Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection

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Diagnosis of HIV Infection in Infants and Children (Last updated November 1, 2012; last reviewed November 1, 2012)

Panel's Recommendations

- Virologic assays that directly detect HIV must be used to diagnose HIV infection in infants younger than 18 months **(AII)**.
- Virologic diagnostic testing in infants with known perinatal HIV exposure is recommended at ages 14 to 21 days, 1 to 2 months, and 4 to 6 months **(AII)**.
- Virologic diagnostic testing at birth should be considered for infants at high risk of HIV infection **(BIII)**.
- HIV DNA polymerase chain reaction and HIV RNA assays are recommended as preferred virologic assays **(AII)**.
- 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 ≥1 month of age and one at ≥4 months of age, or two negative HIV antibody tests from separate specimens obtained at ≥6 months of age **(AII)**.
- Some experts confirm the absence of HIV infection at 12 to 18 months of age in infants with prior negative virologic tests by performing an antibody test to document loss of maternal HIV antibodies **(BIII)**.
- HIV antibody assays alone can be used for diagnosis of HIV infection in children with perinatal exposure who are ≥18 months of age and in children with non-perinatal exposure (see text for exceptions) **(AII)**.

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 and adolescents but not studies limited to postpubertal adolescents

Diagnostic Testing in Infants with Perinatal HIV-1 (HIV) Exposure

HIV infection can be definitively diagnosed through use of virologic assays in most nonbreastfed HIV-exposed infants by 1 month of age and in virtually all infected infants by 4 months of age. Tests for antibodies to HIV, including newer rapid tests, do not establish the presence of HIV infection in infants because of transplacental transfer of maternal antibodies to HIV; therefore a virologic test should be used.[1, 2] A positive virologic test (that is, detection of HIV by DNA polymerase chain reaction (PCR) or RNA assays) indicates 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.

HIV culture is not used for routine HIV diagnostic testing, although it has a sensitivity similar to that of HIV DNA PCR.[3] It is more complex and expensive to perform than DNA PCR or RNA assays and may require 2 to 4 weeks for definitive results; it is generally not available outside of research laboratories. Use of the currently approved HIV p24 antigen assay is not recommended for infant diagnosis in the United States because the sensitivity and specificity of the assay in the first months of life are less than that of other HIV

virologic tests.[4, 5]

An infant who is found to have positive HIV antibody on screening but whose mother's HIV status is unknown (see Identification of Perinatal HIV Exposure), should be assumed to be HIV-exposed and undergo the HIV diagnostic testing described here.

HIV DNA PCR

HIV DNA PCR is a sensitive technique used to detect specific HIV viral DNA in peripheral blood mononuclear cells (PBMCs). The specificity of the HIV DNA PCR is 99.8% at birth and 100% at 1, 3, and 6 months. The sensitivity of the test performed at birth is 55% but increases to more than 90% by 2 to 4 weeks of age, and 100% at 3 months and 6 months of age.[6-8]

HIV RNA Assays

HIV quantitative RNA assays detect extracellular viral RNA in the plasma. Their specificity (for results ≥5,000 copies/mL) has been shown to be 100% at birth, 1, 3, and 6 months of age and is comparable to HIV DNA PCR.[8] HIV RNA levels <5,000 copies/mL may not be reproducible and should be repeated before they are interpreted as documenting HIV infection in an infant. The sensitivity of HIV RNA assays has been shown to be 25% to 58% during the first weeks of life, 89% at 1 month of age, and increases to 90% to 100% by 2 to 3 months of age.[6-11] HIV RNA assays are as sensitive as HIV DNA PCR for early diagnosis of HIV infection in HIV-exposed infants. An HIV RNA assay can be used as the confirmatory test for infants who have an initial positive HIV DNA PCR test. 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. HIV RNA assays may be more sensitive than HIV DNA PCR for detecting HIV non-subtype B (see HIV subtype section below). It is established that HIV DNA PCR remains positive even in individuals receiving highly active antiretroviral therapy (HAART).[12] However, RNA assays can be affected by maternal antenatal therapy with combination antiretroviral (ARV) drugs and/or infant ARV prophylaxis. Among a group of 47 infants who received zidovudine prophylaxis, HIV RNA levels were lower at 1 month of age compared with levels at 3 months of age (median of 5.1 vs. 5.6 logs) and among 9 infants who received combination ARV prophylaxis, the median was 2.5 logs at 1 month of age. However, prenatal and neonatal combination ARV regimens did not affect the sensitivity of the assay to detect the presence of HIV.[8]

