Clinical and Laboratory Monitoring of Pediatric HIV Infection

(Last updated April 16, 2019; last reviewed April 16, 2019)

Panel’s Recommendations

- Absolute CD4 T lymphocyte (CD4) cell count and plasma HIV RNA (viral load) should be measured at the time of HIV diagnosis and, if a child is not started on antiretroviral therapy (ART) after diagnosis, this monitoring should be repeated at least every 3 to 4 months thereafter (AII).

- Absolute CD4 cell count is recommended for monitoring immune status in children of all ages, with CD4 percentage as an alternative for children aged <5 years (AII).

- Antiretroviral (ARV) drug-resistance testing is recommended at the time of HIV diagnosis, before initiation of therapy, in all treatment-naive patients (AII). Genotypic resistance testing is preferred for this purpose (AII).

- After initiation of ART, or after a change in ART regimen, children should be evaluated for clinical adverse effects and should receive support for treatment adherence within 1 to 2 weeks, with laboratory testing for toxicity and viral load response recommended at 2 to 4 weeks after treatment initiation (AIII).

- Children on ART should be monitored for therapy adherence, effectiveness, and toxicities routinely (every 3 to 4 months) (AII*).

- Additional CD4 cell count and plasma viral load monitoring should be performed to evaluate children with suspected clinical, immunologic, or virologic deterioration or to confirm an abnormal value (AII). CD4 cell count can be monitored less frequently (every 6 months–12 months) in children and adolescents who are adherent to therapy, who have CD4 cell count values that are well above the threshold for opportunistic infection risk and sustained virologic suppression, and who have had stable clinical status for more than 2 to 3 years (AII). Viral load measurement every 3 to 4 months is generally recommended to monitor ART adherence and disease progression (AIII).

- Phenotypic resistance testing should be used (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after virologic failure of successive ART regimens (BIII).

- The absence of detectable resistance to a drug does not ensure that use of the drug will be successful, as mutations may not be detected once the drug has been discontinued. A history of all previously used ARV agents and available resistance test results must be reviewed when making decisions regarding the choice of new agents (AII).

- Viral co-receptor (tropism) assays are recommended whenever a CCR5 antagonist is being considered for treatment (AI*). The use of tropism assays should also be considered for patients who demonstrate virologic failure while receiving therapy that contains a CCR5 antagonist (AI*).

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

Laboratory monitoring of children living with HIV poses unique and challenging issues. In particular, the normal ranges of CD4 T lymphocyte (CD4) cell counts and plasma HIV RNA concentrations (viral loads) can vary significantly by age. The CD4 cell counts and viral load values that predict the risk of disease progression also change as a child ages. This section will address immunologic, virologic, general laboratory, and clinical monitoring of children with HIV, with information that is relevant to both those who have recently received an HIV diagnosis and those who are receiving antiretroviral therapy (ART).

Clinical and Laboratory Monitoring of Children Living With HIV

Initial Evaluation of Children Who Recently Received an HIV Diagnosis

Children who have recently received an HIV diagnosis should have their CD4 cell counts and plasma viral loads measured, and their growth and development should be evaluated for signs of HIV-associated abnormalities. They should also undergo a laboratory evaluation that looks for HIV-associated conditions,
including anemia, leukopenia, thrombocytopenia, hypoalbuminemia, nephropathy (urinalysis), and elevated levels of glucose, transaminases, or creatinine. In addition, children with HIV should have a complete, age-appropriate medical history and physical examination (see Table 3). Opportunistic infection (OI) monitoring should follow the guidelines that are appropriate for the child’s exposure history and clinical setting (see the Pediatric Opportunistic Infection Guidelines).

Laboratory confirmation of HIV infection should be obtained if available documentation is incomplete (see Diagnosis of HIV Infection in Infants and Children). Genotypic resistance testing should be performed, even if ART is not initiated immediately. In addition, a full antiretroviral (ARV) drug history should be obtained; this history should include any exposure to ARV drugs for the prevention of perinatal HIV transmission (see Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). If abacavir is being considered as a component of the regimen, HLA-B*5701 testing should be sent prior to initiating abacavir, and an alternative ARV drug should be used if the HLA-B*5701 test result is positive (see the abacavir section in Appendix A: Pediatric Antiretroviral Drug Information).

