Recent developments regarding HIV diagnosis in children

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Basics of Paediatric HIV Infection and TB
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Upper Eastside, Cape Town
Outline

• HIV exposure & HIV infection

• Goals of an HIV testing programme for children

• HIV testing strategies within the PMTCT programme
  – Past, present & future

• HIV testing beyond the PMTCT programme

• Case scenarios
Acknowledgements

• Gayle Sherman
• Max Kroon
• David Le Roux
• Brian Eley
• Abbott (Deborah Shikati)
HIV exposure & HIV infection

• “HIV exposure”
  – An infant born to a mother with HIV infection and indicated by the presence of HIV antibodies in blood of infant/child <18 months of age
  – Ingestion of breastmilk from a woman with HIV infection (HIV antibodies in blood of infant may be absent)
  – Exposure to certain other body fluids of an HIV-infected person (e.g. following sexual abuse)

• HIV infection
  – <18 months of age: PCR or viral load (2)
  – ≥18 months of age: antibody tests (rapid tests, 2)
Timing of vertical HIV infection

• In utero (during pregnancy, before onset of labour)

• Intrapartum (during labour & delivery)

• Postnatal (breastfeeding)
Survival by timing of infection

Goals of an HIV testing programme for children

• Identify HIV-exposed infants

• Confirm or exclude HIV infection in infants
  • Early & reliable identification of in utero & intrapartum infections
  • Use of rapid tests to exclude HIV infections in infants

• Diagnose/exclude HIV infection in older children

• Act on the HIV test results
  – ART
# WHO PMTCT Options A & B

<table>
<thead>
<tr>
<th>Woman receives:</th>
<th>Treatment (for CD4 count ≤350 cells/mm³)</th>
<th>Prophylaxis (for CD4 count &gt;350 cells/mm³)</th>
<th>Infant receives:</th>
</tr>
</thead>
</table>
| **Option A**    | Triple ARVs starting as soon as diagnosed, continued for life | Antepartum: AZT starting as early as 14 weeks gestation  
Intrapartum: at onset of labour, sdNVP and first dose of AZT/3TC  
Postpartum: daily AZT/3TC through 7 days postpartum | Daily NVP from birth through 1 week beyond complete cessation of breastfeeding; or, if not breastfeeding or if mother is on treatment, through age 4–6 weeks |
| **Option B**    | Same initial ARVs for both:  
Triple ARVs starting as soon as diagnosed, continued for life | Triple ARVs starting as early as 14 weeks gestation and continued intrapartum and through childbirth if not breastfeeding or until 1 week after cessation of all breastfeeding | Daily NVP or AZT from birth through age 4–6 weeks regardless of infant feeding method |
<table>
<thead>
<tr>
<th>Option B+</th>
<th>Same for treatment and prophylaxis$^b$:</th>
<th>Daily NVP or AZT from birth through age 4–6 weeks regardless of infant feeding method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regardless of CD4 count, triple ARVs starting as soon as diagnosed,$^c$ continued for life</td>
<td></td>
</tr>
</tbody>
</table>
Guidelines on HIV testing of infants & children

• WHO (2010)
  – HIV virological assays used for clinical diagnostic testing (usually at or after 6 weeks of age) have a sensitivity of at least 95% and ideally greater than 98%, and specificity of 98% or more under quality-assured, standardized and validated laboratory conditions
    (Strong recommendation – Moderate quality of evidence)
  – all HIV-exposed infants have HIV virological testing at 4–6 weeks of age
    (Strong recommendation – High quality of evidence)

• US (2011)
  – PCR at 14-21 days, 1-2 months, 4-6 months
Guidelines on HIV testing of infants & children

• WHO (2010)
  – It is strongly recommended that HIV serological assays used for clinical diagnostic testing have a minimum sensitivity of 99% and specificity of 98% under quality-assured, standardized and validated laboratory conditions.
    • <18 months of age – used as a screening assay to determine HIV exposure
    • >18 months of age – used as a diagnostic assay
      (Strong recommendation – Moderate quality of evidence)

  – It is strongly recommended that well, HIV-exposed infants undergo HIV serological testing at around 9 months of age (or at the time of the last immunization visit). Infants who have reactive serological assays at 9 months should have a virological test to identify HIV-infected infants who need ART. (Strong recommendation – Low quality of evidence)
The role of HIV rapid tests

- Why don’t we just do an HIV PCR test on all infants/children <18 months of age with unknown HIV status?