The HIV qualitative RNA assay (APTIMA HIV-1 RNA Qualitative Assay) is an alternative diagnostic test that can be used for infant testing.[13-17]

Issues Related to Diagnosis of Non-Subtype B HIV-1 Infections

Although HIV-1 subtype B is the predominant viral subtype found in the United States, non-subtype B viruses predominate in some other parts of the world, such as subtype C in regions of Africa and India and subtype CRF01 in much of Southeast Asia.[18-20] Currently available HIV DNA PCR tests have decreased sensitivity for detection of non-subtype B HIV, and false-negative HIV DNA PCR test results have been reported in infants infected with non-subtype B HIV.[21-24] In an evaluation of perinatally infected infants diagnosed in New York State in 2001–2002, 16.7% of infants were infected with a non-subtype B strain of HIV, compared with 4.4% of infants diagnosed between 1998 and 1999.[25]

Some currently available HIV RNA assays have improved sensitivity for detection of non-subtype B HIV infection,[26-31] although even these assays may not detect or properly quantify some non-B subtypes, particularly the more uncommon group O HIV subtypes.[28, 32, 33]

When evaluating an infant whose mother or father (or both) comes from an area endemic for non-subtype B HIV, such as Africa and Southeast Asia, clinicians should consider conducting initial testing using one of the

assays more sensitive for non-subtype B virus.[28, 34] In addition, when non-subtype B perinatal exposure is suspected in infants with negative HIV DNA PCR results, repeat testing using one of the newer RNA assays is recommended. In these situations, the clinician should consult with an expert in pediatric HIV infection. The child should undergo close clinical monitoring and HIV serologic testing at age 18 months to definitively rule out HIV infection.

Issues Related to Diagnosis of HIV-2 Infections

HIV-2 infection is endemic in Angola; Mozambique;West African countries including Cape Verde, Ivory Coast, Gambia, Guinea-Bissau, Mali, Mauritania, Nigeria, Sierra Leone, Benin, Burkina Faso, Ghana, Guinea, Liberia, Niger, Nigeria, Sao Tome, Senegal, and Togo; and in parts of India.[35, 36] It also occurs in countries such as France and Portugal, which have large numbers of immigrants from these regions;[37] HIV-2 is rare in the United States. HIV-2 infection should be suspected in pregnant women who are from—or who have partners from—countries in which the disease is endemic, who are HIV-1 antibody positive on an initial enzyme-linked immunoassay screening test, and who have repeatedly indeterminate results on HIV-1 Western blot and HIV-1 RNA viral loads at or below the limit of detection.[38] This pattern of HIV testing can also be seen in patients who have a false-positive HIV-1 test. HIV-1 and HIV-2 coinfections may also occur further complicating the diagnosis.

The majority of commercially available HIV screening antibody tests can detect both HIV-1 and HIV-2 but cannot distinguish between the two viruses. The only Food and Drug Administration (FDA)-approved antibody test that distinguishes between HIV-1 and HIV-2 is the Bio-Rad Laboratories Multispot HIV-1/HIV-2 test. If HIV-2 is suspected, infection can be confirmed using a supplemental test such as an HIV-2 immunoblot or HIV-2-specific Western blot. HIV-2 immunoblots are available through commercial labs; however, none are FDA-approved for HIV-2 diagnosis. All HIV-2 cases should be reported to the HIV surveillance program of the state or local health department, which can arrange for additional confirmatory testing for HIV-2 by the Centers for Disease Control and Prevention.

Infants born to HIV-2-infected mothers should be tested for HIV-2 infection with HIV-2-specific virologic assays (HIV-2 DNA PCR testing) at time points similar to those used for HIV-1 testing. HIV-2 virologic assays are not commercially available, but the National Perinatal HIV Hotline (1-888-448-8765) can provide a list of sites that perform this testing. Clinicians should consult with an expert in pediatric HIV infection if caring for infants with suspected or known exposure to HIV-2.[36, 39, 40]

Timing of Diagnostic Testing in Infants with Known Perinatal HIV Exposure

Virologic diagnostic testing of the HIV-exposed infant should be performed at age 14 to 21 days, at age 1 to 2 months, and at age 4 to 6 months. Virologic diagnostic testing at birth should be considered for infants at high risk of HIV infection (see below).

