  - immunization
  - growth monitoring
  - feeding center
  - IMCI
Informed consent for testing in children/minors
Recommendation for resource-limited settings
Benefit of Making Early Diagnosis

- Efficiently monitor of PMTCT program
  - lead to improvement of the program
- Facilitate medical Rx, improve outcome
  - PCP prophylaxis
  - ARV
- Society & Mental benefit (*especially for uninfected results*)
  - Family make proper plan for the child and caretakers
Diagnosis of HIV Infection in Children

- In >18 months of age
  - diagnose by Anti-HIV serology

- In <18 months of age
  - Maternal antibody may persist up to 18 month-old
  - Clinical symptoms can guide, but not reliable, and may overlap with other problems
  - Require detection of the virus to confirm infections
    - DNA-PCR
    - RNA-PCR
    - p24 Ag
<table>
<thead>
<tr>
<th>Method of diagnosis</th>
<th>Recommendations for use</th>
<th>Strength of recommendation/level of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virological methods</strong></td>
<td>To diagnose infection in infants and children aged under 18 months; initial testing is recommended from 6 weeks of age</td>
<td>HIV DNA (A(I)) HIV RNA (A(I)) U p24 ag (CII)</td>
</tr>
<tr>
<td>HIV antibody testing</td>
<td>To diagnose HIV infection in mother or identify HIV exposure of infant</td>
<td>A (I)</td>
</tr>
<tr>
<td>HIV antibody testing</td>
<td>To diagnose HIV infection in children aged 18 months or more</td>
<td>A (I)</td>
</tr>
<tr>
<td>HIV antibody testing</td>
<td>To identify HIV-antibody positive children aged under 18 months and support a presumptive clinical diagnosis of severe HIV disease to allow initiation of ART</td>
<td>A (IV)</td>
</tr>
<tr>
<td>HIV antibody testing</td>
<td>To exclude HIV infection where HIV antibody negative in children aged under 18 months who are HIV exposed and never breastfed</td>
<td>A (I)</td>
</tr>
<tr>
<td>HIV antibody testing</td>
<td>To exclude HIV infection where HIV antibody negative in children aged under 18 months who are HIV exposed and discontinued breastfeeding for more than 6 weeks</td>
<td>A (IV)</td>
</tr>
</tbody>
</table>
Anti-HIV Serology

- Should be tested by 2 different HIV tests
- If negative (*and asymptomatic*) >> can exclude infection in any age
  - 74% of HIV-exposed uninfected infants sero-revert by 9 mo, and 96% by 12 mo.
  - Breast feeding may cause infection later >> Need to D'C BF >6 wk before excluding infection

Seronegative result can be “false” in some advanced cases

- Beyond 18 mo => confirm infection
  \[\Rightarrow \text{Do not use “combi” test, it may pos beyond 18 mo.}\]
- Younger than 18 mo => unable to interpret
Seroconversion Sensitivity

HIV combination Ag/Ab kits (4th generation)

HIVAb kits 3rd generation

Roche
<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Ab/Ag I</th>
<th>Ab/Ag II</th>
<th>Ax</th>
<th>Vi</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 yo</td>
<td>+50.43</td>
<td>5.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>1 yo</td>
<td>+5.84</td>
<td>-0.36</td>
<td>-0.65</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>1 yo</td>
<td>+1.3</td>
<td>-0.23</td>
<td>ND</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1 yo</td>
<td>+30</td>
<td>-0.43</td>
<td>+1.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1 yo</td>
<td>+22.5</td>
<td>-0.61</td>
<td>-0.61</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1 yo</td>
<td>+8.25</td>
<td>-0.5</td>
<td>-0.86</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>17 mo</td>
<td>+2.72</td>
<td>+2.31</td>
<td>-0.43</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18 mo</td>
<td>+2.06</td>
<td>+1.35</td>
<td>-0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 yo</td>
<td>ND</td>
<td>ND</td>
<td>+2.25</td>
<td>134</td>
<td>2+</td>
</tr>
<tr>
<td>18 mo</td>
<td>+1.15</td>
<td>-0.19</td>
<td>-0.38</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fourth generation serologic test may be too sensitive to diagnose HIV infection in infants.**