Before initiating therapy or making changes to a patient’s regimen, a clinician should assess potential barriers to adherence and discuss the importance of adherence with the patient (see Adherence to Antiretroviral Therapy in Children and Adolescents Living with HIV).

If a child does not initiate ART after receiving an HIV diagnosis, the child’s CD4 cell count and plasma viral load should be monitored at least every 3 to 4 months.

**Evaluation at Initiation of Antiretroviral Therapy**

At the time of ART initiation, a patient’s CD4 cell count and plasma viral load should be measured to establish a baseline for monitoring the patient’s response to ART. To set the baseline for monitoring ART toxicity (see Management of Medication Toxicity or Intolerance), a complete blood count (CBC), urinalysis, and serum chemistry panel (including levels of electrolytes, creatinine, glucose, hepatic transaminases) should be performed. The levels of serum lipids (cholesterol, triglycerides) should also be measured. A CBC allows monitoring of zidovudine-associated anemia, leukopenia, and macrocytosis (see the zidovudine section in Appendix A: Pediatric Antiretroviral Drug Information). Electrolytes with anion gaps might help identify nucleoside reverse transcriptase inhibitor-associated lactic acidosis. In patients who are receiving tenofovir disoproxil fumarate, creatinine levels may increase, phosphate levels may decrease, and proteinuria can occur (see the tenofovir disoproxil fumarate section in Appendix A: Pediatric Antiretroviral Drug Information). Use of protease inhibitors may be associated with hyperglycemia. Levels of hepatic transaminases (alanine aminotransferase and aspartate aminotransferase) increase with the use of many ARV drugs. Bilirubin should be measured prior to starting atazanavir, because that drug causes an increase in indirect bilirubin (see the atazanavir section in Appendix A: Pediatric Antiretroviral Drug Information). For more information about the adverse effects (AEs) that are associated with a specific ARV drug, see Tables 15a-15k in Management of Medication Toxicity or Intolerance.

**Clinical and Laboratory Monitoring After Initiating or Changing an Antiretroviral Therapy Regimen**

Children who start ART or who change to a new regimen should be monitored to assess the effectiveness, tolerability, and AEs of the regimen and to evaluate medication adherence. Clinicians should schedule frequent clinic visits and monitor patients closely during the first few months after initiating a new ART regimen. These visits are an opportunity for clinicians to provide support and discuss adherence with patients and their caregivers. The first few weeks of ART can be particularly difficult for children and their caregivers; they must adjust their schedules to allow for consistent and routine administration of medication doses. Children may also experience the AEs of medications, and both children and their caregivers need assistance to determine whether the effects are temporary and tolerable or whether they are more serious or long-term and require a visit to the clinician. It is critical that providers speak to caregivers and children in a supportive, nonjudgmental manner and use layman’s terms. This promotes interactive reporting and ensures...
that providers can have a productive dialogue with both children and their caregiver(s), even in situations where medication adherence is reported to be inconsistent.

**Within 1 to 2 Weeks of Initiation of Antiretroviral Therapy**

Within 1 to 2 weeks of initiating therapy, children should be evaluated either in person or by phone. During this evaluation, clinicians should identify clinical AEs and provide support for adherence. Many clinicians plan additional contacts (in person, by telephone, or via email) with children and caregivers to support adherence during the first few weeks of therapy.

**2 to 4 Weeks after Initiation of Antiretroviral Therapy**

Most experts recommend performing laboratory testing at 2 weeks to 4 weeks (and not >8 weeks) after initiation of ART to assess virologic response and laboratory toxicity, though this recommendation is based on limited data. The laboratory chemistry tests that a patient requires will depend on the regimen the patient is receiving (see above). Plasma viral load monitoring is important as a marker of response to ART, because a decline in viral load suggests that the patient is adherent to the regimen, that the appropriate doses are being administered, and that the virus is susceptible to the drugs in the regimen. Some experts favor measuring viral load at 2 weeks to ensure that viral load is declining. A significant decrease in viral load should be observed after 4 weeks to 8 weeks of ART.

**Clinical and Laboratory Monitoring for Children who are Stable on Long-Term Antiretroviral Therapy**

After the initial phase of ART initiation (1 month–3 months), clinicians should assess a patient’s adherence to the regimen and the regimen’s effectiveness (as measured by CD4 cell count and plasma viral load) every 3 months to 4 months. Additionally, clinicians should review a patient’s history of toxicities and evaluate a patient for any new AEs using physical examinations and the relevant laboratory tests. If laboratory evidence of toxicity is identified, testing should be performed more frequently until the toxicity resolves.

