Module 2
Testing of HIV-exposed Infants

Infant HIV Testing
Training Curriculum for Healthcare Providers
Session 2.1
Identifying HIV-exposed Infants

Session Objective
• Identify HIV-exposed infants in the clinical setting
Introduction

• Infant HIV testing is one component of the HIV-exposed infant comprehensive package of care
  • That package starts at birth and extends to 3 months after breastfeeding has ended (or 18 months of age, whichever is later)
• Within HIV testing services, the 4–6 weeks test is one element in the HIV testing cascade
Testing: The Terminology

• *What is meant by nucleic acid testing or NAT?*
Testing: The Terminology

• **Nucleic acid testing (NAT):**
  • An infant virologic testing procedure that diagnoses infection by detection of HIV virus nucleic acid
  • NAT detects DNA, RNA or both
  • NAT uses polymerase chain reaction (PCR) technology, and is sometimes referred to as PCR testing
Testing: The Terminology

• What is the difference between infant HIV testing and EID (or early infant diagnosis)?
Testing: The Terminology

- **Infant HIV testing**: any HIV test included in the testing algorithm; this includes:
  - NAT (virologic)
  - Rapid diagnostic testing or RDT (serologic/antibody testing)

- **Early infant diagnosis (EID)**: a virologic test at 4–6 weeks of age or earlier for diagnosis of HIV infection
  - EID is one component of the infant HIV testing cascade.
Testing: The Terminology

• **Birth testing:**
  • A test at or around birth (0–2 days)
  • Complements current 4–6 week testing but does not replace it
Testing: The Terminology

• What is the difference between point-of-care testing and near point-of-care testing?
Testing: The Terminology

• **PoC testing**: PoC testing is when patients are tested on-site at a health facility and receive their results during the same visit or day
  • Testing at PoC brings test results closer to the patient

• **Near PoC testing**: Near PoC testing is when PoC technology is located at a health facility, district or other non-central laboratory where needed infrastructure (such as electricity) is consistently accessible
Testing: The Terminology

- **Conventional testing** refers to conventional diagnostic technologies located in central or regional laboratories that make up the backbone of national testing services
  - These technologies require sophisticated laboratory infrastructure, stable electricity supply and highly trained technicians

- **HIV-exposed infant care**: a comprehensive package of care that all HIV-exposed infants should receive
  - HIV testing is just one component of HIV-exposed infant care and EID is just one component of the infant HIV testing cascade
Identifying HIV-exposed Infants

• How would you identify an HIV-exposed infant?

• If a mother does not know her HIV status, what should you do?

• How do you obtain consent/agreement for infant testing if the parent is not available?

• When might you test an infant of an uninfected mother for HIV?
Identifying HIV-exposed Infants

- An HIV-exposed infant is an infant whose mother was living with HIV or acquired HIV while pregnant or while breastfeeding that infant.

- Find HIV-exposed infants by identifying mothers with HIV.
- At every patient encounter in any healthcare setting:
  - Review the mother’s health card for HIV testing history.
  - If no HIV status, ask mother when she was tested for HIV.
  - If mother tested HIV-positive, ensure she is on ART and provide retesting for verification.
    - Retesting for verification should never be a barrier to ART initiation.
  - If mother does not have documentation of testing, offer testing as per national guidelines.
HIV Testing should be Routine

- HIV testing of all mothers, HIV-exposed children, children of unknown exposure status, and sick children should be routine
- Inform parents/guardians that testing is urgent
  - Medications that treat HIV infection are life-saving
  - If a child is sick, knowing the HIV status will help to provide the correct treatment
Infants of Mothers of Unknown HIV Status

• Provide mothers of unknown HIV status with the pre-test information and RDT
  • If the mother tests HIV-positive, baby is HIV-exposed (follow national guidelines on re-testing to verify maternal HIV status)

• Test the *mother* rather than the infant to determine whether infant is HIV-exposed:
  • It will provide a diagnosis for the mother
  • Maternal testing is straight-forward and highly accurate