Determine ±
Virological Tests
(PCR for DNA / RNA, p24 Ag)

- Suggest to test at 6-8 wk of age
  - Repeat on a separate sample if possible, *may be*

*at 4 mo (US suggests: HIV is excluded if at least 2 negative PCR (at >1 mo, and > 4 mo)*

Caveat:

- Should confirm virological test with serology
- Some non-subtype B or group “O” can be false negative PCR (*newer assays are better*)
Special Situations

- Diagnosis in breast feeding infants
  
  \(\gg\) Need to quit BF >6 wk before testing, as infection occur at any time via breast milk
  
  \(\gg\) If quit > 6 wk, interpret the results as usual

- Negative serology in symptomatic infants
  
  \(\gg\) Recheck with virological test

- Discrepant PCR result (+ → -, or - → +)
  
  \(\gg\) confirm with serology at 9-18 mo.
WHO: Management of HIV Infection in Infants / Children

- As maternal HIV antibody transferred passively during pregnancy can persist for as long as 18 months in children born to HIV-infected mothers, the interpretation of positive HIV antibody test results is more difficult in children below this age.

- HIV-exposed infants who have a positive HIV antibody test result at ages 9 to <18 months are considered at high risk of having HIV infection but a definitive diagnosis of HIV infection using antibody testing can only be done at ≥18 months of age.

- To diagnose HIV infection definitively in children aged <18 months, assays that detect the virus or its components (i.e. virological tests) are required. A range of laboratory-based techniques is available. These techniques are discussed in detail in the next section. Children who have a positive virological test result at any age are considered HIV-infected.

- Children who are breastfed have an ongoing risk for acquiring HIV infection; therefore, HIV infection can be excluded only after breastfeeding is stopped for >6 weeks.
There are two ways to exclude HIV infection in infants and children:

1. HIV virological test
   - A negative virological test result in an infant 6 weeks of age or more who has never breastfed
   - A negative virological test result in an infant who has completely stopped breastfeeding for at least 6 weeks

2. HIV antibody test
   - A child has a negative HIV antibody test result at $\geq 18$ months of age if not breastfeeding and has completely stopped breastfeeding for $>6$ weeks.
   - A child who has a negative HIV antibody test result at $\geq 9$ months of age and has completely stopped breastfeeding for at least 6 weeks is HIV-uninfected.
   - HIV antibody testing can be done as early as 9–12 months of age. By then, 74% and 96% of HIV-uninfected children will test negative for HIV antibody at 9 and 12 months of age, respectively.
Diagnosing HIV infection in infants and children less than 18 months of age with unknown HIV exposure

Child <18 months with known HIV exposure or sick child with unknown HIV exposure and signs and symptoms suggestive of HIV infection

HIV virological test at 6–8 weeks of age

Positive

Counsel HIV-positive
Follow assessment and management procedures after HIV diagnosis is established (see p. 13)

Negative

Assess for breastfeeding during past 6 weeks

Yes

Ongoing risk for HIV transmission (see p. 8)

No

Counsel HIV-negative
Diagnosing HIV infection in infants and children less than 18 months of age with ongoing breastfeeding

- Child <18 months with negative HIV virological test and ongoing breastfeeding
  - Check for signs and symptoms of HIV at follow-up visits
  - Negative
    - HIV antibody testing at 9–12 months
      - Negative
        - Assess for breastfeeding
          - Yes
            - Repeat HIV antibody testing >6 weeks after stopping breastfeeding
          - No
            - Counsel HIV-negative if no more breastfeeding
  - Positive
    - Recommend to repeat HIV test
  - Positive
    - Likely to be HIV-positive
      - Repeat HIV antibody testing at >18 months and >6 weeks after stopping breastfeeding
Diagnosing HIV infection in infants and children less than 18 months of age with an initial negative HIV virological test and presenting with signs/symptoms of HIV at follow-up visit.