Table 3 provides one proposed general monitoring schedule, which should be adjusted based on the specific ART regimen a child is receiving.

A patient’s baseline CD4 cell count affects how rapidly CD4 cell count improves after ART initiation; children with very low CD4 cell counts may take longer than 1 year to achieve their highest values after viral load suppression.2

Recent studies have critically evaluated the frequency of laboratory monitoring in both adults and children, particularly CD4 cell count and plasma viral load. These studies support less frequent monitoring in stable patients who have been consistently virologically suppressed for ≥1 year.3-9

The current Adult and Adolescent Antiretroviral Guidelines support performing plasma viral load testing every 6 months for individuals who have both:

- Consistent virologic suppression for longer than 2 years
- CD4 cell counts consistently >300 cells/mm³

The Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV finds value in continuing to perform viral load testing every 3 to 4 months to provide enhanced monitoring of adherence or disease progression among children and adolescents. Some experts monitor CD4 cell count less frequently (e.g., every 6 months to 12 months) in children and adolescents who are adherent to therapy, who have CD4 cell count values well above the threshold for OI risk, and who have had sustained virologic suppression and stable clinical status for >2 years to 3 years.10 Some clinicians find value in scheduling visits every 3 months even when lab testing is not performed, in order to review adherence and update drug doses for interim growth.
**Testing at the Time of Switching Antiretroviral Therapy**

When a patient switches regimens in order to simplify ART, clinicians should obtain the appropriate laboratory test results at baseline for the toxicity profile of the new regimen. Follow-up should include a measurement of plasma viral load at 4 weeks (and not >8 weeks) after the switch to ensure that the new regimen is effective. If the regimen is switched because of ART failure (see Recognizing and Managing Antiretroviral Treatment Failure in Management of Children Receiving Antiretroviral Therapy), resistance testing should be performed while a patient is still receiving the failing regimen. This optimizes the chance of identifying resistance mutations, because resistant strains may revert to wild type within a few weeks of stopping ARV drugs (see Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). Clinicians should consider the use of phenotypic resistance testing, including co-receptor tropism testing, in addition to genotypic viral resistance testing in children who have experienced prolonged or repeated periods of viral nonsuppression on multiple ART regimens.11

**Immunologic Monitoring in Children: General Considerations**

When interpreting CD4 cell counts and percentages in children, clinicians must consider age as a factor. CD4 cell count and percentage values in healthy infants without HIV are considerably higher than values observed in adults without HIV; these infant values slowly decline to adult values by age 5 years. An analysis from the HPPM Collaborative Study found that CD4 percentage provided little or no additional prognostic value compared with CD4 cell count regarding short-term disease progression in children aged <5 years; similar results were reported in a study of older children.12 The current pediatric HIV disease classification is based on absolute CD4 cell count, which is the preferred assay for monitoring and estimating the risk for disease progression and OIs.13

In children living with HIV, as in adults living with HIV, CD4 cell count and percentage decline as HIV infection progresses; patients with lower CD4 cell counts or percentage values have a poorer prognosis than patients with higher values (see Tables A–C in Appendix C: Supplemental Information).

Medical practice guidelines now recommend that all people with HIV receive ART, regardless of their CD4 cell count and clinical stage. However, CD4 cell counts are used to determine risk profiles that affect the urgency of recommendations for when to initiate therapy in a treatment-naive child with HIV infection and when to assess the need for OI prophylaxis (see When to Initiate). A meta-analysis from the HPPM Collaborative Study generated plots that can be used to estimate the short-term risk of progression to AIDS or death in the absence of effective ART, according to age and the most recent CD4 percentage/absolute CD4 cell count or HIV RNA viral load measurement.14

CD4 cell counts and percentages can show considerable intrapatient variation.15 Mild intercurrent illness, the receipt of vaccinations, or exercise can produce a transient decrease in CD4 cell count and percentage; thus, CD4 cell count and percentage are best measured when patients are clinically stable. Clinical decisions, especially those regarding therapy changes, should be made in response to confirmed changes in CD4 cell count or percentage in conjunction with a confirmed viral load determination. The CD4 cell count/percentage and viral load measurement should be confirmed by performing the test a second time at least 1 week after the first test.

