Diagnosis of HIV Infection in Infants and Children

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Panel's Recommendations

- Virologic assays (HIV RNA or 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 and HIV antigen/antibody tests should not be used (AII).
- Plasma HIV RNA or cell-associated HIV DNA NATs are generally equally recommended (AII). However, the results of plasma HIV RNA NAT or plasma HIV RNA/DNA NAT can be affected by maternal antiretroviral therapy (ART), or by antiretroviral (ARV) drugs administered to the infant as prophylaxis or presumptive HIV therapy.
- 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 (see Table 3 below) 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 who are at high risk of perinatal HIV infection, additional virologic diagnostic testing is recommended at birth (AII) and at 2 to 6 weeks after ARV drugs are discontinued (BII).
- For infants with perinatal HIV exposure who are being breastfed, virologic diagnostic testing is recommended at birth, 14 to 21 days, 1 to 2 months, and 4 to 6 months of age (AII). An additional virologic test should be performed between the 1-to-2-month and 4-to-6-month time points if the gap between tests is greater than 3 months. See Infant Feeding for Individuals With HIV in the United States.
  - Virologic diagnostic testing should be performed every 3 months during breastfeeding (BII);
  - After cessation of breastfeeding, irrespective of when breastfeeding ends, virologic diagnostic testing should be performed at 4 to 6 weeks, 3 months, and 6 months after cessation (BII).
- A positive virologic test should be confirmed as soon as possible by a repeat virologic test (AII).
- Definitive exclusion of HIV infection in non-breastfed infants is based on two or more negative virologic tests, with one negative test obtained at age ≥1 month (and at least 2 -6 weeks after discontinuation of multi-drug ARV prophylaxis/presumptive HIV therapy) and one at age ≥4 months, or two negative HIV antibody tests from separate specimens that were obtained at age ≥6 months (AII).
- Additional HIV testing (e.g., HIV RNA or HIV DNA NAT, HIV antibody, HIV antigen/antibody) is not needed routinely for non-breastfed infants who meet the criteria for definitive exclusion of HIV and who have had no known or suspected HIV exposure after birth.
- Infants with potential HIV exposure after birth (e.g., from maternal HIV diagnosis during breastfeeding, premasticated feeding, sexual abuse, contaminated blood products, percutaneous exposure) who are aged <18 months require additional testing using HIV RNA/DNA NAT assays to establish their HIV status. Infants aged ≥18 months who have these potential exposures require HIV antigen/antibody testing.
- Age-appropriate HIV testing also is recommended for infants and children with signs and/or symptoms of HIV, even in the absence of documented or suspected HIV exposure.

- For children aged >24 months and for children aged 18 to ≤ 24 months with non-perinatal HIV exposure only, HIV antibody (or HIV antigen/antibody) tests are recommended for diagnostic testing (AII).
- When acute HIV infection is suspected, additional testing with an HIV NAT may be necessary to diagnose HIV infection (AII).

Note: The National Clinician Consultation Center- Perinatal HIV/AIDS provides consultations on issues related to the management of perinatal HIV infection, including diagnostic testing (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† 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† 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† 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† from one or more similar nonrandomized trials or cohort studies with clinical outcome data; III = Expert opinion*

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

Diagnosis of HIV in Infants and Children

HIV can be diagnosed definitively by virologic testing in most non-breastfed infants with perinatal HIV exposure by age 1 to 2 months and in almost all perinatally-exposed infants by age 4 to 6 months. Antibody tests, including 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.[1,2] 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. Plasma HIV RNA and HIV DNA NATs are generally equally recommended. However, both tests can be affected by maternal antiretroviral therapy (ART) through transplacental transfer of antiretroviral (ARV) drugs from the pregnant person to the fetus or by ARV drugs administered to the infant as prophylaxis or presumptive HIV therapy. In general, qualitative HIV proviral DNA PCR assays from whole blood detecting cell-associated virus are less affected by ARVs.

