

# Module 2

## Testing of HIV-exposed Infants

Infant HIV Testing  
Training Curriculum for  
Healthcare Providers

# Session 2.1

## Identifying HIV-exposed Infants

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### **Session Objective**

- Identify HIV-exposed infants in the clinical setting
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# Introduction

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- 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

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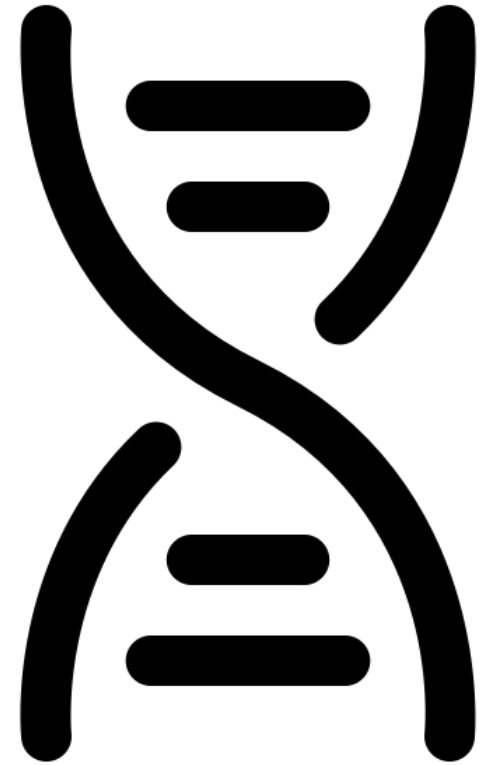
- *What is meant by nucleic acid testing or NAT?*

# Testing: The Terminology

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- **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

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- *What is the difference between infant HIV testing and EID (or early infant diagnosis)?*

# Testing: The Terminology

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- **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

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- **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

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- *What is the difference between point-of-care testing and near point-of-care testing?*

# Testing: The Terminology

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- **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

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- **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

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- *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

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- 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

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- 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

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- 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

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- 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



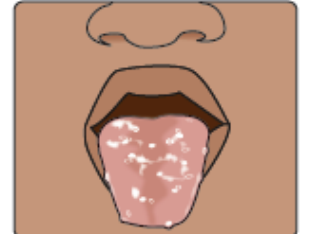
Cough



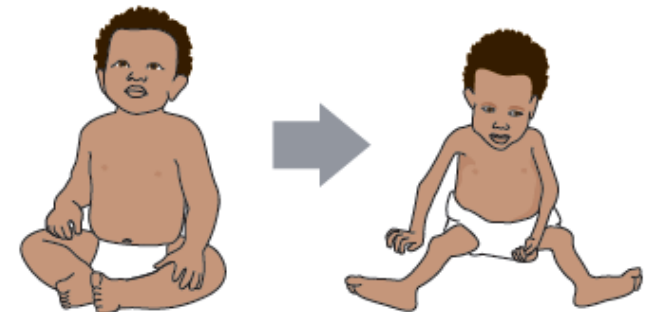
Fever



Diarrhea



Thrush



Weight loss

# Session 2.2: Recommendations on Timing of Infant HIV Testing

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## **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
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# Two Categories of Testing Procedures for Infant HIV Testing

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- *What are “maternal antibodies”?*

# Two Categories of Testing Procedures for Infant HIV Testing

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- **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

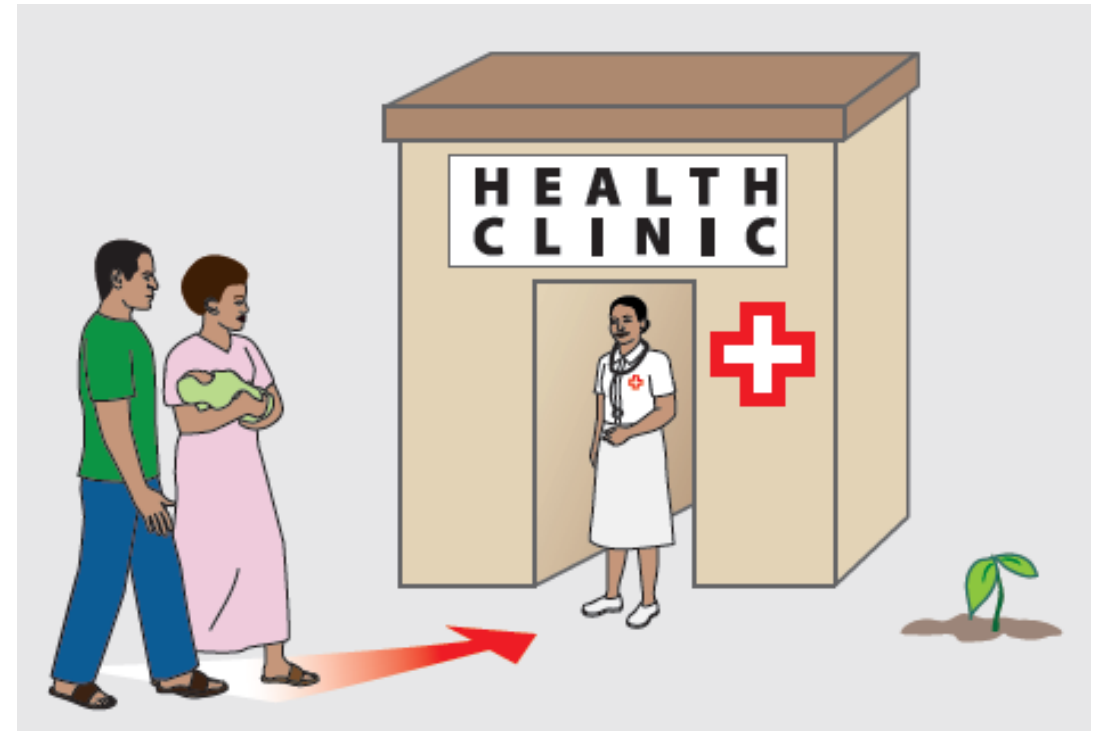
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- Serological tests are not accurate for diagnosing HIV infection in infants and young children
- Maternal antibodies can be present until 18 months of age
- Diagnosis of HIV in those less than 18 months of age requires NAT
- RDT *can* be used to identify 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?

