



**Recommendations for the Use of Antiretroviral Drugs in  
Pregnant Women with HIV Infection and Interventions to Reduce  
Perinatal HIV Transmission in the United States**

Downloaded from <https://aidsinfo.nih.gov/guidelines> on 9/13/2019

Visit the *AIDSinfo* website to access the most up-to-date guideline.

Register for e-mail notification of guideline updates at <https://aidsinfo.nih.gov/e-news>.

## Diagnosis of HIV Infection in Infants and Children (Last updated December 7, 2018; last reviewed December 7, 2018)

### Panel's Recommendations

- Virologic assays (i.e., HIV RNA and HIV DNA nucleic acid tests [NATs]) that directly detect HIV must be used to diagnose HIV in infants and children aged <18 months with perinatal and postnatal HIV exposure; HIV antibody tests should not be used (AII).
- HIV RNA or HIV DNA NATs are generally equally recommended (AII).
- An assay that detects HIV non-B subtype viruses or Group O infections (e.g., an HIV RNA NAT or a dual-target total DNA/RNA test) is recommended for use in infants and children who were born to mothers with known or suspected non-B subtype virus or Group O infections (AII).
- Virologic diagnostic testing is recommended for all infants with perinatal HIV exposure at the following ages:
  - 14 to 21 days (AII)
  - 1 to 2 months (AII)
  - 4 to 6 months (AII)
- For infants at higher risk of perinatal HIV transmission, additional virologic diagnostic testing is recommended at birth (AII) and at 2 to 4 weeks after cessation of antiretroviral prophylaxis (BII).
- A positive virologic test should be confirmed as soon as possible by a repeat virologic test on a second specimen (AII).
- Definitive exclusion of HIV infection in nonbreastfed infants is based on two or more negative virologic tests, with one obtained at age ≥1 month and one at age ≥4 months, or two negative HIV antibody tests from separate specimens obtained at age ≥6 months (AII).
- Some experts confirm the absence of HIV at 12 to 18 months of age in children with prior negative virologic tests by performing an HIV antibody test to document loss of maternal HIV antibodies (BIII).
- Since children aged 18 to 24 months with perinatal HIV exposure occasionally have residual maternal HIV antibodies, definitive exclusion or confirmation of HIV infection in children in this age group who are HIV antibody-positive should be based on an HIV NAT (AII).
- Diagnostic testing in children with nonperinatal exposure only or children with perinatal exposure aged >24 months relies primarily on the use of HIV antibody (or antigen/antibody) tests; when acute HIV infection is suspected, additional testing with an HIV NAT may be necessary to diagnose HIV (AII).

**Note:** The [National Clinician Consultation Center](#) provides consultations on issues related to the management of perinatal HIV infection (1-888-448-8765; 24 hours a day, 7 days a week).

**Rating of Recommendations:** A = Strong; B = Moderate; C = Optional

**Rating of Evidence:** I = One or more randomized trials in children<sup>†</sup> with clinical outcomes and/or validated endpoints; I\* = One or more randomized trials in adults with clinical outcomes and/or validated laboratory endpoints with accompanying data in children<sup>†</sup> from one or more well-designed, nonrandomized trials or observational cohort studies with long-term clinical outcomes; II = One or more well-designed, nonrandomized trials or observational cohort studies in children<sup>†</sup> with long-term outcomes; II\* = One or more well-designed, nonrandomized trials or observational studies in adults with long-term clinical outcomes with accompanying data in children<sup>†</sup> from one or more similar nonrandomized trials or cohort studies with clinical outcome data; III = Expert opinion

<sup>†</sup> Studies that include children or children and adolescents, but not studies limited to post-pubertal adolescents

## Diagnosis of HIV in Infants and Children

HIV can be definitively diagnosed through use of virologic assays in most non-breastfed infants with **perinatal** HIV exposure by age 1 month to 2 months, and in virtually all infants with HIV infection by age 4 months to 6 months. Antibody tests, including the newer antigen-antibody combination immunoassays (sometimes referred to as fourth- and fifth-generation tests), do not establish the presence of HIV in infants because of transplacental transfer of maternal HIV antibodies; therefore, a virologic test must be used.<sup>1,2</sup> Positive virologic tests (i.e., nucleic acid tests [NATs]—a class of tests that includes HIV RNA and HIV DNA polymerase chain reaction [PCR] assays, and related RNA qualitative or quantitative assays) indicate likely HIV infection. The first test result should be confirmed as soon as possible by a repeat virologic test on a second specimen, because false-positive results can occur with both RNA and DNA assays.<sup>3</sup> For additional information on HIV and RNA assays and diagnosis of Group M non-subtype B and Group O HIV-1

*Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States*

Downloaded from <https://aidsinfo.nih.gov/guidelines> on 9/13/2019

infections and HIV-2 infections, see the relevant sections below.

Antigen/antibody combination immunoassays which detect HIV-1/2 antibodies as well as HIV-1 p24 antigen are not recommended for diagnosis of HIV in infants. The sensitivity of the antigen component in the first months of life is less than that of an HIV NAT, and antibody tests should not be used for diagnosis in infants and children <18 months of age.<sup>4-6</sup> Children with perinatal HIV exposure who are aged 18 to 24 months occasionally have residual maternal HIV antibodies; definitive confirmation of HIV infection in children in this age group who are HIV antibody-positive should be based on a NAT (see [Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations](#)). Diagnosis in children aged >24 months relies primarily on HIV antibody and antigen/antibody tests (see [Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal Exposure Aged >24 Months](#)).<sup>1</sup>

An infant who has a positive HIV antibody test but whose mother’s HIV status is unknown (see [Maternal HIV Testing and Identification of Perinatal HIV Exposure](#)) should be assumed to have been exposed to HIV. The infant should undergo HIV diagnostic testing as described below<sup>7</sup> **and receive antiretroviral (ARV) prophylaxis or empiric HIV therapy as soon as possible**. For ARV management of HIV-exposed newborns **and newborns with HIV infection (including those who do not yet have confirmed infection)**, see the [Antiretroviral Management of Newborns with Perinatal HIV Exposure or Perinatal HIV](#) section.<sup>8,9</sup>

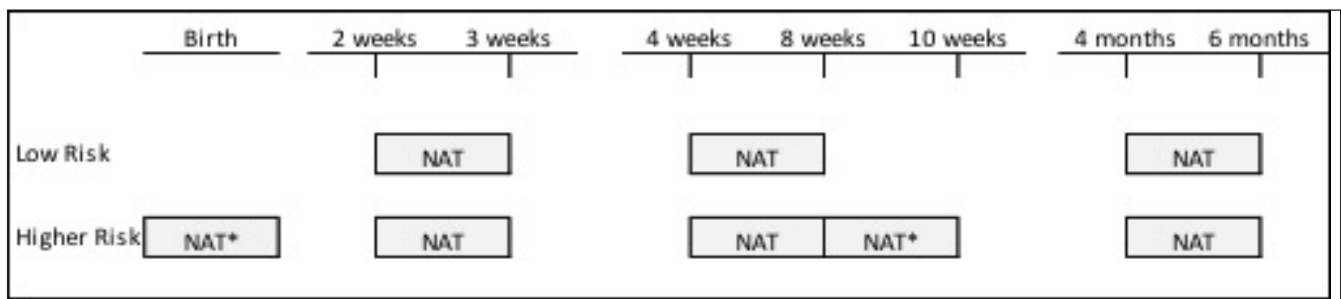
### Timing of Diagnostic Testing in Infants with Perinatal HIV Exposure

Confirmation of HIV infection is based on the results of two positive virologic tests from separate blood samples in infants and children younger than 18 months. Figure 1 summarizes the timing of recommended virologic diagnostic testing for infants at low risk of transmission (based on maternal antiretroviral therapy [ART] and viral suppression) with additional time points to be considered for infants at higher risk and those on combination ARV prophylaxis regimens.