• Refusal of routine testing is rare
  • If a mother declines testing and her baby is ill, ask permission to test the infant
  • If mother/caregiver refuses testing for self and infant:
    • Provide information and reassurance; focus on benefits of testing
    • Never withhold services because testing is refused
    • If infant is strongly suspected of having HIV infection, follow national guidelines
    • Document refusal in health card and discuss at next visit
Mother Unavailable & HIV Status Unknown
Infants younger than 18 months

- Provide legal guardian with pre-test information
- Obtain agreement to test, and
- Test infant using RDT

- RDT can indicate if child is HIV-exposed,
  - In infants over the age of 4 months, RDT is not an accurate indicator of exposure status, these infants need follow-up
  - RDT does not provide an HIV diagnosis

See **Appendix 2A: Pre-test Counselling Session, Maternal HIV Status Unknown**
And in **Module 5, Appendix 5A: Post-test Counselling Session for Infants Less than 18 Months Tested by RDT**
Infants of HIV-uninfected Mothers

• Women who test negative early in pregnancy should be tested again in the third trimester and during the postpartum period.

• Infants whose mothers test HIV negative would not normally be tested for HIV unless:
  • The infant shows signs of chronic illness, severe acute illness, growth retardation, poor milestone development, chronic diarrhoea, repeated chest infections, or TB
  • The mother has a history consistent with acute HIV infection
Session 2.2: Recommendations on Timing of Infant HIV Testing

Session Objectives

• List the recommended ages for testing of HIV-exposed infants and the tests recommended at each age

• Explain the importance of national testing algorithms
Two Categories of Testing Procedures for Infant HIV Testing

• *What are “maternal antibodies”?*
Two Categories of Testing Procedures for Infant HIV Testing

- **Serological testing:**
  - The testing procedure used to diagnose HIV in anyone 18 months of age or older
  - Includes RDT

- **Virological testing:**
  - The diagnosis of HIV in those younger than 18 months requires virological testing using nucleic acid testing (NAT) technologies
  - Until recently, NAT was conducted *only* at central laboratories using DBS samples
  - With PoC NAT technology, virological tests can be conducted in the health clinic or at local laboratories
Two Categories of Testing Procedures for Infant HIV Testing

• Serological tests are not accurate for diagnosing HIV infection in infants and young children
• Maternal antibodies can be present until 18 months of age
• Diagnosis of HIV in those less than 18 months of age requires NAT
• RDT can be used to identify infants who are HIV-exposed
  • In infants over the age of 4 months, RDT is not an accurate indicator of exposure status, these infants need follow-up
When and which test?

• *At what age are HIV-exposed infants tested for HIV?*
When and which test?

<table>
<thead>
<tr>
<th>Category and age</th>
<th>Recommended test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-exposed infant, at birth (0–2 days), provide testing if recommended by national guidelines</td>
<td>HIV virological testing using NAT, as per national guidelines</td>
</tr>
<tr>
<td>HIV-exposed infant, at 4–6 weeks of age, or as soon as possible thereafter</td>
<td>HIV virological testing, using NAT</td>
</tr>
<tr>
<td>HIV-exposed infant, at 9 months of age</td>
<td>HIV virological testing, using NAT</td>
</tr>
<tr>
<td>HIV-exposed infant, at 18 months of age or 3 months after breastfeeding ends (whichever is later) for final assessment of HIV status</td>
<td>HIV serological testing if 18 months of age or older; HIV virologic testing if final test prior to 18 months of age (requires breastfeeding cessation prior to 15 months of age)</td>
</tr>
</tbody>
</table>
Birth Testing

• What do you think are the advantages of birth testing?
• How about the disadvantages?
• How would you minimize the disadvantages?
Birth Testing

- HIV testing at birth is most likely to identify infants infected *in utero*
  - These infants are at greatest risk for early death
- Birth testing will not detect infections during or shortly after delivery
- 4–6 week testing will identify infants who acquired the infection *in utero*, during delivery, or in the early postpartum period
- NAT at birth can be **added to** a routine 4–6 week test
  - It will **not replace** a 4–6 week test
  - A high-functioning system for early infant diagnosis at 4–6 weeks of age and excellent follow up is important to ensure that all HIV-exposed infants who acquired HIV in utero and during delivery are identified
Birth Testing, Advantages

• Birth testing provides an earlier opportunity to diagnose HIV in infants who acquired the infection *in utero*