A positive HIV test result should be confirmed as soon as possible by repeat virologic testing, because false-positive results can occur with both RNA and DNA assays.[3] For additional information on the diagnosis of Group M non-subtype B infections, Group O HIV-1 infections, and HIV-2 infections, see the relevant sections below and the HIV Sequence Database. Newer real-time HIV RNA PCR assays and the qualitative diagnostic RNA assay are better at detecting non-subtype B HIV infection and Group O strains than older RNA assays.[4-9] (See Clinical and Laboratory Monitoring of Pediatric HIV Infection.) One example is the COBAS® AmpliPrep/COBAS® TaqMan-HIV-1 qualitative test (a dual-target DNA/RNA, sometimes called total nucleic acid or TNA test), which also can identify non-subtype B and Group O infections.[10-12]

Antigen/antibody combination immunoassays that detect HIV-1/2 antibodies and HIV-1 p24 antigen **are not recommended** for diagnosis of HIV infection in infants. In the first months of life, the antigen component of antigen/antibody tests is less sensitive than an HIV NAT, and antibody tests should not be used for HIV diagnosis in infants and children <18 months of age.[13-15] 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 remain HIV antibody–positive should be based on a NAT (see Diagnostic Testing in Children With Perinatal HIV Exposure in Special Situations below). Diagnosis in children aged >24 months relies primarily on HIV antibody and antigen/antibody tests (see Diagnostic Testing in Children with Non-Perinatal HIV Exposure or Children With Perinatal HIV Exposure Aged >24 Months below).[1]

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 in Timing of Diagnostic Testing in Infants with Perinatal HIV Exposure below,[16] and receive ARV prophylaxis or presumptive HIV therapy as soon as possible. For ARV management of newborns who have been exposed to HIV and newborns with HIV infection (including those who do not yet have confirmed infection), see Antiretroviral Management of Newborns With Perinatal HIV Exposure or HIV Infection.

Timing of Diagnostic Testing in Infants With Perinatal HIV Exposure

Confirmation of HIV infection is based on the results of positive virologic tests from two separate blood samples in infants and children younger than 18 months. Table 3 below summarizes the timing of recommended virologic diagnostic testing for infants based on HIV transmission risk. Infants at high risk of perinatal HIV transmission, may require additional virologic testing, given the increased risk of infection and concern that ARV prophylaxis, particularly combination ARV prophylaxis or presumptive HIV therapy, may reduce the sensitivity of diagnostic testing. The risk of transmission is determined based on whether a mother is receiving ART and virally suppressed.

HIV infection can be **presumptively** excluded in non-breastfed infants with two or more negative virologic tests (one at age ≥2 weeks and one at age ≥4 weeks) or one negative virologic test at age ≥8 weeks at least 2 weeks after discontinuing multi-drug ARV prophylaxis/presumptive therapy, or one negative HIV antibody test at age ≥6 months.[1,16]

Definitive exclusion of HIV infection in non-breastfed infants is based on two or more negative virologic tests, with one negative test obtained at age ≥1 month (and at least 2 -6 weeks after discontinuation of multi-drug ARV prophylaxis/presumptive therapy) and one at age ≥4 months, or two negative HIV antibody tests from separate specimens that were obtained at age ≥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 cell count/percentage) or clinical evidence of HIV infection and must not be breastfeeding. No additional HIV testing of any kind (e.g., NAT, antibody, antigen/antibody) is needed routinely for non-breastfed infants who meet the criteria for definitive exclusion of HIV and who have had no known or suspected HIV exposure after birth.

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 infection.[17] Thus, PCP prophylaxis can be avoided or discontinued if HIV infection is presumptively excluded (see Initial Postnatal Management of the Neonate Exposed to HIV and *Pneumocystis jirovecii* Pneumonia in the Pediatric Opportunistic Infection Guidelines).

Virologic Testing at Birth for Newborns at High Risk of Perinatal HIV Transmission

Virologic testing at birth should be considered for newborns who are at high risk of perinatal HIV transmission,[18-23] such as infants born to women with HIV who—

- Did not receive prenatal care;
- Received no antepartum ARVs or only intrapartum ARV drugs;
- Initiated ART late in pregnancy (during the late second or third trimester);
- Received a diagnosis of acute HIV infection during pregnancy or in labor; and/or
- Had detectable HIV viral loads (≥50 copies/mL) close to the time of delivery, including those who received ART and did not have sustained viral suppression.

All infants at high risk of perinatal HIV transmission should have specimens obtained for HIV testing at birth before initiating an ARV drug regimen; however, presumptive HIV therapy should not be delayed.

Blood samples from the umbilical cord should not be used for diagnostic evaluation because of the potential for contamination with maternal blood.