**Figure 1. Recommended Virologic Testing Schedules for Infants Exposed to HIV by Perinatal HIV Transmission Risk**

**Low Risk:** Infants born to mothers living with HIV who received standard ART during pregnancy and had sustained viral suppression (usually defined as confirmed HIV RNA level below the lower limits of detection of an ultrasensitive assay) and no concerns related to maternal adherence.

**Higher Risk:** Infants born to mothers living with HIV who did not receive prenatal care, did not receive antepartum or intrapartum ARVs, received intrapartum ARV drugs only, mothers who initiated ART late in pregnancy (during the late second or third trimester), received a diagnosis of acute HIV infection during pregnancy, or had detectable HIV viral loads close to the time of delivery, including those who received combination ARV drugs and did not have sustained viral suppression.



\* For higher-risk infants, additional virologic diagnostic testing **is recommended** at birth and 2 to 4 weeks after cessation of ARV prophylaxis (i.e., at 8–10 weeks of life).

**Key to Acronyms:** ART = antiretroviral therapy; ARV = antiretroviral; NAT = nucleic acid test

HIV infection can be **presumptively** excluded in nonbreastfed infants with two or more negative virologic

tests (one at age  $\geq 14$  days and one at age  $\geq 4$  weeks) or one negative virologic test (i.e., negative NAT [RNA or DNA]) at age  $\geq 8$  weeks, or one negative HIV antibody test at age  $\geq 6$  months.<sup>1,7</sup>

**Definitive** exclusion of HIV infection in a nonbreastfed infant is based on two or more negative virologic tests (i.e., negative NATs [RNA or DNA]), one at age  $\geq 1$  month and one at age  $\geq 4$  months, or two negative HIV antibody tests from separate specimens obtained at age  $\geq 6$  months.

For both **presumptive** and **definitive** exclusion of HIV infection, a child must have no other laboratory evidence (i.e., no positive virologic test results or low CD4 T lymphocyte [CD4] cell count/percent) or clinical evidence of HIV infection and should not be breastfeeding. Many experts confirm the absence of HIV infection in infants with negative virologic tests by performing an antibody test at age 12 to 18 months to document seroreversion to HIV antibody-negative status.

*Pneumocystis jirovecii* pneumonia (PCP) prophylaxis is recommended for infants with **indeterminate** HIV infection status starting at age 4 to 6 weeks until they are determined to be **definitively** or **presumptively** without HIV.<sup>10</sup> Thus, PCP prophylaxis can be avoided or discontinued if HIV infection is presumptively excluded (see the [Pediatric Opportunistic Infection Guidelines](#) and [Initial Postnatal Management of the Neonate Exposed to HIV](#)).

The case definition for **indeterminate** HIV infection status is an HIV-exposed child aged  $< 18$  months who was born to a woman living with HIV and who does not meet the criteria for having HIV infection or for having not contracted HIV. This includes infants who do not meet the minimum requirement for **presumptively** uninfected (e.g., having one negative test result at 4 weeks of age).

### ***Virologic Testing at Birth for Newborns at Higher Risk of Perinatal HIV Transmission***

Virologic testing at birth should be considered for newborns who are at higher risk of perinatal HIV transmission,<sup>11-16</sup> such as infants born to women with HIV who:

- Did not receive prenatal care
- Did not receive antepartum or intrapartum ARV drugs
- Received intrapartum ARV drugs only
- Initiated ART late in pregnancy (late second or third trimester)
- Received a diagnosis of acute HIV infection during pregnancy
- Had detectable HIV viral load close to the time of delivery
- Received combination ARV drugs and did not have sustained viral suppression

Testing infants who have been exposed to HIV close to the time of birth only identifies 20% to 58% of infants with HIV infection. However, in one study that specifically evaluated infants born to mothers who had not received ARV drugs during pregnancy, and hence were at higher risk of *in utero* infection, birth testing identified 66.4% of infants with HIV infection.<sup>17</sup> Prompt diagnosis of infant HIV infection is critical to allow for discontinuing ARV prophylaxis and instituting early ART (see [When to Initiate Therapy in Antiretroviral-Naive Children](#) in the [Pediatric ARV Guidelines](#)). Blood samples from the umbilical cord should not be used for diagnostic evaluation because of the potential for contamination with maternal blood. Infants who have a positive virologic test result at or before age 48 hours are considered to have early (i.e., intrauterine) infection, whereas infants who have a negative virologic test result during the first week of life and subsequent positive tests are considered to have late (i.e., intrapartum) infection.<sup>11,12,18</sup>

### ***Virologic Testing at Age 14 to 21 Days***

The diagnostic sensitivity of virologic testing increases rapidly by age 2 weeks,<sup>7</sup> and early identification of infection permits discontinuation of neonatal ARV prophylaxis and initiation of ART (see the [Infants Younger than 12 Months](#) section and [Table 5](#) in [When to Initiate Therapy in Antiretroviral-Naive Children](#) in the [Pediatric ARV Guidelines](#)).

## ***Virologic Testing at Age 1 to 2 Months***

Testing performed at age 1 month to 2 months is intended to maximize the likelihood of detecting HIV infection in infants.<sup>19,20</sup> Two studies found that the type of maternal or infant prophylaxis used did not affect the sensitivity of diagnostic HIV testing. However, the sensitivity of diagnostic HIV testing was lower during the period of infant ARV prophylaxis than during the subsequent testing interval at 3 months of age, when the infant was no longer receiving prophylaxis. Overall, in both studies, 89% of infants with HIV infection were identified by 4 to 6 weeks of age. Repeat testing was performed at  $\geq 4$  to 6 weeks of age during the period of neonatal ARV prophylaxis on infants who had negative test results in the first 7 days of life. This repeat testing determined that 76% of those infants had HIV infection in one study<sup>19</sup> and 68% of those infants had HIV infection in the second study.<sup>17</sup> In both studies, all infants who had negative test results in the first 7 days of life received an HIV diagnosis when the next diagnostic test was performed at 3 months of age.

For infants at higher risk of perinatal HIV transmission, the Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission suggests an additional virologic test 2 to 4 weeks after cessation of ARV prophylaxis (i.e., at 8–10 weeks of age), given the increased risk of infection and concern that ARV prophylaxis, particularly combination ARV prophylaxis, may reduce the sensitivity of testing during prophylaxis.<sup>7,17,19</sup> In these situations, many experts recommend one test at age 4 weeks to 6 weeks to allow prompt recognition of infants with HIV, with an additional test at 8 weeks of life (2 weeks after cessation of prophylaxis at 6 weeks of life) to capture additional cases. For infants at low risk of transmission, a single test obtained at 1 to 2 months of age may be timed to occur 2 to 4 weeks after cessation of ARV prophylaxis.