• This provides an earlier opportunity to start ART

• Important because infants infected *in utero* or intrapartum are at a higher risk of early death
  • 30–40% of these babies will die by 3 months of age
Birth Testing, Disadvantages

• Potential of reducing the uptake of 4–6 week testing
  • Where birth testing is established, emphasize to caregivers the importance of repeat testing as per national algorithm

• Cannot detect all perinatal infections: Birth testing will only detect in utero infections

• The presence of ARVs (maternal or infant) may reduce the sensitivity of the NAT to detect infant HIV infection

• A study found that birth testing with NAT identifies only about 2 of every 3 infants who are infected

• This highlights the importance of retention in care and repeat testing, particularly at 4–6 weeks
HIV Testing for Sick Infants

• If you were providing care for an 8 month old HIV-exposed infant who had symptoms that might suggest he was infected with HIV, would you wait a month to test him as per recommendations?
HIV Testing for Sick Infants

• Do not wait to test a sick baby. If an infant is sick before the standard age for conducting the test, test earlier!

• IMPORTANT!! Retesting for Verification (also called Confirmatory Testing)
  • A positive virological test result should always be confirmed with a virological test using a second specimen
  • The second specimen should be collected before starting ART, but never delay treatment initiation pending the result of the confirmatory test!
  • Ideally, start ART on same day that the initial test result is given to caregiver
HIV Testing Algorithm

• *What is an HIV testing algorithm?*
• *Why is it important to follow our national testing algorithm?*
HIV Testing Algorithm

- **Algorithms**: the combination and sequence of specific tests used in a given strategy
- Testing algorithms are typically developed at a national level and based on global guidance
- Interpretation of the algorithm for clinical use requires consideration of: HIV treatment criteria, age of the child, ongoing exposure to HIV through breastfeeding, and point of contact within the healthcare system
Advantages of National Testing Algorithms

Nationally adopted algorithms facilitate:

• Country-level standardization of tests: Supporting a limited number of tests is more feasible and practical than many tests

• Procurement and supply management: Using standardized tests allows for bulk procurement

• Training: Easier when test sites follow the same testing algorithm, and it allows trained staff to move between sites/regions without re-training

• Quality assurance: National oversight of quality of testing operations is easier when test sites use the same tests and have similar operations.

• It is important that programme staff adhere to the national testing algorithm

See WHO infant testing algorithm in Figure 2.1
Session 2.3: Overview of NAT

Session Objectives

• Describe how and why NAT is used to diagnose HIV in infants
• Interpret NAT results, whether positive or negative
Laboratory Diagnosis of HIV Infection—NAT

• HIV infection in children under 18 months of age can be diagnosed only by virological testing using NAT technologies

• Different manufacturers use different techniques. One of these techniques is called PCR

• Two types of PCR testing:
  • **Qualitative PCR**: NAT procedure that detects presence of HIV virus
    • Extensive experience using DNA PCR testing for infant diagnosis
    • PCR works well on DBS samples
  • **Quantitative PCR** tells how much of the virus is present
    • Used for viral load (VL) testing
Window Period

• *What does “window period” refer to?*
Window Period

• “Window period” is the time it takes from HIV infection to detection on a diagnostic test

• This can refer to:
  • The time it takes to develop enough antibodies to be detectable using an antibody test, or
  • The time it takes to develop enough virus to be detectable using NAT
Laboratory Diagnosis of HIV Infection—NAT

• Once infected with HIV, it takes about 10 days for HIV to replicate so that there is enough virus in the blood to be detectable by DNA PCR.