- Child <18 months with negative HIV virological test and developing signs and symptoms of HIV during follow up.

1. **Repeat HIV virological test**
   - **Negative**
     - **Assess for breastfeeding**
       - **Yes**
         - **Repeat HIV antibody testing >6 weeks after stopping breastfeeding**
       - **No**
         - **Counsel HIV-negative if no more breastfeeding**
   - **Positive**
     - **Counsel HIV-positive**
Diagnosing HIV infection in infants and children aged 18 months or more

Child >18 months with known HIV exposure or sick child with unknown HIV exposure and signs and symptoms suggestive of HIV infection

- HIV antibody test:
  - Negative: Breastfeeding during past 6 weeks:
    - No: Counsel HIV-negative
    - Yes: Repeat HIV antibody testing >6 weeks after stopping breastfeeding
  - Positive: Confirmatory HIV antibody test:
    - Negative: Inconclusive. Continue according to national HIV testing guidelines for adults
    - Positive: Signs/symptoms consistent with HIV persist:
      - Yes: Counsel HIV-positive
      - No: Confirmatory third HIV antibody test:
        - Negative: Inconclusive. Continue according to national HIV testing guidelines for adults
        - Positive: Counsel HIV-positive
WHO Recommendations Methods for Establishing HIV Infection in Infants and Children < 18 mo

Non breastfed child

- Diagnostic virological test from 6 week of age
  - Negative test result
    - Child is uninfected
      - Refer for HIV treatment and care including initiation of ART
    - Child develops signs or symptoms suggestive of HIV
      - Diagnostic HIV testing (9-18 mo)
        - Virology test available
          - Virology test positive
            - Child is infected
              - Refer for assessment for HIV treatment and care including initiation of ART
          - Virology test not readily available
            - HIV antibody test (18 mo)
              - HIV antibody positive, Presumptive severe HIV disease
                - Refer for assessment for HIV treatment and care including initiation of ART
        - Virology test not readily available
          - HIV antibody test (18 mo)
            - HIV antibody positive, Presumptive severe HIV disease
              - Refer for assessment for HIV treatment and care including initiation of ART
  - Positive test result
    - Child is infected

Breastfed child

- Diagnostic virological test from 6 week of age
  - Negative test result
    - Child remains well
    - Routine follow-up testing as per national programme recommendations (9-18 mo)
      - Not breasted
      - Breasted
  - Positive test result
    - Children remains at risk of acquiring HIV infection until complete cessation of breastfeeding

- Child is uninfected
- Child is infected
- Child develops signs or symptoms suggestive of HIV
Does DNA-PCR Equal RNA-PCR?

- Most of the time RNA-PCR is as sensitive (90-100%) especially by 2-3 mo, because all of perinatal infection have very high viral load by 2-3 month of age.
- RNA-PCR may be “undetectable” from the effect of perinatal ART & neonatal prophylaxis, and may be, ART in breast milk.
- p24 Ag may be affected by perinatal ART.
- DNA-PCR is not affected by perinatal ART and ART in breast milk.
# Rate of Positivity in HIV-Infected Infants

<table>
<thead>
<tr>
<th>Age</th>
<th>DNA-PCR</th>
<th>RNA-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>38%</td>
<td>63%</td>
</tr>
<tr>
<td>4 weeks</td>
<td>71%</td>
<td>100%</td>
</tr>
<tr>
<td>7-8 weeks</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Sensitivity and Specificity of RNA and DNA polymerase chain reaction (PCR)