Virologic testing at birth is critical for early HIV diagnosis (see When to Initiate Therapy in Antiretroviral-Naive Children in the Pediatric Antiretroviral Guidelines). Infants who have a positive virologic test result at or before age 48 hours are considered to have early (intrauterine) infection, whereas non-breastfed infants who have a negative virologic test result during the first week of life and subsequently have positive test results are considered to have late (intrapartum) infection.[18,19,24] Testing at birth also might be considered in instances when there are concerns that a newborn at low risk of perinatal HIV transmission may be lost to follow-up without testing.

Virologic Testing at Age 14 to 21 Days

The diagnostic sensitivity of virologic testing increases rapidly by age 2 weeks,[16] and early identification of infection permits transition from presumptive HIV therapy to treatment doses of ART (see When to Initiate Therapy in Antiretroviral-Naive Children in the Pediatric Antiretroviral Guidelines).

Virologic Testing at Age 1 to 3 Months

Testing performed at age 1 to 3 months is intended to maximize the likelihood of detecting HIV infection in perinatally exposed infants. In the HIV Prevention Trials Network 040 study, 93 of 140 infants with HIV (66.4%) were identified at birth. Infants who received 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.[25] For infants at high risk of perinatal HIV transmission, the Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV and the Panel on Treatment of HIV During Pregnancy and Interventions to Reduce Perinatal Transmission suggests performing an additional virologic test 2 to 6 weeks after ARV drugs are discontinued (i.e., at age 8–12 weeks), given the increased risk of infection and concern that ARV prophylaxis, particularly combination ARV prophylaxis or presumptive HIV therapy, may reduce the sensitivity of diagnostic testing.[25,26]

In these situations, many experts recommend one test at age 4 to 6 weeks to allow prompt diagnosis of HIV in infants with an additional test at 8 to 12 weeks of life (i.e., 2–6 weeks after cessation of prophylaxis or presumptive HIV therapy) to capture additional cases (see Table 3 below). For infants at low risk of HIV 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 (the first at age ≥14 days and the other at age ≥4 weeks), or one negative test result at age ≥8 weeks at least 2 weeks after discontinuing multi-drug ARV prophylaxis/presumptive therapy, can be viewed as presumptively HIV uninfected, assuming the child has not had a positive prior virologic test result or clinical evidence indicative of HIV infection and is not breastfed.

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, who have had no positive virologic tests, who have no clinical evidence of HIV infection, and who are not breastfed should be retested at age 4 to 6 months for definitive exclusion of HIV infection.

Virologic Testing for Infants With Perinatal HIV Exposure Who Are Being Breastfed.

Some individuals with HIV may choose to breastfeed their infants (see Infant Feeding for Individuals With HIV in the United States). Infants with perinatal HIV exposure who are being breastfed should have virologic diagnostic testing at the standard time points: 14 to 21 days, 1 to 2 months, and 4 to 6 months (see Table 3 below). In addition, a virologic test at birth is recommended. In some cases, an additional virologic test should be performed between the 1-to-2 month and 4-to-6-month time points if the gap between tests is greater than 3 months. Infants continuing to be breastfed beyond 6 months of age should have virologic diagnostic testing every 3 months during breastfeeding. At cessation of breastfeeding, virologic diagnostic testing should be performed at 4 to 6 weeks, 3 months, and 6 months after breastfeeding has ended, regardless of the age of the child when breastfeeding is discontinued. If an infant's virologic test result is positive, a repeat test should be performed as soon as possible and ART should be initiated.

Maternal viral load monitoring is recommended every 1 to 2 months during breastfeeding. Additional infant virologic testing, including immediate NAT testing, is indicated if maternal viral load becomes detectable during breastfeeding. If the mother has a detectable viral load and continues breastfeeding, some Panel members would recommend monthly virologic testing of the infant as an approach to early detection of HIV infection during ongoing exposure. After cessation of breastfeeding, virologic testing should be performed at least 2 weeks after cessation of presumptive HIV therapy or ARV prophylaxis (see Antiretroviral Management of Newborns of Newborns With Perinatal HIV Exposure or HIV Infection) and at 4 to 6 weeks, 3 months, and 6 months after cessation of breastfeeding. Consultation with an expert and/or the Perinatal HIV Hotline (888-448-8765) is recommended in these situations and for questions about HIV diagnostic testing for infants with perinatal HIV exposure who are being breastfed. For additional information see Infant Feeding for Individuals With HIV in the United States.