• The time to detection, or window period, can vary depending on the individual and the test:
  • Presence of the virus using NAT: 1–3 weeks
  • Presence of antibodies using serological testing: 3–5 weeks
Analysers Validated for Infant HIV Testing

High throughput, laboratory-based testing
• Conventional method of infant HIV virological testing
• Specimens collected in the clinic by DBS & transported to a central or regional laboratory for testing by trained laboratory technicians
• Turn-around time can be 4 weeks or longer

Point-of-care and near point-of-care technologies
• Becoming widely available
PoC and Near PoC Technologies

Two testing procedures have earned the CE-IVD Marking and WHO prequalification:

1. **Alere™ q HIV-1/2 Detect**:
   - Blood is collected by heel/toe or fingerstick into a sample capillary in a testing cartridge
   - Portable, runs on a battery for up to eight hours

2. **Cepheid AB Xpert® HIV-1 Qual Assay**
   - Blood is collected by heel/toe, fingerstick or venipuncture in a sterile tube using EDTA (lavender top) as the anticoagulant
   - Can be used on DBS
   - Runs on same technology that diagnoses tuberculosis
   - Not portable, considered “near PoC”
   - Needs continuous power supply and temperature control
   - Reduced maintenance needs; few training requirements

• Both tests can diagnose at point-of-care (or near to the point-of-care) in as little as an hour
Meaning of HIV Test Results, Virological Testing

• **HIV-positive test result:**
  • Child has HIV and will require initiation of ART with confirmatory testing

• **HIV-negative test result:**
  • **In the child who has not been exposed to HIV in the past 3 months:** child is not HIV-infected
  • **In the child who has been exposed to HIV either during pregnancy, delivery, or through breastfeeding at any time in the past 3 months:** child is either not infected with HIV or infected and still in the window period
  • Retest as per national guidelines

• Retest, regardless of age, if the child is sick
Session 2.4
Overview of Serological Testing

Session Objectives

• Describe when serological testing is used in the context of infant HIV testing

• Interpret serological testing results, whether positive or negative, in the context of infant HIV testing
Laboratory Diagnosis of HIV Infection—Serological Testing

• Detects antibodies, such as HIV antibodies, in blood or saliva
  • HIV antibodies are produced by the immune system in response to infection with HIV
• Can diagnose HIV in adults and children 18 months of age or older

• Types of serological testing procedures
  • RDT (PoC or Lab-based)
  • Enzyme-linked immunosorbent assay (ELISA or EIA) (Lab-based)
  • Western blot (WB) testing (Lab-based)
• ELISA requires a larger blood sample -- specimens taken by venipuncture
• RDT needs only a drop of blood, samples obtained by finger, toe or heel prick
• RDT results available in 20 minutes
Serological Testing, Notes

• RDT does not detect the virus, it detects antibodies

• In children less than 18 months, RDT cannot differentiate between the child’s own antibodies and maternal antibodies
  • Maternal HIV antibodies are passed to the infant through the placenta before birth, but they are not passed during breastfeeding.
Serological Testing, Notes

• Usual: Most infants clear maternal antibodies between 6–9 months of age
• Maximum: It may take as long as 18 months for some infants to fully clear all maternal antibodies
  • HIV antibody positive result in infant less than 18 months of age, does not necessarily indicate the infant is HIV-infected
  • It means that the infant is HIV-exposed
• Minimum: Many HIV-exposed infants will clear maternal antibody before 5 months
  • Some HIV-exposed infants may have a negative RDT result at this age
  • RDT in infants age 4–18 months will not identify all who are HIV-exposed
  • A negative RDT might occur in an infant that is HIV-exposed but has lost all or most of the maternal antibodies
Window Period

• It can take the body a few weeks or more to develop antibodies in response to an infection, and so it can take the RDT a similar amount of time to become reactive after HIV infection has been acquired

• WHO recommends final testing of HIV-exposed infants with a serological test at 18 months of age or 3 months after breastfeeding has ended, whichever is later
Window Period

• The “window period” of 3 months is different from the typical 3–5 week window period to detect antibodies:
  • 3-5 weeks is based on when *most* people will test HIV-positive (after infection)
  • 3 months is the amount of time it takes for *almost everyone* to test HIV-positive after infection
RDT: Interpreting the Test Result

• In the context of infant HIV testing, RDT is used to identify infants and children under 18 months of age who are HIV-exposed

• An HIV-positive RDT result means
  • Child < 18 months of age: HIV-exposed
  • Child ≥ 18 months of age: HIV-infected

• An HIV-negative RDT result means:
  • Infant < 4 months of age: not HIV-exposed
  • Child 4–18 months of age: HIV exposure cannot be ruled out. Child could have been HIV exposed but cleared maternal antibodies. Retest as per national guidelines
  • Adult or child 18 months of age or older: HIV-uninfected, unless breastfed within the past 3 months. Repeat RDT 3 months after stopping breastfeeding
RDT: Interpreting the Test Result