<table>
<thead>
<tr>
<th>Age</th>
<th>RNA-PCR</th>
<th>95% CI</th>
<th>DNA-PCR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>25/53 (47%)</td>
<td>33-61</td>
<td>20/53 (38%)</td>
<td>25-52</td>
</tr>
<tr>
<td>2 months</td>
<td>47/47 (100%)</td>
<td>92-100</td>
<td>47/47 (100%)</td>
<td>92-100</td>
</tr>
<tr>
<td>6 months</td>
<td>35/35 (100%)</td>
<td>90-100</td>
<td>35/35 (100%)</td>
<td>90-100</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>100/100 (100%)</td>
<td>96-100</td>
<td>329/329 (100%)</td>
<td>99-100</td>
</tr>
<tr>
<td>2 months</td>
<td>Not evaluated</td>
<td></td>
<td>325/325 (100%)</td>
<td>99-100</td>
</tr>
<tr>
<td>6 months</td>
<td>100/100 (100%)</td>
<td>96-100</td>
<td>282/282 (100%)</td>
<td>99-100</td>
</tr>
</tbody>
</table>

Young NL. JAID 2000;24:401-7.
Other Tests

- HIV-culture
  - Not better than PCR
  - Need facilities, more expensive, take longer time
- HIV-IgM: non-specific and cross react with RF
- HIV-IgA: not sensitive in younger than 6 mo.
- P24 Ag by Immune Complex Dissociation Assay
  - Highly specific but less sensitive
    (sens = 81%, spec = 100% at 15 d -3 mo)

*NEJM 1993;328:297-302.*
Experiences With Dry Blood Spot Technique: Advantages

- Small volume required
- Ease of sample collection, storage and shipment
- Noninfectious transport medium
- Safety/ handling exposure
- Stability of sample – stable in room T > 1 month
- Allows for centralization of testing facilities
- Facilitates systemic, unbiased surveillance
Experiences With Dry Blood Spot Technique: Limitations

- Live viral isolates cannot be determined from DBS.
- Sample processing is more difficult, require more steps.
- Lymphocytes subsets cannot be measured.
- It is difficult to obtain long PCR fragment (>1.2 kb) DBS – based genetic screening.
## Performance characteristics between whole blood and DBS for HIV-1 infant diagnosis and viral load monitoring

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Whole blood</th>
<th>DBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability of samples</td>
<td>- Up to 4 days at 2-25°C</td>
<td>- Up to at least 3 months at room temperature</td>
</tr>
<tr>
<td>Sample collection</td>
<td>- More difficult especially in infant age less than 1 year</td>
<td>- Easy to collect sample in young infant</td>
</tr>
<tr>
<td>Transportation of specimen</td>
<td>- Require cold chain storage to ensure specimen integrity</td>
<td>- Can be transport at room temperature but avoid from heat and humidity</td>
</tr>
<tr>
<td>Assay to be used</td>
<td>- In-house or commercial kit</td>
<td>- In-house or commercial kit</td>
</tr>
<tr>
<td></td>
<td>- Standard procedure, less complexity</td>
<td>- Modified procedure, more complexity</td>
</tr>
<tr>
<td>Volume of blood sample required</td>
<td>- At least 100µL</td>
<td>- At least 10µL</td>
</tr>
</tbody>
</table>
FDA Approved Filter papers

- Whatman
  - BFC 180
- Scheicher & Schuell
  - Grade 903
### HIV-1 proviral DNA Detection in Whole Blood and DBS by Multiplex DNA-PCR (In house) and Commercial Standard Method Amplicor HIV-1 test

<table>
<thead>
<tr>
<th>HIV-1 proviral DNA detection in:</th>
<th>Sample with known HIV status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (50 cases)</td>
</tr>
<tr>
<td>WB by Amplicor</td>
<td>47/50 (94%)</td>
</tr>
<tr>
<td>WB by Multiplex</td>
<td>50/50 (100%)</td>
</tr>
<tr>
<td>S&amp;S IsoCode</td>
<td>42/47* (89.4%)</td>
</tr>
<tr>
<td>Whatman</td>
<td>47/50 (94%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
</table>