Table 3. Recommended Virologic Testing Schedules for Infants Who Were Exposed to HIV According to Risk of Perinatal HIV Acquisition at and After Birth[a]

Infants at High Risk	
Criteria for Infants at High Risk	**Age at HIV NAT[b] Testing for Infants at High Risk**
Infants born to mothers with HIV who— • Did not receive prenatal care; • Received no antepartum ARVs or only intrapartum ARV drugs; • Initiated ART late in pregnancy (during the late second or third trimester); • Received a diagnosis of acute HIV infection during pregnancy or in labor; and/or • Had detectable HIV viral loads (≥50 copies/mL) close to the time of delivery, including those who received ART but did not achieve sustained viral suppression	Birth 14–21 days 1–2 months 2–3 months[c] 4–6 months All infants at high risk of perinatal HIV transmission should have specimens obtained for HIV testing at birth before initiating an ARV drug regimen; however, presumptive HIV therapy should not be delayed. If an infant's NAT test result is positive, a repeat test should be performed as soon as possible and ART should be initiated.
Infants at Low Risk	
Criteria for Infants at Low Risk	**Age at HIV NAT[b] Testing for Infants at Low Risk**
Infants born to mothers who— • Received ART during pregnancy; • Had sustained viral suppression (usually defined as <50 copies/mL); *and* • Were adherent to their ARV regimens	14–21 days 1–2 months[d] 4–6 months
Infants With Perinatal HIV Exposure Who Are Being Breastfed	
Age at HIV NAT[b] Testing for Infants With Perinatal HIV Exposure Who Are Being Breastfed	
Birth 14–21 days 1–2 months 2–4 months[e] 4–6 months If breastfeeding continues beyond 6 months of age, NAT testing should be performed every 3 months during breastfeeding. In addition to the standard time points after birth, NAT testing also should be performed at 4 to 6 weeks, 3 months, and 6 months after cessation of breastfeeding, regardless of the age at when breastfeeding ends. Consultation with an expert is recommended to determine additional testing time points that may be needed for infants with risk factors for HIV acquisition at birth who are being breastfed. Prompt NAT testing of the infant is indicated if maternal viral load becomes detectable during breastfeeding.	

If the mother has a detectable viral load and continues breastfeeding, some Panel members would recommend monthly virologic testing of the infant as an approach to early detection of HIV infection during ongoing exposure.

See Infant Feeding for Individuals With HIV in the United States. Consultation with an expert and/or the Perinatal HIV Hotline (888-448-8765) is recommended for questions about HIV diagnostic testing for infants with perinatal HIV exposure who are being breastfed.

[a] This table summarizes standard time points for HIV virologic diagnostic testing of infants according to risk of perinatal acquisition.

[b] HIV RNA or HIV DNA NATs that directly detect HIV.

[c] For high-risk infants, virologic diagnostic testing is recommended at birth. For infants treated with multiple ARVs in the first 2 to 4 weeks of life, additional virologic testing is recommended 2 to 6 weeks after ARV drugs are discontinued (i.e., at 8–12 weeks of life).

[d] For low-risk infants, testing may be timed to occur at least 2 weeks after cessation of ARV prophylaxis.

[e] An additional virologic test should be performed at age 2 to 4 months if the gap between the tests at ages 1 to 2 months and 4 to 6 months is greater than 3 months.

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

Antibody Testing at Age 6 Months and Older

Two or more negative results of HIV antibody tests that were performed in non-breastfed infants at age ≥6 months also can be used to exclude HIV infection definitively in children with no clinical or virologic laboratory-documented evidence of HIV infection.[27,28]

Antibody Testing at Age 18 to 24 Months to Document Seroreversion

In general, no additional HIV testing of any kind (e.g., NAT, antibody, antigen/antibody) is needed routinely for non-breastfed infants who meet the criteria for definitive exclusion of HIV and who have had no known or suspected HIV exposure after birth. However, infants with potential HIV exposure after birth (e.g., maternal diagnosis during breastfeeding, premasticated feeding, sexual abuse, contaminated blood products, percutaneous exposure) who are aged <18 months require additional testing using HIV RNA/DNA NAT assays to establish their HIV status. Infants aged ≥18 months who have these potential exposures require HIV antigen/antibody testing.