   ▶ HIV antibody testing in these children:
     - Identifies HIV exposure within minutes
       - Can make decision to start co-trimoxazole prophylaxis and useful in management of infant/young child with acute illness e.g. pneumonia

     - Can exclude HIV infection in infant/young child (HIV-unexposed or seroreversion) within minutes & thereby avoid the need for PCR test: delay in getting result & cost
WHO HIV Testing Algorithm

6 week PCR

Post cessation of breastfeeding

9 month rapid test

18 month rapid test
HIV-exposed infants
Current prophylaxis & testing algorithm
SA NDOH, 2013

Mother on lifelong ART or antenatal prophylaxis with ART or → NVP at birth and then daily for 6 weeks
antenatal AZT

If mother is breastfeeding and not virally suppressed e.g. late booking or established poor adherence, continue NVP for infant throughout breastfeeding until one week post cessation of breastfeeding

6 week PCR

6 weeks post cessation of breastfeeding
18 month rapid test

± 75% coverage
Unknown (low) coverage <30% coverage
# Early diagnosis: 2009 - 2011

<table>
<thead>
<tr>
<th>Province</th>
<th>Total PCR tests: all ages</th>
<th>Total PCR tests: &lt;2 months</th>
<th>% of total PCR: &lt;2 months</th>
<th>% positivity: &lt;2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>2,838</td>
<td>2,571</td>
<td>1,053</td>
<td>1,474</td>
</tr>
<tr>
<td>FS</td>
<td>1,175</td>
<td>1,207</td>
<td>435</td>
<td>751</td>
</tr>
<tr>
<td>GP</td>
<td>5,748</td>
<td>5,066</td>
<td>2,779</td>
<td>3,408</td>
</tr>
<tr>
<td>KZN</td>
<td>5,991</td>
<td>3,399</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>1,699</td>
<td>1,855</td>
<td>676</td>
<td>1,136</td>
</tr>
<tr>
<td>MP</td>
<td>1,820</td>
<td>2,043</td>
<td>790</td>
<td>1,256</td>
</tr>
<tr>
<td>NC</td>
<td>298</td>
<td>330</td>
<td>123</td>
<td>165</td>
</tr>
<tr>
<td>NW</td>
<td>1,600</td>
<td>1,448</td>
<td>635</td>
<td>874</td>
</tr>
<tr>
<td>WC</td>
<td>1,673</td>
<td>1,428</td>
<td>912</td>
<td>1,071</td>
</tr>
<tr>
<td>Total</td>
<td>16,851</td>
<td>21,939</td>
<td>7,422</td>
<td>13,534</td>
</tr>
</tbody>
</table>

Courtesy of Gayle Sherman, NHLS routine statistics, January 2012
The 6-week HIV PCR test

• Why do we do the PCR test at 6 weeks of age?
  – Coincides with routine 6-week immunisation visit
  – Aim to detect all in utero and intra partum infections
  – A single DNA PCR test was 98.8% sensitive & 99.4% specific in 627 infants tested at 6 weeks of age (58 HIV-infected and 569 HIV-uninfected).
  – **PMTCT: sd NVP or nothing (no prolonged NVP)**
    – Repeat testing of all positive HIV PCR tests minimized false positive results. (Sherman et al. 2005)

• Is 6 weeks still the best time to do the PCR test
  – To detect HIV infection and
  – To rule out HIV infection?
Case 1

- A 3-month old exclusively formula fed HIV-exposed infant was admitted with acute gastroenteritis.

