RNA PCR, Proviral DNA and Emerging Trends in Infant HIV Diagnosis

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OUTLINE

- Charlie (1)

- Background: Infants vs adults

- Proviral DNA PCR

- RNA PCR

- Other methods

- Charlie (2)
Charlie

- Charlie is 4 days old

- His mother, Sue, has been on ARVs throughout pregnancy and viral load is undetectable

  - *How likely is Charlie to be infected?*

  - *How can infection be excluded?*
Mother-to-child Transmission (MTCT) of HIV

- The mechanism of infection for the vast majority of children with HIV
- Without intervention MTCT is between 25-40%
- With appropriate interventions, risk of transmission is <<1%
Standard adult HIV Testing

- Screening with 4\textsuperscript{th} generation ELISA

- Confirmation with supplementary tests and Western Blot

- Subsequent testing for HIV RNA viral load and resistance genotyping

- Other options include rapid tests
  - (screening)
Diagnostic issues in infants

[Diagram showing the interaction of IgG and FcRn in maternal and fetal blood.]

Infant HIV testing

- Virologic only (prior to 18 months)
- Serologic testing (after ~18 months)

Ciaranello et al. BMC Medicine 2011, 9:59
HIV-exposed uninfected children (APSU)

HIV exposed, uninfected
HIV exposed, infected

- 2004
- 2005
- 2006
- 2007
- 2008
- 2009
- 2010
- 2011
- 2012
HIV Groups and Subtypes

Medscape

Source: HIV Ther © 2009 Future Medicine Ltd
HIV Proviral DNA PCR

- Qualitative PCR
- Target is HIV-1 *gag* gene
- Limit of detection can be 10 copies/mL (assay and sample-dependent)

**Limitations**
- Transport
- Access to testing
- Non-subtype B virus
HIV RNA PCR

- Designed as viral load PCR
- Levels relatively low at birth but high by 1-2 months of age
- Appears as sensitive and specific as DNA PCRs
- More widely available than DNA PCRs

Limitations
- Potential interference from antiretroviral therapy
- Possible false positives with low copy numbers
- Blood volume requirements
BHIVA 2014  diagnosis of HIV infection in infants

- Gold standard test in infancy was HIV DNA PCR on peripheral blood lymphocytes
  - Equal or increased early sensitivity RNA PCR

- Infants infected intrapartum may have low peripheral blood HIV levels, so HIV DNA/RNA may not be amplified from all infected infants at birth.
  - Positive HIV DNA/RNA <72 hours = presumptive intrauterine transmission
  - Within first weeks sensitivity of the viral diagnostic tests increase dramatically - by 3 months 100% of non-breastfed HIV-positive infants are likely to be detected
BHIVA 2014 diagnosis of HIV infection in infants

- **Situations when infant should be tested using DNA PCR include**
  - Low volume sample: RNA assays commonly require greater volume (at least 1mL plasma).
  - MTCT may have occurred in utero, and subsequent maternal antiretroviral therapy could lead to a false-negative RNA result in an infected infant. (eg high risk such as late-presenting mother)

- Maternal sample should always be obtained for HIV DNA or RNA amplification with the first infant sample to confirm primers used detect the maternal virus
US NIH guidelines – August 2015

- HIV infection in infants should be diagnosed using HIV nucleic acid amplification virologic assays, include DNA and RNA PCR.

- Virologic diagnostic testing at birth should be considered for infants at high risk

- Virologic diagnostic testing should be considered 2-4 weeks after cessation of antiretroviral (ARV)

- Non-subtype B HIV may not be detected by commercial NAT, particularly HIV DNA PCR
  - Newer HIV RNA assays have improved detection of non-subtype B HIV, but there are still variants that are poorly detected - use newer HIV RNA assays should be used in this case
### HIV proviral DNA PCR
- HIV DNA PCR detects specific HIV viral DNA in peripheral blood mononuclear cells.
- Specificity of the HIV DNA PCR is 99.8% at birth and 100% at ages 1, 3, and 6 months.
- Sensitivity of test at birth is 55% but increases to >90% by 2-4wks and 100% at 3mths.

### HIV RNA PCR
- HIV quantitative RNA assays detect extracellular viral RNA in plasma.
- Specificity (for results ≥5,000 copies/mL)* is 100% at birth and 1, 3, 6mths and comparable to HIV DNA PCR.
- Sensitivity is 25% to 58% during the first weeks of life, 89% at age 1 month, and 90% to 100% by age 2 to 3 months.