In a study from 2012, the median age at seroreversion was 13.9 months.[29] Although the majority of infants who do not have HIV will serorevert by age 15 months to 18 months, late seroreversion after 18 months has been reported (see Diagnostic Testing in Children With Perinatal HIV Exposure in Special Situations below). Factors that might influence the time to seroreversion include maternal disease stage and assay sensitivity.[29-32]

Diagnostic Testing in Children With Perinatal HIV Exposure in Special Situations

Breastfeeding at the Time of New Maternal HIV Diagnosis

Infants may be exposed to HIV through breastfeeding if the mother develops acute or primary HIV infection or when pre-existing maternal HIV infection was not diagnosed during pregnancy or immediately postpartum.[33] People who are diagnosed with HIV during breastfeeding should be counseled to discontinue breastfeeding immediately to reduce the risk of postnatal transmission to the

infant, see Situations to Consider Stopping or Modifying Breastfeeding in Infant Feeding for individuals With HIV in the United States. In these situations, infant virologic diagnostic testing by HIV DNA or RNA PCR is recommended as soon as possible with the schedule for subsequent tests affected by the infant's age when breastfeeding is discontinued. Virologic tests should be conducted at ages 14 to 21 days, 1 to 2 months, and 4 to 6 months. A virologic test also should be performed at least 2 weeks after cessation of ARV prophylaxis or presumptive HIV therapy (see Antiretroviral Management of Newborns With Perinatal HIV Exposure or HIV Infection) and at 4 to 6 weeks, 3 months, and 6 months after cessation of breastfeeding. Duplicate tests are not needed if some of these time points overlap. For additional information, consult the Perinatal HIV Hotline (1-888-448-8765).

Late Seroreversion (Aged ≤24 Months)

Non-breastfed children with perinatal HIV exposure, no other HIV transmission risk factor, and no clinical or virologic laboratory evidence of HIV infection may have residual maternal HIV antibodies up to age 24 months. These children are called late seroreverters.[29-32] In one study, 14% of children with HIV exposure who did not have HIV infection seroreverted after age 18 months.[29] More recent data from Thailand associated late seroreversion with the antenatal use of protease inhibitors in pregnant women with HIV. In this study, late seroreversion also was associated with the use of fourth-generation combination antigen/antibody immunoassays.[34] These children may have had positive immunoassay results, but supplemental antibody test results indicated indeterminate HIV status. In such cases, repeat antibody testing at a later date confirmed seroreversion. Due to the possibility of residual maternal HIV antibodies, virologic testing is necessary to definitively exclude or confirm HIV infection in children with perinatal HIV exposure who have a positive HIV antibody (or antigen/antibody) test at age 18 months to 24 months. Virologic testing will distinguish late-seroreverting children who do not have HIV but have residual antibodies from children who have antibodies due to underlying HIV infection. Age-appropriate HIV testing also is recommended for infants and children with signs and/or symptoms of HIV, even in the absence of documented or suspected HIV exposure.

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 test results. This occurs in children who acquire HIV through an additional risk factor after completion of testing (see Diagnostic Testing in Children With Non-Perinatal HIV Exposure or Children with Perinatal HIV Exposure Aged >24 Months below).

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.[35-37] The diagnostic approach in these situations is discussed below in Virologic Assays to Diagnose Group M Non-Subtype B and Group O HIV-1 Infections and in Virologic Assays to Diagnose HIV-2 Infections.

Diagnostic Testing in Children With Non-Perinatal HIV Exposure or Children With Perinatal HIV Exposure Aged >24 Months

Premastication

Receipt of solid food that has been premasticated or prewarmed (in the mouth) by a caregiver with HIV is associated with risk of HIV transmission.[38-43] If this occurs in children with perinatal HIV exposure aged ≤24 months with prior negative virologic tests, it will be necessary for such children to undergo virologic diagnostic testing because they may have residual maternal HIV antibodies (see Diagnostic Testing in Children With Perinatal HIV Exposure in Special Situations above).

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. It may be difficult to obtain a history of HIV exposure. Therefore, age-appropriate HIV testing is recommended for infants and children with signs and/or symptoms of HIV infection, even in the absence of documented or suspected perinatal or non-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 reported cases of HIV transmission from these activities have been documented.[44]

Diagnostic Testing

Diagnosis of HIV-1 infection in infants and children with non-perinatal HIV exposure only or in children with perinatal HIV exposure who are aged >24 months relies primarily on HIV antibody and antigen/antibody tests.[1,45] U.S. Food and Drug Administration (FDA)-approved diagnostic tests include the following:

- Antigen/antibody combination immunoassays, which detect HIV-1/2 antibodies and HIV-1 p24 antigen. These tests are 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.[46] Recent data suggest that the use of immunoassays and rapid diagnostic test combination algorithms that have limited HIV antigen breadth may not be adequate for diagnosis of HIV infection in children following early treatment with ART.[47]
- HIV-1/HIV-2 antibody differentiation immunoassay, which differentiates HIV-1 antibodies from HIV-2 antibodies. This immunoassay is recommended for supplemental testing.
- HIV-1 NAT. A NAT always is indicated as an additional test to diagnose acute HIV infection.