An infant with two negative virologic test results (one at age  $\geq 14$  days and the other at age  $\geq 4$  weeks) or one negative test result at age  $\geq 8$  weeks can be viewed as presumptively HIV uninfected, assuming the child has not had a positive **prior** virologic test result, **laboratory evidence of** CD4 immunosuppression, or clinical evidence **indicative** of HIV infection.

## ***Virologic Testing at Age 4 to 6 Months***

Infants with HIV exposure who have had negative virologic assays at age 14 to 21 days and at age 1 to 2 months, have no clinical evidence of HIV infection, and are not breastfed should be retested at age 4 to 6 months for definitive exclusion of HIV infection.

## ***Antibody Testing at Age 6 Months and Older***

Two or more negative results of HIV antibody tests that were performed in nonbreastfed infants at age  $\geq 6$  months can also be used to definitively exclude HIV infection in children with no clinical or virologic laboratory-documented evidence of HIV infection.<sup>21,22</sup>

## ***Antibody Testing at Age 12 to 18 Months to Document Seroreversion***

Some experts confirm the absence of HIV infection in infants and children with negative virologic test results (when there has not been prior confirmation of two negative antibody test results) by repeat serologic testing between 12 months and 18 months of age to confirm that maternal HIV antibodies transferred *in utero* have cleared.<sup>1</sup> In a study from 2012, the median age at seroreversion was 13.9 months.<sup>23</sup> Although the majority of infants who are without HIV will serorevert by age 15 months to 18 months, there are reports of late seroreversion after 18 months (see below). Factors that might influence the time to seroreversion include maternal disease stage and assay sensitivity.<sup>23-26</sup>

## **Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations**

### ***Late Seroreversion ( $\leq 24$ Months of Age)***

Nonbreastfed children with **perinatal** HIV exposure, no other HIV transmission risk, and no clinical or virologic laboratory evidence of HIV infection may have residual HIV antibodies up to age 24 months. These children are called late seroreverters.<sup>23-26</sup> In one study, 14% of children with HIV exposure who

were uninfected seroreverted after age 18 months.<sup>23</sup> These children may have had positive immunoassay results but supplemental antibody test results that indicated indeterminate HIV status (such as Western blot or immunofluorescence assay [IFA]). In such cases, repeat antibody testing at a later date confirmed seroreversion. Due to the possibility of residual HIV antibodies, virologic testing (i.e., with a NAT) is necessary to definitively exclude or confirm HIV infection in children with perinatal HIV exposure, who continue to have a positive HIV antibody (or antigen/antibody) test at age 18 months to 24 months.

### ***Postnatal HIV Infection in Children with Perinatal HIV Exposure and Prior Negative Virologic Test Results for Whom There Are Additional HIV Transmission Risks***

In contrast to late seroreverters, in rare situations postnatal HIV infections have been reported in children with HIV exposure who had prior negative HIV virologic tests. This occurs in children who **acquire HIV** through an additional risk factor after completion of testing (see [Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal Exposure Aged >24 Months](#)). If an HIV antibody test is positive at age 18 to 24 months, repeated virologic testing will distinguish late-seroreverting (uninfected) children with residual antibodies from children with antibodies due to **underlying HIV** infection.

### ***Suspicion of HIV-2 or Non-Subtype B HIV-1 Infections with False-Negative Virologic Test Results***

Children with non-subtype B HIV-1 and children with HIV-2 may have false-negative virologic tests but persistent positive immunoassay results and indeterminate HIV-1 Western blot results.<sup>27-29</sup> The diagnostic approach in these situations is discussed below in the sections on Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections and on Virologic Assays to Diagnose HIV-2 Infections.

## **Diagnostic Testing in Children with Nonperinatal HIV Exposure or Children with Perinatal HIV Exposure Aged >24 Months**

### ***Breastfeeding***

**Women with HIV should be encouraged to avoid breastfeeding (see [Antiretroviral Management of Newborns with Perinatal HIV Exposure or Perinatal HIV](#)). Monitoring of infants born to women with HIV who opt to breastfeed should include immediate HIV diagnostic testing with a NAT and virologic HIV testing at the standard time points (see Figure 1 above). Many experts then recommend testing every 3 months throughout breastfeeding, followed by monitoring at 4 weeks to 6 weeks, 3 months, and 6 months after cessation of breastfeeding. Clinicians caring for a woman with HIV who is considering breastfeeding should consult with an expert and, if necessary, the Perinatal HIV Hotline (888-448-8765). See [Antiretroviral Management of Newborns with Perinatal HIV Exposure or Perinatal HIV](#) and [Guidance for Counseling and Managing Women Living with HIV in the United States Who Desire to Breastfeed](#).<sup>30-32</sup>**

### ***Premastication***

Receipt of solid food that has been premasticated or prewarmed (**in the mouth**) by a caregiver living with HIV is associated with risk of HIV transmission.<sup>33-38</sup> If this occurs in children with perinatal HIV exposure aged 24 months or younger with prior negative virologic tests, it will be necessary for such children to undergo virologic diagnostic testing, as they may have residual maternal HIV antibodies (see [Diagnostic Testing in Children with Perinatal HIV Exposure in Special Situations](#)).

### ***Additional Routes of HIV Transmission***

Additional routes of HIV transmission in children include sexual abuse, receipt of contaminated blood products, **and needlestick with contaminated needles**. In such cases, maternal HIV status may be negative. If the maternal HIV status is unknown, age-appropriate testing should be performed as described for children with perinatal HIV exposure. Acquisition of HIV in older children is possible through accidental

needlestick injuries, sexual transmission, or injection drug use. Medical procedures performed in settings with inadequate infection control practices may pose a potential risk; although tattooing or body piercing presents a potential risk of HIV transmission, no cases of HIV transmission from these activities have been documented.<sup>39</sup>

## ***Diagnostic Testing***

Diagnosis of HIV-1 infection in infants and children with nonperinatal HIV exposure only or children with perinatal HIV exposure aged >24 months relies primarily on HIV antibody and antigen/antibody tests.<sup>1,40</sup> Food and Drug Administration (FDA)-approved diagnostic tests include:

- Antigen/antibody combination immunoassays, which detect HIV-1/2 antibodies as well as HIV-1 p24 antigen.
- Recommended for initial testing to screen for established infection with HIV-1 or HIV-2 and for acute HIV-1 infection. However, p24 antigen from HIV-1 non-B strains, HIV-1 non-M strains, and HIV-2 strains may not be detected.<sup>41</sup>
- HIV-1/2 immunoassays (third-generation antibody tests) are alternatives for initial testing.
- HIV-1/HIV-2 antibody differentiation immunoassay, which differentiates HIV-1 antibodies from HIV-2 antibodies is recommended for supplemental testing.
- HIV-1 NAT may be necessary as an additional test to diagnose acute HIV infection.
- HIV-1 Western blot and HIV-1 indirect IFAs (first-generation tests) are alternatives for supplemental testing, but will not detect acute HIV infection.