**HIV RNA Monitoring in Children: General Considerations**

Quantitative HIV RNA assays measure the plasma concentration of HIV RNA as copies/mL. Without therapy, plasma viral load initially rises to high peak levels during the period of primary infection in adults and adolescents, and then it declines by as much as 2 to 3 log10 copies to reach a stable lower level (the virologic set point) approximately 6 months to 12 months after acute infection.16,17 In adults with HIV, the stable lower level (or virologic set point) correlates with the subsequent risk of disease progression or death in the absence of therapy.18
The pattern of change in plasma viral load in untreated infants with perinatal HIV infection differs from that in adults and adolescents with HIV infection. High plasma viral loads persist in untreated children for prolonged periods. In one prospective study of infants with perinatal infection who were born prior to ARV drug availability for children, plasma viral loads generally were low at birth (i.e., <10,000 copies/mL), increased to high values by age 2 months (most infants had values >100,000 copies/mL, ranging from undetectable to nearly 10 million copies/mL), and then decreased slowly, with a mean plasma viral load of 185,000 copies/mL during the first year of life. After the first year of life, plasma viral load slowly declined during the next few years. Viral load during the first 12 months to 24 months after birth showed an average decline of approximately 0.6 log₁₀ copies/mL per year, followed by an average decline of 0.3 log₁₀ copies/mL per year until age 4 years to 5 years. This pattern probably reflects the lower efficiency of a developing immune system in containing viral replication, and possibly the rapid expansion of HIV-susceptible cells that occurs with somatic growth.

Despite the established association between high plasma viral load and disease progression, a specific HIV RNA concentration has only moderate predictive value for disease progression and death in an individual child. Plasma viral load may be difficult to interpret during the first year of life because values are high and are less predictive of disease progression risk than those in older children. In both children and adults with HIV, CD4 cell count or percentage and plasma viral load are independent predictors of disease progression and mortality risk, and using the two markers together more accurately defines prognosis.

**Methodological Considerations When Interpreting and Comparing HIV RNA Assays**

Based on accumulated experience with currently available assays, the current definition of virologic suppression is a plasma viral load that is below the detection limit of the assay used (generally <20 copies/mL to 75 copies/mL). This definition of suppression has been much more thoroughly investigated in adults with HIV than in children with HIV (see the Adult and Adolescent Antiretroviral Guidelines). Temporary viral load elevations (“blips”) that are between the level of detection and 500 copies/mL often are detected in adults and children who are on ART; these temporary elevations do not represent virologic failure, as long as the values have returned to below the level of detection when testing is repeated. For definitions and management of virologic treatment failure, see Recognizing and Managing Antiretroviral Treatment Failure in Management of Children Receiving Antiretroviral Therapy. These definitions of virologic suppression and virologic failure are recommended for clinical use. Research protocols or surveillance programs may use different definitions.

Several different methods can be used for quantitating HIV RNA, each of which has a different level of sensitivity (see Table 4). Although the results of the assays are correlated, the absolute HIV RNA copy number obtained from a single specimen tested by two different assays can differ by 0.3 log₁₀ copies/mL or more. Because different assays use different methods to measure HIV RNA, and because the tests have different levels of sensitivity, clinicians should consistently use a single HIV RNA assay method to monitor an individual patient when possible.

The predominant HIV-1 subtype in the United States is subtype B, and early assays were designed to detect this subtype. Current kit configurations for all companies have been designed to detect and quantitate essentially all viral subtypes (see Diagnosis of HIV Infection in Infants and Children). This is important for many regions of the world where non-B subtypes are predominant, as well as for the United States, where a small subset of individuals contract non-B viral subtypes. It is particularly relevant for children who are born outside the United States or to foreign-born parents.

Biologic variation in plasma viral load within one person is well documented. In adults, repeated measurements of plasma viral load using the same assay can produce results that vary by as much as 0.5 log₁₀ copies/mL in either direction during the course of a day or on different days. This biologic variation may be greater in infants and young children with HIV. This inherent biologic variability must be considered when interpreting changes in plasma viral load in children. Thus, after repeated testing, only differences >0.7
log_{10} copies/mL in infants aged <2 years and differences >0.5 log_{10} copies/mL in children aged ≥2 years should be considered reflective of plasma viral load changes that are biologically and clinically significant.