The diagnosis of HIV-2 in children with non-perinatal exposure only or in children with perinatal exposure aged >24 months relies on the 2014 Centers for Disease Control and Prevention (CDC)/Association of Public Health Laboratories laboratory testing guidelines. These guidelines recommend using an HIV-1/HIV-2 antibody differentiation immunoassay that distinguishes between HIV-1 and HIV-2 antibodies for supplemental testing. When used as a supplemental test, the results of the HIV-1 Western blot are more ambiguous than those of the HIV-1/HIV-2 antibody

differentiation immunoassay; >60% of individuals with HIV-2 are misclassified as having HIV-1 by the HIV-1 Western blot.[1,48] 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 by CDC if an HIV-1/HIV-2 antibody differentiation immunoassay is inconclusive. HIV-2 DNA PCR testing may be necessary for definitive diagnosis, although this assay is not commercially available.[49,50]

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 ages 1 month, 3 months, and 6 months and is comparable to the specificity of HIV DNA PCR.[26] Testing at birth will detect HIV RNA in infants who acquire HIV *in utero* and not in those who acquire HIV from exposure during delivery or immediately before delivery (i.e., during 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. These results are similar to the results of HIV DNA PCR for early diagnosis of HIV.[3,26,51]

The sensitivity of HIV RNA assays is affected by maternal antenatal ART or ARV drugs administered to the infant as prophylaxis or presumptive therapy.[52] In one study, the sensitivity of HIV RNA assays was 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 who were receiving multidrug prophylaxis. In contrast, the median HIV RNA levels were high by age 3 months in both groups after stopping prophylaxis.[26] 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 ARV regimens during those years.[53] Further studies are necessary to evaluate the sensitivity of HIV RNA assays during receipt of multidrug ARV prophylaxis or presumptive HIV therapy in infants whose mothers also received antenatal ART.

An HIV quantitative RNA assay can be used as a confirmatory test for infants who have an initial positive HIV DNA PCR test result. In addition to providing virologic confirmation of infection status, an HIV RNA measurement assesses baseline viral load. An HIV genotype can be performed on the same sample to 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 below).

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.[24,54-57]

HIV DNA PCR and Related Assays

HIV DNA PCR is a sensitive technique that is 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 detects only *in utero* HIV infection and not infection in those infants who acquire HIV during the intrapartum period. This percentage increases to >90% by age 2 weeks to 4 weeks and to 100% at ages 3 months and 6 months.[24,26,51]

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. One study noted that among 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.[25] Another study from Cape Town evaluated the sensitivity of HIV DNA assays within 8 days of life during and after initiating ART in infants with HIV. The infants had been exposed to a combination of maternal ART *in utero* and ARV drugs for prophylaxis and treatment. In seven infants who achieved 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 noted that one infant had undetectable HIV DNA after 6 days on treatment, another had undetectable HIV DNA after 3 months, and a third had undetectable HIV DNA after 4 months, suggesting that rapid decline of HIV-1 RNA and DNA may complicate definitive diagnosis.[58] More recent studies from the same authors suggest that ART initiation within the first week of life reduces persistence of long-lived infected cells and that delaying ART initiation is associated with slower decay of infected cells.[59] A data set of 38,043 infants from the Western Cape province of South Africa who were tested at a median age of 45 days showed that infants who received the World Health Organization Option B+ ARV regimen had fewer indeterminate DNA PCR results than infants who were receiving older ARV regimens.[60] Another group of South African investigators reported similar findings in a study of a cohort of 5,743 neonates from Johannesburg who were exposed to HIV.[61]

The AMPLICOR® HIV-1 DNA test has been used widely for diagnosis of HIV in infants born to mothers with HIV-1 infection since it was introduced in 1992. However, it is no longer commercially available in the United States. The sensitivity and specificity of noncommercial HIV-1 DNA tests that use individual laboratory reagents may differ from the sensitivity and specificity of an FDA-approved commercial test. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0 qualitative test (which detects both HIV-1 RNA and proviral DNA in plasma, whole blood, and dried blood spots) may be used for early HIV diagnosis in infants, but it is not approved by the FDA.[10,11,61] These considerations underscore the importance of testing with HIV NATs at 4 months—well after neonatal ARV prophylaxis or presumptive HIV therapy has stopped.