Diagnosis of HIV-2 in children with nonperinatal exposure or children with perinatal exposure aged >24 months relies on the Centers for Disease Control and Prevention (CDC)/Association of Public Health Laboratories 2014 laboratory testing guidelines, which recommend using an HIV-1/HIV-2 antibody differentiation immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies for supplemental testing. This is not subject to the same testing ambiguity as when the HIV-1 Western blot is used as a supplemental test; >60% of individuals with HIV-2 are misclassified as having HIV-1 by the HIV-1 Western blot.<sup>1,42</sup> All HIV-2 cases should be reported to the HIV surveillance program of the state or local health department; additional HIV-2 DNA PCR testing can be arranged by a local public health laboratory or the CDC if an HIV-1/HIV-2 antibody differentiation immunoassay is inconclusive. HIV-2 DNA PCR testing may be necessary for definitive diagnosis, though this assay is not commercially available.<sup>43,44</sup>

## **Virologic Assays to Diagnose HIV in Infants Younger Than 18 Months with Perinatal HIV-1 Exposure**

### ***HIV RNA Assays***

HIV quantitative RNA assays detect extracellular viral RNA in plasma. Their specificity has been shown to be 100% at birth and at 1, 3, and 6 months of age and is comparable to HIV DNA PCR.<sup>19</sup> Results of quantitative assays that show HIV RNA levels <5,000 copies/mL may not be reproducible, and the test should be repeated before these results are interpreted as documentation of HIV infection in an infant.<sup>45,46</sup> Testing at birth will detect infants who acquire HIV *in utero* and not those who acquire HIV from exposure during or immediately prior to delivery (i.e., in the intrapartum period). Studies have shown that HIV RNA assays identify 25% to 58% of infants with HIV infection from birth through the first week of life, 89% at age 1 month, and 90% to 100% by age 2 months to 3 months (similar to results of HIV DNA PCR for early diagnosis of HIV).<sup>3,7,19,47</sup>

HIV RNA undergoes reverse transcription to double-stranded DNA, which persists intracellularly within an infected cell. HIV DNA PCR assays detect intracellular DNA, and an individual receiving ART will continue to have a positive result even with a suppressed viral load. In contrast, HIV RNA assays are affected by

maternal antenatal treatment or infant combination ARV prophylaxis.<sup>48</sup> In one study, the sensitivity of HIV RNA assays were not associated with the type of maternal ART or infant ARV prophylaxis, but HIV RNA levels at 1 month were significantly lower in infants with HIV infection receiving multidrug prophylaxis (n = 9) compared to levels among infants receiving single-drug zidovudine prophylaxis (n = 47) (median HIV RNA 2.5 log<sub>10</sub> copies/mL vs. 5.4 log copies/mL, respectively). In contrast, the median HIV RNA levels were high (median HIV RNA 5.6 log copies/mL) by age 3 months in both groups after stopping prophylaxis.<sup>19</sup> Between 2010 and 2016, a significant decline in baseline viremia was noted in South Africa's Early Infant Diagnosis program, with loss of detectability documented among some infants with HIV. This decline may have reflected the administration of various prophylactic regimens during those years, including Option A, Option B, and Option B+, as recommended by the World Health Organization.<sup>49</sup> Further studies are necessary to evaluate the sensitivity of HIV RNA assays in infants during receipt of three-drug ARV prophylaxis or empiric therapy.

An HIV quantitative RNA assay can be used as a supplemental test for infants who have an initial positive HIV DNA PCR test result. In addition to providing virologic confirmation of infection status, the expense of repeat HIV DNA PCR testing is spared, and an HIV RNA measurement is available to assess baseline viral load. This viral load can also be used to determine HIV genotype and guide initial ARV treatment in an infant with HIV. HIV RNA assays may be more sensitive than HIV DNA PCR for detecting non-subtype B HIV (see Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections).

The HIV qualitative RNA assay (APTIMA HIV-1 RNA Qualitative Assay) is an alternative diagnostic test that can be used for infant testing. It is the only qualitative RNA test that is approved by the FDA.<sup>18,50-53</sup>

### ***HIV DNA PCR and Related Assays***

HIV DNA PCR is a sensitive technique used to detect intracellular HIV viral DNA in peripheral blood mononuclear cells. The specificity of the HIV DNA PCR is 99.8% at birth and 100% at ages 1 month, 3 months, and 6 months. Studies have shown that HIV DNA PCR assays identify 20% to 55% of infants with HIV infection from birth through the first week of life, with the same caveat as for RNA testing—testing at birth only detects *in utero* HIV infection and not infection in those infants who acquire HIV during the intrapartum period. This percentage increases to >90% by 2 weeks to 4 weeks of age and to 100% at ages 3 months and 6 months.<sup>7,18,19,47</sup>

Two studies provided data on diagnostic testing at different time points in infants with confirmed HIV infection, including those who had negative test results at birth (i.e., infants considered to have acquired HIV during the intrapartum period). A randomized, international study of 1,684 infants evaluated the efficacy of three different regimens of neonatal prophylaxis consisting of 6 weeks of zidovudine either alone or with two or three other ARV drugs; none of the infants' mothers had received prenatal ARV drugs. Infant testing was performed at birth, 10 to 14 days, 4 to 6 weeks, and 3 and 6 months (no testing was performed between 6 weeks and 3 months). Ninety-three of 140 infants (66.4%) with HIV infection were identified at birth, and by 4 to 6 weeks of age, 89% of the 140 infants were identified. Of the 47 infants with HIV infection who had negative DNA PCR test results at birth, 68% were identified during the period of neonatal ARV prophylaxis at 4 to 6 weeks; by 3 months, all 47 infants were identified.<sup>17</sup> Data from Thailand showed that, in nonbreastfed infants, receiving a prophylaxis regimen of zidovudine/lamivudine/nevirapine for 6 weeks was associated with a delay in first HIV DNA detection. In this cohort, up to 20% of HIV-exposed infants had their first positive DNA PCR test after 2 months of age, prompting the authors to recommend infant testing at 4 months of age, after neonatal prophylaxis had been discontinued for at least 4 to 6 weeks.<sup>54</sup>

A recent study from Cape Town evaluated the sensitivity of HIV DNA assays within 8 days of life, during and after initiation of infant ART in infants with HIV. The infants had been exposed to a combination of maternal ART *in utero* and early ART for prophylaxis and treatment. The study noted that one infant subsequently had undetectable HIV DNA after 6 days on treatment, another was undetectable after 3 months, and a third was undetectable after 4 months. In seven infants who had virologic suppression (defined as a



continuous downward trend in plasma HIV RNA, with <100 copies/mL after 6 months) total HIV DNA continued to decay over 12 months. The authors suggested that rapid decline of HIV-1 RNA and DNA may complicate definitive diagnosis.<sup>55</sup> A dataset of 38,043 infants from the Western Cape province of South Africa who were tested at a median age of 45 days of life in the setting of intensified vertical HIV transmission prevention regimens, particularly with the Option B+ program, showed that indeterminate PCRs decreased in frequency. These findings should be regarded with a high index of suspicion since many patients had positive results representative of true HIV infections on subsequent samples. These findings point out the need for additional virologic testing for definitive diagnosis.<sup>56</sup> Another group of South African investigators reported similar conclusions in a study of a cohort of 5,743 HIV-exposed neonates from Johannesburg.<sup>57</sup>

Although the AMPLICOR<sup>®</sup> HIV-1 DNA test has been widely used for diagnosis of infants born to mothers with HIV-1 infection since it was introduced in 1992, it is no longer commercially available in the United States. The sensitivity and specificity of noncommercial HIV-1 DNA tests (using individual laboratory reagents) may differ from the sensitivity and specificity of the FDA-approved commercial test.

The COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 qualitative test (which detects both HIV-1 RNA and proviral DNA in plasma, whole blood, and dried blood spots) may be used for infant diagnosis, but it is not approved by the FDA.<sup>57-59</sup>

## Other Issues

### *Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections*

Although HIV-1 Group M subtype B is the predominant viral subtype found in the United States, multiple subtypes and recombinant forms are found in the United States with a widespread geographic distribution.<sup>60</sup> Recent data from the CDC National HIV Surveillance System showed that the number of foreign-born children with HIV has exceeded the number of U.S.-born children with HIV since 2011, with 65.5% of foreign-born children with HIV being born in sub-Saharan Africa and 14.3% in Eastern Europe.<sup>61</sup> In an evaluation of infants that received a perinatal HIV infection diagnosis in New York state in 2001 and 2002, 16.7% of infants had acquired a non-subtype B strain of HIV, compared with 4.4% of infants born in 1998 and 1999.<sup>62</sup> Among a group of 40 children attending a pediatric HIV clinic in Rhode Island between 1991 and 2012, 14 (35%) acquired HIV with non-B HIV-1 subtypes. All 14 children with non-B subtypes were either born outside the United States or their parents were of foreign origin.<sup>63</sup> In an analysis of 1,277 unique sequences collected in Rhode Island from 2004 to 2011, 8.3% were non-B subtypes (including recombinant forms). Twenty-two percent of non-B subtypes formed transmission clusters, including individuals with perinatally acquired infection.<sup>64</sup> In an analysis of 3,895 HIV-1 sequences collected between July 2011 and June 2012 in the United States, 5.3% were determined to be non-B subtypes (including recombinant forms). Among individual states, the percentage of non-B subtypes ranged from 0% (in 12 states) to 28.6% in South Dakota, with seven states having percentages that were greater than 10%.<sup>65</sup>

Evolving immigration patterns may be contributing to local and regional increases in HIV-1 subtype diversity. Non-subtype B viruses predominate in other parts of the world, such as subtype C in regions of Africa and India and subtype CRF01 in much of Southeast Asia. Group O HIV strains are seen in West-Central Africa.<sup>66</sup> Non-subtype B and Group O strains may also be seen in countries with links to these geographical regions.<sup>67-71</sup> Geographical distribution of HIV groups is available at the [HIV Sequence Database](#).

Currently available real-time HIV RNA PCR assays and the qualitative diagnostic RNA assay have improved sensitivity for detection of non-subtype B HIV infection and the less common Group O strains, compared to older RNA assays that did not detect or appropriately amplify many non-B subtypes and Group O HIV<sup>72-77</sup> (see [Clinical and Laboratory Monitoring of Pediatric HIV Infection](#)). Similarly, the COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 qualitative test (a dual-target DNA/RNA test) can identify non-subtype B and Group O infections.<sup>58,59</sup>

Thus, a real-time PCR assay, qualitative RNA assay **or a dual-target total DNA/RNA test** should be used for infant testing instead of a DNA PCR assay when evaluating an infant born to a mother whose HIV infection is linked to an area endemic for non-subtype B HIV or Group O strains, such as Africa or Southeast Asia. Another indication is when initial testing is negative using a HIV DNA PCR test and non-subtype B or Group O perinatal exposure is suspected. Two negative HIV antibody tests obtained at age  $\geq 6$  months provide further evidence to definitively rule out HIV infection. Clinicians should consult with an expert in pediatric HIV infection; state or local public health departments or the CDC may be able to assist in obtaining referrals for diagnostic testing.

### ***Virologic Assays to Diagnose HIV-2 Infections***

HIV-2 infection is endemic in Angola; Mozambique; West African countries, including Cape Verde, Ivory Coast, the Gambia, Guinea-Bissau, Mali, Mauritania, Nigeria, Sierra Leone, Benin, Burkina Faso, Ghana, Guinea, Liberia, Niger, Nigeria, Sao Tome, Senegal, and Togo; and parts of India.<sup>78-80</sup> It also occurs in countries such as France and Portugal, which have large numbers of immigrants from these regions.<sup>81,82</sup> HIV-1 and HIV-2 coinfections may also occur, but these are rare outside areas where HIV-2 is endemic. HIV-2 is rare in the United States. Although accurately diagnosing HIV-2 can be difficult, it is clinically important because HIV-2 strains are resistant to several ARV drugs developed to suppress HIV-1.<sup>83-85</sup>

Infant testing with HIV-2-specific DNA PCR tests should be performed at time points similar to those used for HIV-1 testing when evaluating an infant born to a mother with a known or suspected HIV-2 infection. A mother should be suspected of having HIV-2 if her infection is linked to an area endemic for HIV-2 infection or if her HIV test results are suggestive of HIV-2 infection (i.e., the mother has a positive initial HIV 1/2 immunoassay test result, repeatedly indeterminate results on HIV-1 Western blot, and HIV-1 RNA viral loads that are at or below the limit of detection; however, the current recommendation to use an HIV-1/HIV-2 antibody differentiation immunoassay for supplemental testing is not subject to the same testing ambiguity as when the HIV-1 Western blot is used as a supplemental test).<sup>1,86</sup> HIV-2 DNA PCR testing can be arranged by the HIV surveillance program of the state or local health department through their public health laboratory or the CDC, because this assay is not commercially available.<sup>43,44</sup> Clinicians should consult with an expert in pediatric HIV infection when caring for infants with suspected or known exposure to HIV-2.<sup>78,87</sup>

### **References**

1. Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory testing for the diagnosis of HIV infection: updated recommendations. 2014. Available at: <http://dx.doi.org/10.15620/cdc.23447>.
2. Donovan M, Palumbo P. Diagnosis of HIV: challenges and strategies for HIV prevention and detection among pregnant women and their infants. *Clin Perinatol*. 2010;37(4):751-763, viii. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21078448>.
3. Read JS, Committee on Pediatric AIDS, American Academy of Pediatrics. Diagnosis of HIV-1 infection in children younger than 18 months in the United States. *Pediatrics*. 2007;120(6):e1547-1562. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18055670>.
4. Tamhane M, Gautney B, Shiu C, et al. Analysis of the optimal cut-point for HIV-p24 antigen testing to diagnose HIV infection in HIV-exposed children from resource-constrained settings. *J Clin Virol*. 2011;50(4):338-341. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21330193>.
5. Wessman MJ, Theilgaard Z, Katzenstein TL. Determination of HIV status of infants born to HIV-infected mothers: a review of the diagnostic methods with special focus on the applicability of p24 antigen testing in developing countries. *Scand Journal of Infect Dis*. 2012;44(3):209-215. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22074445>.
6. Bhowan K, Sherman GG. Performance of the first fourth-generation rapid human immunodeficiency virus test in children. *Pediatr Infect Dis J*. 2013;32(5):486-488. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23190776>.
7. Havens PL, Mofenson LM, American Academy of Pediatrics Committee on Pediatric AIDS. Evaluation and management of the infant exposed to HIV-1 in the United States. *Pediatrics*. 2009;123(1):175-187. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19117880>.