* Low DNA template 3 cases  **Low DNA template 2 cases

*Uttayamakul S. J* *of Virological Methods 2005;128:128-134*
Prospective Field-Collected Dried Blood Spot DNA & RNA PCR for Infant Diagnosis and Viral load Monitoring of HIV-1 Infection in Thailand

Young NL\textsuperscript{1-2}, Chokephaibulkit K\textsuperscript{3}, Chotpitayasunondh T\textsuperscript{4}, Chaowanachan T\textsuperscript{1}, Teeratkul A\textsuperscript{1}, Jetsawang B\textsuperscript{1}, Neeyapun K\textsuperscript{1}, Simonds RJ \textsuperscript{1-2}

1 HIV/AIDS Collaboration, Nonthaburi, Thailand
2 CDC, Atlanta, GA, USA
3 Siriraj Hospital, Mahidol Univ., Bangkok, Thailand
4 Queen Siririkit NICHI, MOPH, Bangkok, Thailand

: Poster Presentation at 6\textsuperscript{th} International Congress on AIDS in Asia and the Pacific, October 5-10, 2001; Melbourne, Australia
: Oral Presentation in XIV International AIDS Conference, July 5-12, 2002; Barcelona, Spain
Specificity and sensitivity of DBS compared to venipuncture whole blood samples for diagnosis of HIV-1 infection

<table>
<thead>
<tr>
<th>Assay</th>
<th>Whole blood</th>
<th></th>
<th>DBS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Amplicor DNA PCR(^a)</td>
<td>56/56</td>
<td>106/106</td>
<td>56/56</td>
<td>106/106</td>
</tr>
<tr>
<td>Biomerieux RNA NASBA(^b)</td>
<td>56/56</td>
<td>106/106</td>
<td>56/56</td>
<td>106/106</td>
</tr>
<tr>
<td>In-house real-time PCR(^c)</td>
<td>Not done</td>
<td>Not done</td>
<td>54/56</td>
<td>25/25</td>
</tr>
</tbody>
</table>

Sensitivity = 96-100%, specificity = 100%

\(^a\) whole blood samples assayed was white blood cell pellet
\(^b\) whole blood samples assayed was plasma
\(^c\) In the real-time PCR assay, only 25 negative samples were tested
Entry Points for Testing

- It is best to screen at well baby clinic that include:
  - immunization
  - growth monitoring
  - feeding center
  - Anticipatory guidance
  - Educational activities
- Integrated management of childhood illness:
  - Integrate preventive and curative intervention to improve practices both in health facilities and at home; aim against 5 common diseases: ARI, diarrhea, measles, malaria, and malnutrition
PHYSICAL EXAMINATION ROOM

VACCINATION ROOM

BLOOD COLLECTING ROOM
Informed Consent for Testing in Children / Minors

- Depend on local law
- In Thailand, under 18 years old require parental (or legal guardian, or caregiver’s) consent, unless married

Principal of Interest Balancing

- In symptomatics: to help with treatment
- In asymptomatics, esp. young children: to decide for ARV and PCP prophylaxis
Recommendation for Resource-Limited Settings

- Need to develop an easier, cheaper, and more practical test for early diagnosis.
  - Boosted p24 Ag assay?
    - Commercial by Perkin Elmer
    - Very promising, cheaper ($5-10), but need more study and standardization
    - Sensitivity 100% if VL > 30,000, 46% if VL < 30,000

- Corporate HIV diagnosis into the “one stop service” for routine well child care
- Develop a specific national policy and guidelines
- Secure adequate supplies and resources
Boosted p24 Ag Correlate Well With HIV-RNA

Brinkhof M WG. JAIDS 2006; 41:557-62.

Boosted p24 Ag may be an alternative marker for treatment monitoring