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 also are found in the United States.[62] Data from the CDC National HIV Surveillance System (NHSS) showed that the number of non-U.S.-born children with HIV has exceeded the number of U.S.-born children with HIV since 2011, with 65.5% of non-U.S.-born children with HIV born in sub-Saharan Africa and 14.3% in Eastern Europe.[63] In an evaluation of infants who 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.[64] Among a group of 40 children who visited a pediatric HIV clinic in

Rhode Island between 1991 and 2012, 14 (35%) acquired HIV with non-B HIV-1 subtypes. All 14 children were either born outside the United States or their parents were of foreign origin.[65] 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 participants with non-B subtypes formed transmission clusters, including individuals with perinatally acquired infection.[66] In an analysis of 3,895 HIV-1 sequences that were collected between July 2011 and June 2012 in the United States, 5.3% were determined to be non-B subtypes (including recombinant forms).

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.[67] Non-subtype B and Group O strains may be seen in countries with links to these geographical regions.[68-72] The geographical distribution of HIV groups is available at the HIV Sequence Database.

Real-time HIV RNA PCR assays and the qualitative diagnostic RNA assay are better at detecting non-subtype B HIV infection and the less-common Group O strains than older RNA assays[4-9] (see Clinical and Laboratory Monitoring of Pediatric HIV Infection). An example includes the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 qualitative test (a dual-target DNA/RNA test), which also can identify non-subtype B and Group O infections.[10,11]

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 that is 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 an HIV DNA PCR test and non-subtype B or Group O perinatal exposure is suspected. Two negative HIV antibody test results obtained at age ≥6 months provide further evidence to rule out HIV infection definitively. Clinicians should consult with an expert in pediatric HIV infection; state or local public health departments or CDC may be able to assist in obtaining referrals for diagnostic HIV testing.

Chimeric Antigen Receptor T-Cell and Lentiviral-Based Gene Therapy May Give Rise to False-Positive HIV NAT Results

Chimeric antigen receptor (CAR) T-cell immunotherapy is a major advancement in cancer therapeutics, including for pediatric B-cell acute lymphoblastic leukemia. Reprogramming of T cells is achieved by using gammaretroviral or lentiviral vectors. Recent reports indicate that these vectors may interfere with long terminal repeat genomes in HIV NAT results and, thus, produce false-positive results. As CAR T-cell therapy becomes more widely available for multiple indications, it will be important for clinicians to recognize that routine HIV-1 NAT results may give rise to false results. In addition, lentiviral vector–based gene therapy as treatment for severe combined immunodeficiency can give rise to false-positive HIV NAT results. Laboratories should, therefore, have appropriate alternate HIV-1 NAT resulting platforms made available for this emerging patient population.[73-77]

Virologic Assays to Diagnose HIV-2 Infections

HIV-2 infection is endemic in Angola; Mozambique; West African countries, including Benin, Burkina Faso, Cape Verde, the Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Mali, Mauritania, Niger, Nigeria, Sao Tome, Senegal, Sierra Leone, and Togo; and parts of India.[78-80]

HIV-2 infection also is well documented in France and Portugal, which have large numbers of immigrants from these regions.[81,82] HIV-1 and HIV-2 coinfection may occur, but this rarely is described 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 that were developed to suppress HIV-1.[83-85] (See HIV-2 Infection and Pregnancy.)

A mother should be suspected of having HIV-2 if her infection is linked to an area that is 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 and HIV-1 RNA viral loads that are at or below the limit of detection in the absence of treatment). The current recommendation is to use an HIV-1/HIV-2 antibody differentiation immunoassay for supplemental testing.[1] Between 2010 and 2017, an increase in the number of HIV-1/HIV-2 differentiation test results was reported to the CDC's NHSS. More than 99.9% of all HIV infections identified in the United States were categorized as HIV-1, and the number of HIV-2 diagnoses (mono-infection or dual-infection) remained extremely low (<0.03% of all HIV infections).[86]

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 known or suspected HIV-2 infection. 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.[49,50] Clinicians should consult with an expert in pediatric HIV infection when caring for infants with suspected or known exposure to HIV-2.[78,87]

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