8. Ferguson W, Goode M, Walsh A, Gavin P, Butler K. Evaluation of 4 weeks' neonatal antiretroviral prophylaxis as a component of a prevention of mother-to-child transmission program in a resource-rich setting. *Pediatr Infect Dis J*. 2011;30(5):408-412. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21266939>.
9. Sollai S, Noguera-Julian A, Galli L, et al. Strategies for the prevention of mother to child transmission in Western countries: an update. *Pediatr Infect Dis J*. 2015;34(5 Suppl 1):S14-30. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25894973>.
10. Panel on Opportunistic Infections in HIV-Exposed and HIV-Infected Children. Guidelines for the prevention and treatment of opportunistic infections in HIV-exposed and HIV-infected children. 2018. Available at [http://aidsinfo.nih.gov/contentfiles/lvguidelines/oi\\_guidelines\\_Pediatrics.pdf](http://aidsinfo.nih.gov/contentfiles/lvguidelines/oi_guidelines_Pediatrics.pdf).
11. Lilian RR, Kalk E, Technau KG, Sherman GG. Birth diagnosis of HIV infection on infants to reduce infant mortality and monitor for elimination of mother-to-child transmission. *Pediatr Infect Dis J*. 2013. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23574775>.
12. Jourdain G, Mary JY, Coeur SL, et al. Risk factors for *in utero* or intrapartum mother-to-child transmission of human immunodeficiency virus type 1 in Thailand. *J Infect Dis*. 2007;196(11):1629-1636. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18008246>.
13. Tubiana R, Le Chenadec J, Rouzioux C, et al. Factors associated with mother-to-child transmission of HIV-1 despite a maternal viral load <500 copies/mL at delivery: a case-control study nested in the French perinatal cohort (EPF-ANRS CO1). *Clin Infect Dis*. 2010;50(4):585-596. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20070234>.
14. Katz IT, Shapiro DE, Tuomala R. Factors associated with lack of viral suppression at delivery. *Ann Intern Med*. 2015;162(12):874-875. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26075762>.
15. Momplaisir FM, Brady KA, Fekete T, Thompson DR, Diez Roux A, Yehia BR. Time of HIV diagnosis and engagement in prenatal care impact virologic outcomes of pregnant women with HIV. *PLoS One*. 2015;10(7):e0132262. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26132142>.
16. Mandelbrot L, Tubiana R, Le Chenadec J, et al. No perinatal HIV-1 transmission from women with effective antiretroviral therapy starting before conception. *Clin Infect Dis*. 2015;61(11):1715-1725. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26197844>.
17. Nielsen-Saines K, Watts DH, Veloso VG, et al. Three postpartum antiretroviral regimens to prevent intrapartum HIV infection. *N Engl J Med*. 2012;366(25):2368-2379. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22716975>.
18. Lilian RR, Kalk E, Bhowan K, et al. Early diagnosis of *in utero* and intrapartum HIV infection in infants prior to 6 weeks of age. *J Clin Microbiol*. 2012;50(7):2373-2377. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22518871>.
19. Burgard M, Blanche S, Jasseron C, et al. Performance of HIV-1 DNA or HIV-1 RNA tests for early diagnosis of perinatal HIV-1 infection during anti-retroviral prophylaxis. *J Pediatr*. 2012;160(1):60-66 e61. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21868029>.
20. Lilian RR, Johnson LF, Moolla H, Sherman GG. A mathematical model evaluating the timing of early diagnostic testing in HIV-exposed infants in South Africa. *J Acquir Immune Defic Syndr*. 2014;67(3):341-348. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25118910>.
21. Kuhn L, Schramm DB, Shiau S, et al. Young age at start of antiretroviral therapy and negative HIV antibody results in HIV-infected children when suppressed. *AIDS*. 2015;29(9):1053-1060. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25870988>.
22. Payne H, Mkhize N, Otjombe K, et al. Reactivity of routine HIV antibody tests in children who initiated antiretroviral therapy in early infancy as part of the children with HIV early antiretroviral therapy (CHER) trial: a retrospective analysis. *Lancet Infect Dis*. 2015;15(7):803-809. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26043884>.
23. Gutierrez M, Ludwig DA, Khan SS, et al. Has highly active antiretroviral therapy increased the time to seroreversion in HIV exposed but uninfected children? *Clin Infect Dis*. 2012;55(9):1255-1261. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22851494>.
24. Gulia J, Kumwenda N, Li Q, Taha TE. HIV seroreversion time in HIV-1-uninfected children born to HIV-1-infected mothers in Malawi. *J Acquir Immune Defic Syndr*. 2007;46(3):332-337. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17786126>.
25. Alcantara KC, Pereira GA, Albuquerque M, Stefani MM. Seroreversion in children born to HIV-positive and AIDS mothers from Central West Brazil. *Trans R Soc Trop Med Hyg*. 2009;103(6):620-626. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19339030>.