• An HIV-infected infant initiated on ART at a very early age (before 12 weeks of age) may have a negative RDT test
• This is because ART can stop the antibody response if initiated very early in life
• Children on ART should not be re-tested using RDT
## RDT for Identification of HIV-exposed Infants

<table>
<thead>
<tr>
<th>Age group</th>
<th>Unknown HIV exposure status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4 months</td>
<td>Test mother</td>
</tr>
<tr>
<td></td>
<td>If mother is not available:</td>
</tr>
<tr>
<td></td>
<td>• RDT in the child can reliably assess exposure</td>
</tr>
<tr>
<td>5–18 months</td>
<td>Test mother</td>
</tr>
<tr>
<td></td>
<td>If mother is not available:</td>
</tr>
<tr>
<td></td>
<td>• A positive RDT establishes exposure. Infants with positive RDT should get NAT to confirm infection.</td>
</tr>
<tr>
<td></td>
<td>• A negative RDT for the child does not fully rule out exposure. Perform NAT to assess HIV infection status in any sick child**</td>
</tr>
<tr>
<td></td>
<td>• Infants with negative RDT who are still breastfeeding will need testing 3 months after cessation of breastfeeding</td>
</tr>
<tr>
<td></td>
<td>• If sick, or index of suspicion is high, conduct virologic testing.</td>
</tr>
<tr>
<td>&gt;18 months</td>
<td>Serological testing (including RDT) is recommended to assess HIV infection status unless breastfed within the last 3 months or still breastfed.</td>
</tr>
<tr>
<td></td>
<td>• If still breastfed, RDT should be provided 3 months after cessation of breastfeeding.</td>
</tr>
</tbody>
</table>

**Consider initiating ART for presumed HIV infection if there is high degree of suspicion while waiting for NAT results, especially if RDT positive.**

NAT = Nucleic acid testing, a virological test
Testing HIV-exposed Sick Infants and Children

• If an infant is sick with signs & symptoms that could be HIV:
  • Test child using the correct test for age (virological or serological, see Table 2.1)
Exercise 1

Making sense of RDT results: Group game
Exercise 2

Making sense of virological testing results: Group game, re-match
Module 2: Key Points

• Most HIV-exposed infants are identified through follow-up with the mother who is already enrolled in PMTCT services.
  • When screening infants in other clinical settings (OPD, hospital, immunization clinic, well child) for HIV exposure, review mother’s antenatal card or child health card, for mother’s HIV test results.
  • If mother’s HIV status is unknown or she has not been tested recently (according to national guidelines), she should be tested using RDT.
  • If mother is not available, then test the infant for HIV exposure using RDT.
Module 2: Key Points

• WHO recommends that HIV-exposed infants are tested for HIV at 4–6 weeks of age using NAT.
  • All HIV-exposed infants who tested HIV-negative should be retested at 9 months of age and again at 18 months or 3 months after cessation of breastfeeding (whichever is later).
Module 2: Key Points

• Some countries may also recommend testing at birth of all or some HIV-exposed infants. Birth testing should only be implemented in parallel with efforts to strengthen and expand existing testing strategies for infants age 4–6 weeks.
Module 2: Key Points

• Testing algorithms define the sequence of specific HIV tests used for a particular population.
  • Each country will have their own HIV testing algorithm.
  • It is important that all health providers follow the national algorithm for infant HIV testing.

• Virological testing using NAT is used to diagnose HIV infection in HIV-exposed infants and children under the age of 18 months.
Module 2: Key Points

• A negative RDT result in an infant less than 4 months of age means that the infant is not HIV-exposed.
  • However, in children, 4-18 months of age, RDT is not reliable for determining HIV exposure.
  • These children should be retested according to national guidelines.

• In children over the age of 18 months, RDT can be used to diagnose HIV infection.
• “DNA” icon by Christopher T. Howlett from the Noun Project
• Finger prick photo (slide 14) by unknown author is licensed under CC BY-NC-ND
• “HIV Test” icon by Andrei Yushchenko from the Noun Project