- His mother did not access ante-natal PMTCT, but tested HIV positive post-partum; she demised 2 weeks after delivery.

- The baby completed 6 weeks of daily nevirapine prophylaxis.

- Blood drawn at the local clinic on day 44 of life tested HIV DNA PCR negative.

- After admission to hospital on day 82 of life, DNA PCR was positive; confirmatory HIV RNA viral load was >10 million copies/ml (>log 6.7).

- The original specimen had been saved on a dried blood spot card. This card was retrieved and re-tested, and confirmed DNA PCR negative.
Case 2

- A 5-week old exclusively formula-fed infant was admitted with acute gastro-enteritis, dehydration and hypovolaemic shock.

- He had received daily NVP prophylaxis since birth.

- HIV DNA PCR tests on day 37 and day 39 were reported as equivocal with low viral loads.

- After nevirapine was discontinued on day 42, the HIV RNA viral load increased substantially and DNA PCR was strongly positive.
**Age and HIV DNA PCR results of infants during and after daily nevirapine prophylaxis**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (days)</th>
<th>HIV DNA PCR result</th>
<th>HIV RNA viral load</th>
<th>Anti-retroviral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>Negative</td>
<td></td>
<td>Daily nevirapine</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>Positive</td>
<td>&gt;10 million (&gt;log 6.7)</td>
<td>(none)</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Equivocal</td>
<td>4650 (log 3.67)</td>
<td>Daily nevirapine</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>Equivocal</td>
<td>2575 (log 3.41)</td>
<td>Daily nevirapine</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Positive</td>
<td>266 956 (log 5.43)</td>
<td>(none)</td>
</tr>
</tbody>
</table>
HPTN 040 study

• 1684 infants in Brazil, South Africa, Argentina, United States from 2004-2010

• No antenatal PMTCT (except IV AZT in labour)

• Randomised to 1 of 3 regimens
  – AZT twice daily for 6 weeks
  – AZT + 3 doses of nevirapine in the first week
  – AZT + nelfinavir + lamivudine

• HIV DNA PCR done at study visits: birth, day 10-14, 4-6 weeks, 3 months, 6 months

• Exclusive formula feeding
HPTN 040 study

• 93 (5.7%) infected in utero
• 32 (2.1%) PCR positive at 4-6 weeks
• 15 (1.1%) became PCR positive at 3 months

→ While receiving prolonged ARV prophylaxis, PCR at 6 weeks missed 1/3 of perinatal transmissions
Proportion with HIV transmission

HPTN 040

Zidovudine alone
Zidovudine plus nelfinavir and lamivudine
Zidovudine plus nevirapine

Nielsen Saines NEJM 2012;366: 2368-79
Sensitivity of HIV PCR test with prolonged ARV prophylaxis (≥4 wks)

- French cohort (Burgard et al. J Pediatr 2012)
  - 1567 infants undergoing PCR testing, receiving prolonged postnatal prophylaxis, no breastfeeding. Performance of PCR assessed in relation to 6-month HIV RNA result.
  - Sensitivity was 58% (RNA) and 55% (DNA) at birth, and 89% at 1 month, 100% at 3 months for both, and 100% at 6 months (DNA)
  - At 1 month during prophylaxis, 11% of infected children had negative PCR results.

- French guidelines recommended PCR screening for HIV at birth, 1, 3 and 6 months
Shortcomings of the 6-week PCR test

• Too late for early ART initiation
  – Median age at ART initiation in CHER study was 7.4 weeks
  – 20% death rate/100 person years by 13 weeks in CHER study deferred ART group
  – Loss to follow-up or death before 6 weeks

• Too early to detect all intrapartum infections
  – Maternal ARV and infant daily NVP prophylaxis delay detection of HIV at 6 weeks because of a low target of virus failing to detect 10-20% of early infections

• May miss up to 30-40% of all HIV + infants!
Performance of PCR testing at birth compared to 6 weeks

- Dried blood spots (DBS) were collected at birth and at 2, 4, and 6 weeks from HIV exposed infants receiving various postnatal prophylaxis regimens enrolled in an observational cohort study in Johannesburg, South Africa.