26. Sohn AH, Thanh TC, Thinh le Q, et al. Failure of human immunodeficiency virus enzyme immunoassay to rule out infection among polymerase chain reaction-negative Vietnamese infants at 12 months of age. *Pediatr Infect Dis J*. 2009;28(4):273-276. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19289981>.
27. Kline NE, Schwarzwald H, Kline MW. False negative DNA polymerase chain reaction in an infant with subtype C human immunodeficiency virus 1 infection. *Pediatr Infect Dis J*. 2002;21(9):885-886. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12380591>.
28. Zaman MM, Recco RA, Haag R. Infection with non-B subtype HIV type 1 complicates management of established infection in adult patients and diagnosis of infection in newborn infants. *Clin Infect Dis*. 2002;34(3):417-418. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11774090>.
29. Obaro SK, Losikoff P, Harwell J, Pugatch D. Failure of serial human immunodeficiency virus type 1 DNA polymerase chain reactions to identify human immunodeficiency virus type 1 clade A/G. *Pediatr Infect Dis J*. 2005;24(2):183-184. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15702052>.
30. Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission. Recommendations for the use of antiretroviral drugs in pregnant women with HIV infection and interventions to reduce perinatal HIV transmission in the United States. 2018. Available at <http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf>.
31. Committee on Pediatric AIDS. Infant feeding and transmission of human immunodeficiency virus in the United States. *Pediatrics*. 2013;131(2):391-396. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23359577>.
32. King CC, Kourtis AP, Persaud D, et al. Delayed HIV detection among infants exposed to postnatal antiretroviral prophylaxis during breastfeeding. *AIDS*. 2015;29(15):1953-1961. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26153671>.
33. Centers for Disease Control and Prevention. Premastication of food by caregivers of HIV-exposed children—nine U.S. sites, 2009-2010. *MMWR Morb Mortal Wkly Rep*. 2011;60(9):273-275. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21389930>.
34. Gaur AH, Freimanis-Hance L, Dominguez K, et al. Knowledge and practice of prechewing/prewarming food by HIV-infected women. *Pediatrics*. 2011;127(5):e1206-1211. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21482608>.
35. Hafeez S, Salami O, Alvarado M, Maldonado M, Purswani M, Haggmann S. Infant feeding practice of premastication: an anonymous survey among human immunodeficiency virus-infected mothers. *Arch Pediatr Adolesc Med*. 2011;165(1):92-93. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21199989>.
36. Maritz ER, Kidd M, Cotton MF. Premasticating food for weaning African infants: a possible vehicle for transmission of HIV. *Pediatrics*. 2011;128(3):e579-590. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21873699>.
37. Ivy W, 3rd, Dominguez KL, Rakhmanina NY, et al. Premastication as a route of pediatric HIV transmission: case-control and cross-sectional investigations. *J Acquir Immune Defic Syndr*. 2012;59(2):207-212. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22027873>.
38. Gaur AH, Cohen RA, Read JS, et al. Prechewing and prewarming food for HIV-exposed children: a prospective cohort experience from Latin America. *AIDS Patient Care STDS*. 2013;27(3):142-145. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23477456>.
39. Centers for Disease Control and Prevention. HIV transmission. 2018. Available at: <https://www.cdc.gov/hiv/basics/transmission.html>.
40. Alexander TS. Human immunodeficiency virus diagnostic testing: 30 years of evolution. *Clin Vaccine Immunol*. 2016;23(4):249-253. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26936099>.
41. Ly TD, Plantier JC, Leballais L, Gonzalo S, Lemee V, Laperche S. The variable sensitivity of HIV Ag/Ab combination assays in the detection of p24Ag according to genotype could compromise the diagnosis of early HIV infection. *J Clin Virol*. 2012;55(2):121-127. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22795598>.
42. Centers for Disease Control and Prevention. HIV-2 infection surveillance—United States, 1987–2009. *MMWR Morb Mortal Wkly Rep*. 2011;60(29):985-988. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21796096>.
43. Shanmugam V, Switzer WM, Nkengasong JN, et al. Lower HIV-2 plasma viral loads may explain differences between the natural histories of HIV-1 and HIV-2 infections. *J Acquir Immune Defic Syndr*. 2000;24(3):257-263. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10969350>.
44. Damond F, Benard A, Balotta C, et al. An international collaboration to standardize HIV-2 viral load assays: results from the 2009 ACHI(E)V(2E) quality control study. *J Clin Microbiol*. 2011;49(10):3491-3497. Available at: <http://>

[www.ncbi.nlm.nih.gov/pubmed/21813718](http://www.ncbi.nlm.nih.gov/pubmed/21813718).

45. Lilian RR, Bhowan K, Sherman GG. Early diagnosis of human immunodeficiency virus-1 infection in infants with the NucliSens EasyQ assay on dried blood spots. *J Clin Virol: the official publication of the Pan American Society for Clinical Virology*. 2010;48(1):40-43. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/20211580>.
46. Patel JA, Anderson EJ, Dong J. False positive ultrasensitive HIV bDNA viral load results in diagnosis of perinatal HIV-infection in the era of low transmission. *Laboratory Medicine*. 2009;40(10):611-614. Available at: <http://labmed.oxfordjournals.org/content/40/10/611>.
47. American Academy of Pediatrics Committee on Pediatric AIDS. HIV testing and prophylaxis to prevent mother-to-child transmission in the United States. *Pediatrics*. 2008;122(5):1127-1134. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18977995>.
48. Saitoh A, Hsia K, Fenton T, et al. Persistence of human immunodeficiency virus (HIV) type 1 DNA in peripheral blood despite prolonged suppression of plasma HIV-1 RNA in children. *J Infect Dis*. 2002;185(10):1409-1416. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11992275>.
49. Mazanderani AH, Moyo F, Kufa T, Sherman GG. Brief report: declining baseline viremia and escalating discordant HIV-1 confirmatory results within South Africa's early infant diagnosis program, 2010-2016. *J Acquir Immune Defic Syndr*. 2018;77(2):212-216. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/29084045>.
50. Food and Drug Administration. APTIMA HIV-1 RNA qualitative assay. 2006. Available at: <http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm149922.htm>.
51. Pierce VM, Neide B, Hodinka RL. Evaluation of the gen-probe aptima HIV-1 RNA qualitative assay as an alternative to Western blot analysis for confirmation of HIV infection. *J Clin Microbiol*. 2011;49(4):1642-1645. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21346052>.
52. Fiscus SA, McMillion T, Nelson JA, Miller WC. Validation of the gen-probe aptima qualitative HIV-1 RNA assay for diagnosis of human immunodeficiency virus infection in infants. *J Clin Microbiol*. 2013;51(12):4137-4140. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24088864>.
53. Nelson JA, Hawkins JT, Schanz M, et al. Comparison of the gen-probe aptima HIV-1 and abbot HIV-1 qualitative assays with the roche amplicor HIV-1 DNA assay for early infant diagnosis using dried blood spots. *J Clin Virol*. 2014;60(4):418-421. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24929752>.
54. Puthankit T, Rojanwivat T. Delayed HIV DNA PCR detection among infants received combination ART prophylaxis. Presented at: Conference on Retroviruses and Opportunistic Infections. 2017. Seattle, WA.
55. Veldsman KA, Maritz J, Isaacs S, et al. Rapid decline of HIV-1 DNA and RNA in infants starting very early antiretroviral therapy may pose a diagnostic challenge. *AIDS*. 2018;32(5):629-634. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/29334551>.
56. Maritz J, Maharaj JN, Cotton MF, Preiser W. Interpretation of indeterminate HIV-1 PCR results are influenced by changing vertical transmission prevention regimens. *J Clin Virol*. 2017;95:86-89. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/28898704>.
57. Technau KG, Mazanderani AH, Kuhn L, et al. Prevalence and outcomes of HIV-1 diagnostic challenges during universal birth testing - an urban South African observational cohort. *J Int AIDS Soc*. 2017;20(Suppl 6):21761. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/28872276>.
58. Templer SP, Seiverth B, Baum P, Stevens W, Seguin-Devaux C, Carmona S. Improved sensitivity of a dual-target HIV-1 qualitative test for plasma and dried blood spots. *J Clin Microbiol*. 2016;54(7):1877-1882. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/27194686>.
59. Mossoro-Kpinde CD, Jenabian MA, Gody JC, et al. Evaluation of the upgraded version 2.0 of the Roche COBAS((R)) AmpliPrep/COBAS((R)) TaqMan HIV-1 qualitative assay in Central African Children. *Open AIDS J*. 2016;10:158-163. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/27857825>.
60. Pyne MT, Hackett J, Jr., Holzmayer V, Hillyard DR. Large-scale analysis of the prevalence and geographic distribution of HIV-1 non-B variants in the United States. *J Clin Microbiol*. 2013;51(8):2662-2669. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23761148>.
61. Nesheim SR, Linley L, Gray KM, et al. Country of Birth of Children With Diagnosed HIV Infection in the United States, 2008-2014. *J Acquir Immune Defic Syndr*. 2018;77(1):23-30. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/29040167>.