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- *At what age are HIV-exposed infants tested for HIV?*



# When and which test?

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Category and age	Recommended test
HIV-exposed infant, at birth (0–2 days), provide testing if recommended by national guidelines	HIV virological testing using NAT, as per national guidelines
HIV-exposed infant, at 4–6 weeks of age, or as soon as possible thereafter	HIV virological testing, using NAT
HIV-exposed infant, at 9 months of age	HIV virological testing, using NAT
HIV-exposed infant, at 18 months of age or 3 months after breastfeeding ends (whichever is later) for final assessment of HIV status	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)

# Birth Testing

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- *What do you think are the advantages of birth testing?*
- *How about the disadvantages?*
- *How would you minimize the disadvantages?*



# Birth Testing

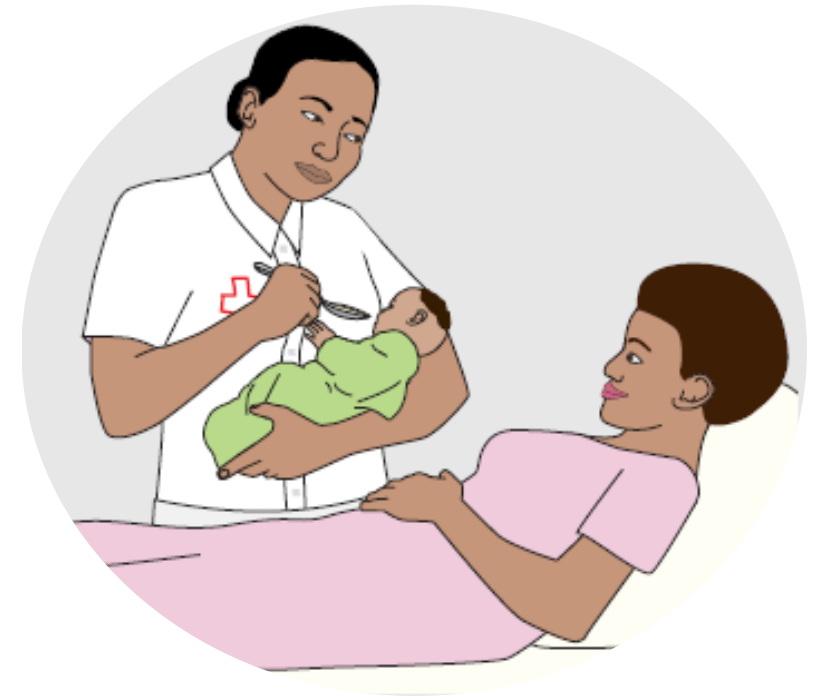
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- HIV testing at birth is most likely to identify infants infected *in utero*
  - These infants are at greatest risk for early death
- Birth testing will not detect infections during or shortly after delivery
- 4–6 week testing will identify infants 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

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- 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



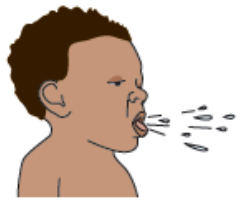
# Birth Testing, Disadvantages

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- 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

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Cough



Fever



Diarrhea



Thrush



Weight loss

- 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

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- 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

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- *What is an HIV testing algorithm?*
- *Why is it important to follow our national testing algorithm?*

# HIV Testing Algorithm

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- **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

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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.
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- 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

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### **Session Objectives**

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

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- 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

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- *What does “window period” refer to?*



# Window Period

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- “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

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- 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

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## **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

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**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

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- **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

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### **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
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# Laboratory Diagnosis of HIV Infection— Serological Testing