- HIV status was determined at 6 weeks by DNA PCR on whole blood. Serial DBS samples from all HIV-infected infants and two HIV-uninfected, age-matched controls were tested with 3 different virological assays.

- Of 710 infants of known HIV status, 38 (5.4%) had in utero (n=29) or intrapartum (n=9) infections.

- By 14 weeks, when treatment should have been initiated, 13 (45%) in utero-infected and 2 (22%) intrapartum-infected infants had died or were lost to follow-up.

- The virological assays identified 68.4-76.3% of all infants with early HIV infections at birth.

- Testing at birth versus 6 weeks of age identifies a higher total number of HIV-infected infants, irrespective of the assay (Lilian et al. J. Clin. Microbiol. 2012)
**EARLY (IU & IP) transmission in 100 HIV-infected infants**

<table>
<thead>
<tr>
<th>In utero</th>
<th>Intrapartum</th>
<th>Postnatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>30</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>6 wk PCR</th>
<th>Birth PCR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>HIV+</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>HIV+</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Intrapartum* transmissions undiagnosed at 6-weeks will reflect as postnatal transmissions.
Proposed Algorithm Options

- Birth PCR
- 10 week PCR

- Post cessation of breastfeeding
- 9 month rapid test
- 18 month rapid test

- 6 week PCR

- Post cessation of breastfeeding
- 9 month rapid test
- 18 month rapid test

- 6 week PCR

- 6 weeks post cessation of breastfeeding
- 18 month rapid test
Current vs Ideal Testing Algorithm

**Current Testing Algorithm:**
- **6 week PCR**
- **10 week PCR**
- **Birth PCR**
- **18 month rapid test**

**Ideal Testing Algorithm:**
- **6 weeks post cessation of breastfeeding**
- **18 month rapid test**
- **Post cessation of breastfeeding**
- **9 month rapid test**
- **18 month rapid test**

**PCR (20%)**
W Cape PMTCT guidelines 2013

HIV-EXPOSED INFANT

Stat NVP

Attending clinician discretion: Infant symptomatic or high risk: Consider birth HIV-1 DNA PCR test and NVP + AZT for infant prophylaxis

Maternal ART ≥8 weeks OR Formula feeding

Infant NVP TTO for 4 weeks

Maternal ART <8 weeks AND breastfeeding

Infant NVP TTO for 12 weeks

Routine HIV-1 DNA PCR test at 6 weeks & Initiate CPT at 6 weeks

Positive

Fast track for ART and continue CPT

It PCR positive:

Confirm positive result with HIV-1 DNA PCR test.

Alere Determine® rapid HIV test at 9 months

Also test infants with unknown HIV status

Negative

HIV test if clinically indicated

<9 months:
HIV-1 DNA PCR test

9-17 months:
Alere Determine® rapid HIV test and confirm positive result with HIV-1 DNA PCR test

≥18 months:
Test as per adult testing algorithm

Negative

HIV test 6 weeks after the final breastfeeding

Stop CPT if breastfeeding

Rapid HIV test at 18 months

Stop CPT if negative

Infant testing

18 MONTHS
Draft indications for HIV DNA PCR test on infant as soon as possible after birth
(W Cape working group)

• Inadequate antenatal ARV prophylaxis
  – Mother diagnosed with HIV infection in labour or immediate post-partum period
  – Mother who has received <8 weeks of antepartum ART

• Potential drug resistance
  – Mother on ART (1st or 2nd line regimen) with viral load >400 copies/ml at ≥36 weeks gestation or during 4 weeks before delivery OR unknown viral load in 8 weeks prior to delivery
  – Mother with poor adherence or interruption of antepartum ART