62. Karchava M, Pulver W, Smith L, et al. Prevalence of drug-resistance mutations and non-subtype B strains among HIV-infected infants from New York State. *J Acquir Immune Defic Syndr*. 2006;42(5):614-619. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16868498>.
63. Rogo T, DeLong AK, Chan P, Kantor R. Antiretroviral treatment failure, drug resistance, and subtype diversity in the only pediatric HIV clinic in Rhode Island. *Clin Infect Dis*. 2015;60(9):1426-1435. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25637585>.
64. Chan PA, Reitsma MB, DeLong A, et al. Phylogenetic and geospatial evaluation of HIV-1 subtype diversity at the largest HIV center in Rhode Island. *Infect Genet Evol*. 2014;28:358-366. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24721515>.
65. Germer JJ, Wu P, Soderberg JD, Mandrekar JN, Yao JD. HIV-1 subtype diversity among clinical specimens submitted for routine antiviral drug resistance testing in the United States. *Diagn Microbiol Infect Dis*. 2015;83(3):257-260. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26302855>.
66. Bush S, Tebit DM. HIV-1 group O origin, evolution, pathogenesis, and treatment: unraveling the complexity of an outlier 25 years later. *AIDS Rev*. 2015;17(3):147-158. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26450803>.
67. Auwanit W, Isarangkura-Na-Ayuthaya P, Kasornpikul D, Ikuta K, Sawanpanyalert P, Kameoka M. Detection of drug resistance-associated and background mutations in human immunodeficiency virus type 1 CRF01\_AE protease and reverse transcriptase derived from drug treatment-naïve patients residing in central Thailand. *AIDS Res Hum Retroviruses*. 2009;25(6):625-631. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19500016>.
68. Deshpande A, Jauvin V, Pinson P, Jeannot AC, Fleury HJ. Phylogenetic analysis of HIV-1 reverse transcriptase sequences from 382 patients recruited in JJ Hospital of Mumbai, India, between 2002 and 2008. *AIDS Res Hum Retroviruses*. 2009;25(6):633-635. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19534630>.
69. Chaix ML, Seng R, Frange P, et al. Increasing HIV-1 non-B subtype primary infections in patients in France and effect of HIV subtypes on virological and immunological responses to combined antiretroviral therapy. *Clin Infect Dis*. 2013;56(6):880-887. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23223603>.
70. Hemelaar J, Gouws E, Ghys PD, Osmanov S, WHO-UNAIDS Network for HIV Isolation Characterisation. Global trends in molecular epidemiology of HIV-1 during 2000–2007. *AIDS*. 2011;25(5):679-689. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21297424>.
71. Dauwe K, Mortier V, Schauvliege M, et al. Characteristics and spread to the native population of HIV-1 non-B subtypes in two European countries with high migration rate. *BMC Infect Dis*. 2015;15:524. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26572861>.
72. Church D, Gregson D, Lloyd T, et al. Comparison of the RealTime HIV-1, COBAS TaqMan 48 v1.0, Easy Q v1.2, and Versant v3.0 assays for determination of HIV-1 viral loads in a cohort of Canadian patients with diverse HIV subtype infections. *J Clin Microbiol*. 2011;49(1):118-124. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21084515>.
73. Cobb BR, Vaks JE, Do T, Vilchez RA. Evolution in the sensitivity of quantitative HIV-1 viral load tests. *J Clin Virol*. 2011;52 Suppl 1:S77-82. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22036041>.
74. Katsoulidou A, Rokka C, Issaris C, et al. Comparative evaluation of the performance of the abbot realtime HIV-1 assay for measurement of HIV-1 plasma viral load on genetically diverse samples from Greece. *Virol J*. 2011;8:10. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21219667>.
75. Gueudin M, Leoz M, Lemee V, et al. A new real-time quantitative PCR for diagnosis and monitoring of HIV-1 group O infection. *J Clin Microbiol*. 2012;50(3):831-836. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22170927>.
76. Xu S, Song A, Nie J, et al. Comparison between the automated Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 assay and its version 1 and Nuclisens HIV-1 EasyQ version 2.0 assays when measuring diverse HIV-1 genotypes in China. *J Clin Virol*. 2012;53(1):33-37. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22051503>.
77. Muenchhoff M, Madurai S, Hempenstall AJ, et al. Evaluation of the NucliSens EasyQ v2.0 assay in comparison with the Roche Amplicor v1.5 and the Roche CAP/CTM HIV-1 Test v2.0 in quantification of C-clade HIV-1 in plasma. *PLoS One*. 2014;9(8):e103983. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25157919>.
78. Torian LV, Eavey JJ, Punsalang AP, et al. HIV type 2 in New York City, 2000-2008. *Clin Infect Dis*. 2010;51(11):1334-1342. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21039219>.
79. Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. *Clin Infect Dis*. 2011;52(6):780-787. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21367732>.
80. Prince PD, Matser A, van Tienen C, Whittle HC, Schim van der Loeff MF. Mortality rates in people dually infected with

HIV-1/2 and those infected with either HIV-1 or HIV-2: a systematic review and meta-analysis. *AIDS*. 2014;28(4):549-558. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/23921613>.

81. Barin F, Cazein F, Lot F, et al. Prevalence of HIV-2 and HIV-1 group O infections among new HIV diagnoses in France: 2003-2006. *AIDS*. 2007;21(17):2351-2353. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18090288>.
82. Thiebaut R, Matheron S, Taieb A, et al. Long-term nonprogressors and elite controllers in the ANRS CO5 HIV-2 cohort. *AIDS*. 2011;25(6):865-867. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21358376>.
83. Menendez-Arias L, Alvarez M. Antiretroviral therapy and drug resistance in human immunodeficiency virus type 2 infection. *Antiviral Res*. 2014;102:70-86. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24345729>.
84. Tchounga BK, Inwoley A, Coffie PA, et al. Re-testing and misclassification of HIV-2 and HIV-1&2 dually reactive patients among the HIV-2 cohort of the West African database to evaluate AIDS collaboration. *J Int AIDS Soc*. 2014;17:19064. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25128907>.
85. Balestre E, Ekouevi DK, Tchounga B, et al. Immunologic response in treatment-naive HIV-2-infected patients: the IeDEA West Africa cohort. *J Int AIDS Soc*. 2016;19(1):20044. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26861115>.
86. Linley L, Ethridge SF, Oraka E, et al. Evaluation of supplemental testing with the multispot HIV-1/HIV-2 rapid test and APTIMA HIV-1 RNA qualitative assay to resolve specimens with indeterminate or negative HIV-1 Western blots. *J Clin Virol*. 2013;58 Suppl 1:e108-112. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/24342469>.
87. Burgard M, Jasseron C, Matheron S, et al. Mother-to-child transmission of HIV-2 infection from 1986 to 2007 in the ANRS French Perinatal Cohort EPF-CO1. *Clin Infect Dis*. 2010;51(7):833-843. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20804413>.