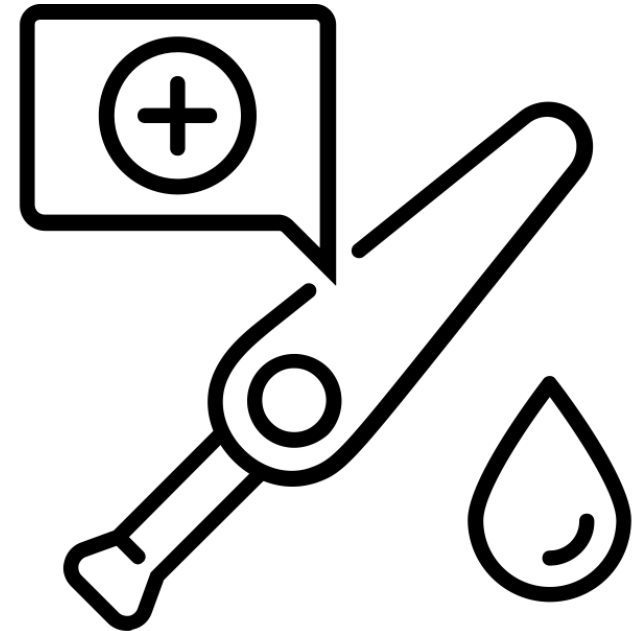
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- Detects antibodies, such as HIV antibodies, in blood or saliva
  - HIV antibodies are produced by the immune system in response to infection with HIV
- Can diagnose HIV in adults and children 18 months of age or older
- **Types of serological testing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

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- 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

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- 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

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- 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

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- 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

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- 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  $\geq$  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

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- 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



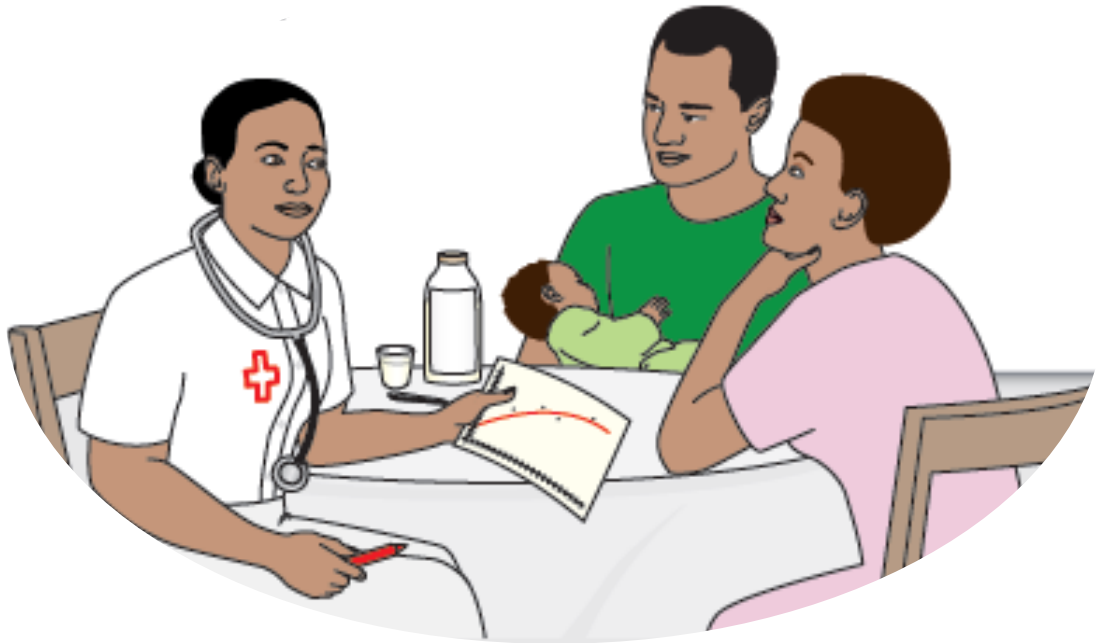
# RDT for Identification of HIV-exposed Infants

Age group	Unknown HIV exposure status
0–4 months	Test mother If mother is not available: <ul style="list-style-type: none"><li>• RDT in the child can reliably assess exposure</li></ul>
5–18 months	Test mother If mother is not available: <ul style="list-style-type: none"><li>• A positive RDT establishes exposure. Infants with positive RDT should get NAT to confirm infection.</li><li>• A negative RDT for the child does not fully rule out exposure. Perform NAT to assess HIV infection status in any sick child**</li><li>• Infants with negative RDT who are still breastfeeding will need testing 3 months after cessation of breastfeeding</li><li>• If sick, or index of suspicion is high, conduct virologic testing.</li></ul>
>18 months	<ul style="list-style-type: none"><li>• Serological testing (including RDT) is recommended to assess HIV infection status unless breastfed within the last 3 months or still breastfed.</li><li>• If still breastfed, RDT should be provided 3 months after cessation of breastfeeding.</li></ul>
<p>**Consider initiating ART for presumed HIV infection if there is high degree of suspicion while waiting for NAT results, especially if RDT positive.</p> <p>NAT = Nucleic acid testing, a virological test</p>	

# Testing HIV-exposed Sick Infants and Children

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- 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

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Making sense of RDT results: Group game

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# Exercise 2

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Making sense of virological testing results:  
Group game, re-match

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# Module 2: Key Points

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- 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

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- 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

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- 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

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- 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

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- 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.

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