• Symptomatic newborn
  – Unwell HIV-exposed infant (e.g. underweight for gestational age, hepatosplenomegaly, pneumonia, thrombocytopenia, pancytopenia)
Not all HIV-exposed infants are identified by our PMTCT programme

• Ideally,
  – PMTCT programmes would identify all HIV-exposed infants by identifying all HIV-infected pregnant women resulting in HIV PCR test on infants at 6 wks of age (and if breastfeeding, repeat PCR testing during and 6 weeks after completion of breastfeeding)

• But,
  – Antenatal HIV testing & PMTCT coverage is incomplete
  – New infections and seroconversions occur during pregnancy & breastfeeding
  – Infants may miss HIV testing at 6 weeks of age
  – Lack of integration between PMTCT & HIV/ART services for children results in late/missed diagnoses of HIV infection in children
Which children should be tested for HIV infection?

- All children with unknown HIV status
- All HIV-exposed infants
- Children with:
  - Clinical features suggestive of HIV infection
  - Acute illnesses, especially if severe
- All children with the following IMCI classifications: Suspected symptomatic HIV infection or possible HIV infection
- All children diagnosed with TB or who have a history of TB treatment
- Family and social history:
  - Parental request to test the child
  - Father or sibling with HIV infection
  - Death of mother, father or sibling
  - When the mother’s HIV status is unknown and her whereabouts are unknown
- When the child may have been wet-nursed or breastfed by a woman of unknown or positive HIV status
- When the child may have experienced or been at risk of sexual assault
- When it is in the best interest of the child where the child is being considered for foster or adoption placement
Suspect HIV infection in the presence of:

- Two or more of:
  - Generalised lymphadenopathy
  - Hepatomegaly
  - Splenomegaly
  - Dermatitis
  - Parotid enlargement
  - Persistent candidiasis
  - Recurrent or persistent upper respiratory infection or otitis media

- Other marker conditions include:
  - Repeated or chronic diarrhoea
  - Bacterial infection – single or multiple episodes
  - Tuberculosis
  - Persistent wasting
  - Chronic lung disease
  - Persistent anaemia, neutropenia or thrombocytopenia
  - Atypical infection incl. Pneumocystis jiroveci pneumonitis, salmonella septicaemia, severe or disseminated chickenpox, shingles, recurrent herpes stomatitis, neurodevelopmental delay, progressive encephalopathy, malignancy & low birth weight

Bottom line: HIV status of all infants and children should be established, testing should not just be on “clinical suspicion”
HIV testing of infants & children: beyond the PMTCT programme

• The HIV rapid test should be the 1st line test for screening any child with unknown HIV status

• The Determine® HIV rapid test is the currently recommended 1st line test for all children (<15 years of age)

• Other HIV rapid tests are under evaluation for use as alternative screening tests in infants & young children
If the Determine test is negative, is the child HIV negative?

• Yes, but

  – Breastfeeding children will require repeat testing at least 6 weeks after stopping breastfeeding or if there are clinical features suggestive of HIV infection during the period of breastfeeding

  – If there is strong clinical suspicion of HIV infection and the rapid test is negative, a PCR test (if child <18 months old) or ELISA test (if child ≥18 months old) should be sent to the laboratory
If the Determine test is positive, what confirmatory test is to be used?

• If child is <18 months old
  – HIV PCR test

• If child is ≥18 months old
  – Different HIV rapid test (First Response) as for adult testing
Can HIV testing be done in infants <6 weeks of age?