Confirmation of HIV infection should be based on two positive virologic tests from separate blood samples, regardless of a child's age. A positive HIV antibody test with confirmatory Western blot (or immunofluorescent antibody [IFA] assay) at age ≥18 months confirms HIV infection, except in rare late seroreverters (see Diagnostic Testing in Exceptional Situations section below).[1]

HIV infection can be *presumptively* excluded in non-breastfed infants with two or more negative virologic tests, with one test obtained at ≥14 days of age and one obtained at ≥4 weeks of age, or one negative virologic test obtained at ≥8 weeks of age, or one negative HIV antibody test obtained at ≥6 months of age.[1, 41] *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis is recommended for infants with indeterminate HIV infection status starting at 4 to 6 weeks of age until they are determined to be HIV uninfected or *presumptively* uninfected with HIV.[42, 43] Thus, initiation of PCP prophylaxis can be avoided or, if prophylaxis was initiated, can be stopped, if an infant has negative virologic tests at 2 weeks of age and at ≥4 weeks of age, or if virologic

testing is negative at ≥8 weeks of age.

Definitive exclusion of HIV infection in a non-breastfed infant is based on 2 or more negative virologic tests, with one obtained at ≥1 month of age and one at ≥4 months of age, or 2 negative HIV antibody tests from separate specimens obtained at ≥6 months of age. For both *presumptive* and *definitive* exclusion of HIV infection, a child must have no other laboratory (meaning, no positive virologic test results or low CD4 T lymphocyte [CD4 cell] count/percent) or clinical evidence of HIV infection and not be breastfeeding. Many experts confirm the absence of HIV infection in infants with negative virologic tests by performing an antibody test at 12 to 18 months of age to document seroreversion to HIV antibody negative status.

Virologic Testing at Birth (Optional)

Virologic testing at birth can be considered for newborns at high risk of HIV infection, such as infants born to HIV-infected mothers who did not receive prenatal care or prenatal antiretroviral therapy (ART), were diagnosed with acute HIV infection during pregnancy, or who had HIV viral loads ≥1,000 copies/mL close to the time of delivery. As many as 30% to 40% of HIV-infected infants can be identified by 48 hours of age.[7] Blood samples from the umbilical cord should not be used for diagnostic evaluations because of the potential for contamination with maternal blood. Working definitions have been proposed to differentiate acquisition of HIV infection during the intrauterine period from the intrapartum period. Infants who have a positive virologic test at or before age 48 hours are considered to have early (that is, intrauterine) infection, whereas infants who have a negative virologic test during the first week of life and subsequent positive tests are considered to have late (that is, intrapartum) infection.[44] Some researchers have proposed that infants with early infection may have more rapid disease progression than those with late infection and, therefore, should receive more aggressive therapy.[44, 45] However, data from prospective cohort studies have demonstrated that although early differences in HIV RNA levels were present between infants with a positive HIV culture within 48 hours of birth and those with a first positive culture after 7 days of age, these differences were no longer statistically significant after 2 months of age.[46] HIV RNA levels after the first month of life were more predictive of rapid disease progression than the time at which HIV culture tests were positive.[46]

Virologic Testing at Age 14 Days to 21 Days

The diagnostic sensitivity of virologic testing increases rapidly by age 2 weeks,[7] and early identification of infection would permit discontinuation of neonatal ARV prophylaxis and further evaluation for initiation of combination ART (see When to Initiate Therapy in Antiretroviral-Naive HIV-Infected Infants Younger than 12 Months and Table 7).

Virologic Testing at Age 1 to 2 Months

Infants with negative virologic tests before 1 month of age should be retested at 1 to 2 months of age. Most HIV-exposed neonates will receive 6 weeks of neonatal ARV prophylaxis. Although ARV agents, in theory, could affect the predictive value of HIV virologic testing in neonates, use of prenatal/intrapartum/neonatal zidovudine single-drug prophylaxis did not delay detection of HIV by culture in infants in Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 and has not decreased the sensitivity and predictive values of many virologic assays.[9-11, 41, 47, 48] In one study, prenatal and neonatal combination ARV regimens lowered HIV RNA levels for HIV-exposed infected infants but did not affect the assay's sensitivity for detecting the presence of HIV (that is, HIV RNA levels remained detectable).[8] Further studies are necessary to confirm this finding. An infant with two negative virologic tests, one at ≥14 days and one at ≥1 month of age, can be viewed as *presumptively* uninfected and would not need PCP prophylaxis, assuming the child has no laboratory (such as, no positive virologic test results or low CD4 cell count) or clinical evidence of HIV infection.

Virologic Testing at Age 4 to 6 Months

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

Antibody Testing at Age 6 Months or Older

Two or more negative HIV antibody tests performed in non-breastfed infants at ≥6 months of age can also be used to *definitively* exclude HIV infection in HIV-exposed children with no clinical or virologic laboratory evidence of HIV infection.