Generally, no change in ARV treatment should be made as a result of a change in plasma viral load unless the change is confirmed by a second measurement. Clinicians should consult an expert in pediatric HIV infection when making clinical decisions based on plasma viral loads, due to the complexities of HIV RNA testing and the age-related changes in plasma viral load in children.

**Genetic Testing for Management of HIV**

Modern disease intervention strategies often employ genetic testing to evaluate the genes of humans and pathogens. This approach to treatment is an important component in the rise of precision medicine. Clinicians who manage HIV have routinely probed HIV’s genetic sequences for mutations that are associated with HIV drug resistance. Some ARV drugs are metabolized differently based on specific human genotypes. For example, studies have shown that certain genotypes can affect efavirenz exposure in young children. In addition, some human genetic polymorphisms are associated with drug toxicity or adverse events (e.g., using HLA-B*5701 testing to predict abacavir hypersensitivity; for more information, see the abacavir section of the drug appendix). Future clinical practice is likely to feature broader applications of multiple forms of genetic testing to guide management of health and disease.

**Table 3. Sample Schedule for Clinical and Laboratory Monitoring of Children Before and After Initiation of Antiretroviral Therapy** (page 1 of 2)

<table>
<thead>
<tr>
<th>Laboratory Testing</th>
<th>Entry Into Care&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pre-Therapy&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ART Initiation&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Weeks 1–2 on Therapy</th>
<th>Weeks 2–4 on Therapy</th>
<th>Every 3–4 Months&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Every 6–12 Months&lt;sup&gt;e&lt;/sup&gt;</th>
<th>When Switching ARV Regimens</th>
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<td>Hepatitis B Screening&lt;sup&gt;h&lt;/sup&gt;</td>
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Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection  

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### Table 3. Sample Schedule for Clinical and Laboratory Monitoring of Children Before and After Initiation of Antiretroviral Therapy (page 1 of 2)

- **a** See text for details on recommended laboratory tests to perform.
- **b** A patient's ability to adhere to an ARV regimen is assessed prior to starting ART. If abacavir is being considered as part of the regimen, send HLA-B*5701 testing prior to initiating abacavir and choose an alternative ARV drug if the patient is HLA-B*5701 positive (see the abacavir section in Appendix A: Pediatric Antiretroviral Drug Information). Genotype resistance testing is recommended if it has not already been performed (see Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). Send tests that are appropriate for the toxicity profile that is associated with a patient's ART regimen and the patient's medical history (see text).
- **c** If ART is initiated within 30 days to 90 days of a pre-therapy lab result, repeat testing may not be necessary.
- **d** CD4 cell count, CBC, and chemistries can be monitored less frequently (every 6 months–12 months) in children and youth who are adherent to therapy, who have CD4 cell values that are well above the threshold for OI risk, and who have had sustained virologic suppression and stable clinical status for more than 2 years to 3 years. Viral load testing every 3 to 4 months is generally recommended to monitor ARV adherence.
- **e** If lipid levels have been abnormal in the past, more frequent monitoring might be needed. For patients treated with TDF, more frequent urinalysis should be considered.
- **f** Chemistries refer to a comprehensive metabolic panel.
- **g** Random plasma glucose is collected in a gray-top blood collection tube or other designated tube.
- **h** This screening is only recommended for individuals who have previously demonstrated no immunity to hepatitis B and who are initiating a regimen that contains ARV drugs with activity against hepatitis B, specifically lamivudine, emtricitabine, TAF, or TDF.

**Key to Acronyms:** ART = antiretroviral therapy; ARV = antiretroviral; CBC = complete blood count; CD4 = CD4 T lymphocyte; TAF = tenofovir alafenamide; TDF = tenofovir disoproxil fumarate; OI = opportunistic infection

### Table 4. Primary, Food and Drug Administration-Approved Assays for Monitoring Viral Load

<table>
<thead>
<tr>
<th>Assay</th>
<th>Abbott Real Time</th>
<th>NucliSens EasyQ v2.0</th>
<th>COBAS AmpliPrep/ TaqMan v2.0</th>
<th>Versant v1.0</th>
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<tr>
<td><strong>Method</strong></td>
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<td>Real-time NASBA</td>
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<td>25–10^7 copies/mL</td>
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<td>Abbott</td>
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</table>

- **Smaller volumes for children can be accommodated.**

**Key to Acronyms:** NASBA = nucleic acid sequence-based amplification; RT-PCR = reverse transcription polymerase chain reaction

### References