• Yes

• HIV screening and testing is encouraged from as early as possible

• For infants who are known to be HIV-exposed (i.e. mother is HIV-positive), omit the Determine test and do the PCR test
  – A negative PCR test in an infant of <6 weeks of age must be repeated once the infant is ≥6 weeks of age

• For infants of unknown HIV status, the first test to do is the Determine test. If the Determine test is positive, then do a PCR test
Blood samples for HIV testing:
EDTA tube (purple-top) or Dried Blood Spot (DBS)

Antibody tests (HIV ELISA or HIV rapid tests):
Capillary blood or venous blood used directly or collected into EDTA (purple-top) or clotted blood tubes (not heparinised tubes)

HIV PCR test:
Capillary blood or venous blood collected into EDTA (purple-top) tube or as dried blood spot sample

In infants, capillary blood may be obtained by finger/toe or heel-prick
HIV Testing scenarios

What would be your management of:

– An 8-week old breastfed infant whose HIV PCR result comes back positive?
  – Post-test counselling of mother, stop dNVP p, urgent initiation of ART (within the next 1-2 weeks after further counselling), send blood for HIV viral load (RNA PCR) as confirmatory diagnostic test along with CD4 count, check that child is receiving co-trimoxazole prophylaxis, encourage mother to continue breastfeeding, check if mother had CD4 count/qualifies for ART & rest of family tested

– A 10-week old infant whose Determine test is positive?
  – Do HIV PCR test, start co-trimoxazole prophylaxis

– A 10-week old formula-fed infant whose HIV PCR result comes back negative?
  – Post-test counselling: child is regarded as HIV uninfected but repeat testing is required at 9 months of age or before if any symptoms or signs suggestive of HIV infection, stop co-trimoxazole, check if mother had HIV test/CD4 count/qualifies for ART & rest of family tested
HIV testing scenarios

What is the appropriate testing process to follow in:

a. A well 1-year old child who has never breastfed and whose mother is HIV-positive?

✓ Determine test (child is ≥9 months of age)

- If Determine test is negative, child is HIV-uninfected (child has “sero-reverted” & delay in getting result/expense of PCR test has been avoided)

- If Determine test is positive, do PCR test
  - If PCR test is negative, child is HIV-uninfected
  - If PCR test is positive, child is HIV-infected & needs ART
HIV testing scenarios

What is the appropriate testing process to follow in:

b. A 3-month old infant whose mother is HIV-positive?

✓ HIV PCR test

– If HIV PCR test is positive, child is HIV-infected. Send confirmatory HIV viral load (RNA PCR) & plan to start ART within 1-2 weeks.

– If HIV PCR test is negative, is the child breastfeeding currently or within the last 6 weeks?

– If yes, infant will require, repeat HIV PCR test ≥6 weeks after last breastfeed or during period of breastfeeding if develops clinical features suggestive of HIV infection. Ensure mother of child is on ART. If new maternal diagnosis, the child will require NVP prophylaxis for 4 weeks if breastfeeding is discontinued or 12 weeks if breastfeeding continued while mother is established on ART. Recommended duration of breastfeeding is 12 months.

– If no, child is HIV-uninfected
HIV testing scenarios

What is the appropriate testing process to follow in:

c. A 1-week old abandoned infant?

- Determine test
  - If Determine test is negative, Determine test should be repeated at least 6 weeks later
  - If Determine test is positive do PCR test on child
    - if PCR test is positive, child is HIV-infected
    - if PCR test is negative, PCR test should be repeated when the child is ≥6 weeks of age
HIV testing scenarios

• What is the appropriate testing process to follow in:

  d. A 9-month old breastfeeding infant of HIV-infected mother and infant had negative PCR test at 6 weeks of age?

  ✓ Determine test

  • Maternal antibodies may still be present in infant circulation or may have disappeared.
  • If Determine test is positive, do PCR test
  • If Determine test is negative, child will need repeat Determine test 6 weeks after stopping breastfeeding
Key messages

• Early identification of HIV status among children is essential to facilitate early ART initiation and prevent rapid HIV progression and death

• Priority is identification of intrapartum infection in high-risk infants: role of HIV PCR test at birth

• The 6-week PCR test in the context of prolonged (≥4-6 weeks) NVP prophylaxis may be less sensitive in excluding HIV infection

• Routine HIV testing at 9 & 18 months of age, including HIV-exposed children who tested PCR negative at 6 weeks of age is recommended