Antibody Testing at Age 12 to 18 Months to Document Seroreversion

If there has not been previous confirmation of two negative antibody tests, many experts confirm the absence of HIV infection in infants with negative virologic tests by repeat serologic testing between 12 and 18 months of age to confirm that maternal HIV antibodies transferred *in utero* have disappeared. The proportion of infants who serorevert by 15 to 18 months of age is close to 100%, with as many as 95% seroreverting by 12 months of age. Factors that might influence the time to seroreversion include maternal disease stage and assay sensitivity.[1, 49-52]

Diagnostic Testing in Children with Perinatal HIV Exposure in Exceptional Situations

- Late seroreversion up to 24 months of age
- Postnatal HIV infection in HIV-exposed children with prior negative virologic tests for whom there are additional HIV transmission risks
- HIV-2 and non-subtype B HIV-1

On rare occasions, non-breastfed perinatally HIV-exposed infants with no other HIV transmission risk and no clinical or virologic laboratory evidence of HIV infection may have residual HIV antibodies for up to 24 months (these infants are called late seroreverters).[52-54] These children may have positive enzyme-linked immunosorbent assay (EIA) results but indeterminate confirmatory antibody tests (Western Blot or IFA). In such cases, repeat antibody testing at a later time would document seroreversion.

In contrast to late seroreverters, in rare situations, postnatal HIV infections have been reported in HIV-exposed infants who had prior negative HIV virologic tests. This occurs in infants who become infected through an additional risk after completion of testing (see Diagnostic Testing in Children with Non-Perinatal HIV Exposure section below). If a confirmatory HIV antibody test is positive at 18 months of age, repeated virologic testing will distinguish between residual antibodies in uninfected, late seroreverting children and true infection.

Postnatal HIV exposure can occur if an HIV-infected mother breastfeeds her infant. Typical scenarios in the US include women who have not been adequately counseled about infant feeding, women who breastfeed despite being counseled not to do so, and women who learn of their HIV diagnosis only after initiating breastfeeding. Diagnostic testing to rule out acquisition of HIV through breast milk will only be accurate after breastfeeding has completely ceased. The timing of testing in such situations is discussed below in Diagnostic Testing in Children with Non-Perinatal HIV Exposure.

Another example where there can be postnatal HIV exposure is when an HIV-infected caregiver premasticates or prechews solid food before feeding it to an infant. This practice has been documented to result in HIV transmission.[53, 54] In such exposed children, both screening EIA and confirmatory antibody tests (EIA, Western Blot or IFA) may be positive at 18 months. Another study documented very rare cases of late postnatal infection without identified risk factors, suggesting the possibility of intrafamilial HIV transmission.[55]

Children with non-subtype B HIV-1 infection and children with HIV-2 infection may have persistent positive EIA tests and indeterminate confirmatory antibody tests.[21-24] Situations in which such infections may be suspected and the diagnostic approach to them are discussed above in the sections Issues Related to Diagnosis of Non-Subtype B Infection and Issues Related to Diagnosis of HIV-2 Infection.

Diagnostic Testing in Children with Non-Perinatal HIV Exposure

Breastfeeding is a known route of HIV transmission. Infants who are breastfed by an HIV-infected woman, including those diagnosed with acute HIV infection during breastfeeding or who breastfed before knowing their HIV diagnosis, should undergo immediate HIV virologic testing and breastfeeding should be discontinued. Follow-up virologic testing should be performed at 4 to 6 weeks, 3 and 6 months after breastfeeding cessation if the initial tests are negative.[56] HIV antibody testing of an infant to assess for HIV exposure would not be helpful if the mother acquired HIV infection after giving birth. In that situation, an infant would be HIV antibody-negative but still at risk of acquiring HIV infection through breastfeeding and counseling to cease breastfeeding should be provided.

Perinatal HIV acquisition accounts for the majority of HIV infections in children, but providers may need to evaluate children exposed to HIV through other routes, such as sexual abuse, or because they were adopted from countries in which parenteral exposure to HIV via contaminated blood products is a possibility. In such cases, maternal HIV status may be negative or unknown. Receipt of solid food premasticated or prechewed by an HIV-infected caregiver also has been documented to be associated with risk of HIV transmission.[53, 54] Finally, acquisition of HIV is possible through accidental needle sticks or behavioral risks, such as sexual activity or injection drug use in older children.

HIV antibody testing should be performed on children who are suspected to have HIV infection because of clinical or laboratory findings consistent with HIV. Additional virologic testing may be necessary if acute HIV infection or end-stage AIDS is suspected because antibody testing can be negative in these situations.

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