*HIV RNA levels <5,000 copies/mL may not be reproducible and should be repeated.
HIV RNA can also:
- be used as supplemental test for infants who with initial positive HIV DNA
- provide virologic confirmation of infection and are less expensive and assesses baseline viral load
- potentially be more sensitive than HIV DNA PCR for detecting HIV non-subtype B

HIV DNA:
- remains positive in most on ART
- Whereas HIV RNA could potentially be affected by maternal ART or infant cART
HIV PCR DNA or RNA can be used – highly specific and equivalent sensitivity

<table>
<thead>
<tr>
<th>TIME OF TESTING</th>
<th>PCR – Proviral DNA or HIV RNA (^c)</th>
<th>HIV Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>Week 6</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>3 months</td>
<td>+</td>
<td>No</td>
</tr>
<tr>
<td>6 months</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12 months</td>
<td>No (clinical visit only)</td>
<td>No</td>
</tr>
<tr>
<td>18 months</td>
<td>Yes (to document clearance of maternal HIV antibodies and confirm infant’s HIV-ve status)</td>
<td>No</td>
</tr>
</tbody>
</table>

- Testing should occur at least 2 weeks and 2 months after antiretroviral prophylaxis is ceased, hence testing at 6 weeks and 3 months.
- Whilst testing at 6 and 12 months is no longer recommended, clinical visits here provide the opportunities for clinical assessment, routine childhood immunisations and maintenance of contact with family.
DNA or RNA?

- **Sensitivity/Specificity**
  - Both effective strategies in standard-risk settings
  - Additional issues if high-risk, infant cART, unusual genotype

- **Practicalities**
  - Sample volume
  - Local or send-away test
  - Turn-around time
What about serology?

- **Case report:**
  - Infant born to HIV-positive mother
  - Maternal VL negative at delivery
  - Infant completed 6 weeks zidovudine
  - Negative DNA PCRs at birth, 6 weeks, 4 months
  - Reportedly no breastfeeding/premastication
  - Declared uninfected

  - 20 months – diarrhoea, failure to thrive, oral thrush, lymphadenopathy – diagnosed with HIV1 with reactive serology and HIV1 RNA VL of 1.3 million/mL
  - Need for routine recommendation about confirmatory 18-month serology
## International comparison

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Australia</th>
<th>UK</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>DNA/RNA</td>
<td>DNA/RNA</td>
<td>(High risk)</td>
</tr>
<tr>
<td>2-3 weeks</td>
<td>No</td>
<td>(High risk)</td>
<td>DNA/RNA</td>
</tr>
<tr>
<td>6 weeks</td>
<td>DNA/RNA</td>
<td>DNA/RNA</td>
<td>DNA/RNA (1-2m)</td>
</tr>
<tr>
<td>3 months</td>
<td>DNA/RNA</td>
<td>DNA/RNA</td>
<td>No</td>
</tr>
<tr>
<td>6 months</td>
<td>No</td>
<td>No</td>
<td>DNA/RNA</td>
</tr>
<tr>
<td>18 months</td>
<td>Ag/Ab</td>
<td>Ag/Ab (Breastfed)</td>
<td>Not routine</td>
</tr>
</tbody>
</table>

**Recommendations with limited evidence:**
*Use RNA if non-B subtype HIV
*Use DNA if mother on cART
*Additional testing if high-risk
DBS and Diagnosis

- Use of dried blood spots for testing
  - Infant diagnosis
  - Remote settings
  - Home collection

- Total Nucleic Acid PCR (Xpert®)

- Qualitative RNA PCR (Aptima™)
Cepheid Xpert® HIV-1 Qual

- “Total” Nucleic Acid PCR = RNA and proviral DNA
  - 100μl of whole blood or 1 dried blood spot
  - 90 minute run-time
- Charlie is 18 months old now

- His HIV RNA tests were negative at 5 days, 6 weeks and 3 months and Ag/Ab test was non-reactive at 18 months

- He is uninfected and discharged from HIV follow-up
Acknowledgements

- Dr Emma Best, Starship Hospital, Auckland
- SEALS Virology
- Dr Alex Carrera, Sydpath HIV Laboratory


• Puertolas, Late ELISA Testing in Infants Born to HIV-Positive Mothers. Clin Pediatrics 2015 ePub ahead of print
Further information and useful resources

- http://aidsinfo.nih.gov/guidelines
- www.bhiva.org.uk