### Panel's Recommendations

- Absolute CD4 T lymphocyte (CD4) cell count and plasma HIV RNA (viral load) should be measured at the time of diagnosis of HIV infection 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 (AIII).

- 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 (AIII).

- After initiation of ART, or after a change in ART regimen, children should be evaluated for clinical adverse effects and to support 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 for evaluation of children with suspected clinical, immunologic, or virologic deterioration or to confirm an abnormal value (AIII). CD4 cell count can be monitored less frequently (every 6–12 months) in children and youth who are adherent to therapy and have CD4 cell value well above the threshold for opportunistic infection risk, sustained viral suppression, and stable clinical status for more than 2 to 3 years (AII).

- 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 coreceptor (tropism) assays should be used whenever the use of a CCR5 antagonist is being considered (AI*). Tropism assays should also be considered for patients who demonstrate virologic failure while receiving therapy that contains a CCR5 antagonist (AI*).

- 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).

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**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

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Laboratory monitoring of children living with HIV poses unique and challenging issues. In particular, normal ranges and the value of CD4 T lymphocyte (CD4) cell count and plasma HIV-1 RNA concentration (viral load) for prediction of risk of disease progression vary significantly by age. This section will address immunologic, virologic, and general laboratory monitoring as well as clinical monitoring of children with HIV infection, relevant to both those who are newly diagnosed and those who are receiving antiretroviral therapy (ART).

## Clinical and Laboratory Monitoring of Children Living With HIV

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

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 (AIII).
**Initial Evaluation of Newly Diagnosed Children**

Children recently diagnosed with HIV should be evaluated with measurement of CD4 cell count and plasma viral load; evaluation of growth and development for signs of HIV-associated change; and laboratory evaluation of HIV-associated conditions including anemia, leukopenia, thrombocytopenia, hypoalbuminemia, elevated glucose, transaminases, or creatinine, and HIV-associated nephropathy (urinalysis). In addition, children with HIV infection should have a complete age-appropriate medical history and physical examination (see Table 3). Opportunistic infection (OI) monitoring should follow guidelines appropriate for the child’s exposure history and clinical setting (see the Pediatric Opportunistic Infections Guidelines).

Laboratory confirmation of HIV infection should be obtained if available documentation is incomplete (see Diagnosis of HIV Infection). Genotypic resistance testing should be performed, even if ART is not initiated immediately. In addition, a full ARV drug history including exposure to medications for prevention of mother-to-child transmission should be obtained (see Antiretroviral Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). If abacavir is being considered as part of the regimen, HLA-B*5701 testing should be sent prior to initiation of that ARV drug, and an alternative ARV drug should be used if HLA-B*5701 testing is positive (see Abacavir in Appendix A: Pediatric Antiretroviral Drug Information).

Readiness for ARV drug adherence should be assessed prior to starting ART and associated discussion/counseling implemented.

In the event that a child is not placed on ART after HIV diagnosis, monitoring of CD4 count and plasma viral load should be implemented at least every 3 to 4 months.

**Evaluation at Initiation of Combination Antiretroviral Therapy**

At the time of ART initiation, CD4 cell count and plasma viral load should be measured to establish a baseline to monitor ART benefit. To set the baseline for monitoring ART toxicity (see Management of Medication Toxicity or Intolerance), complete blood count (CBC) and differential, serum chemistries (including electrolytes, creatinine, glucose, hepatic transaminases), urinalysis, and serum lipids (cholesterol, triglycerides) should be measured. CBC allows monitoring of zidovudine-associated anemia, leukopenia, and macrocytosis (see Zidovudine in Appendix A: Pediatric Antiretroviral Drug Information). Electrolytes with anion gap might help identify nucleoside reverse transcriptase inhibitor-associated lactic acidosis. With use of tenofovir disoproxil fumarate, creatinine may increase, phosphate decrease, and proteinuria can occur (see Tenofovir Disoproxil Fumarate in Appendix A: Pediatric Antiretroviral Drug Information). Use of protease inhibitors may be associated with hyperglycemia. Hepatic transaminases (alanine aminotransferase and aspartate aminotransferase) increase with many ARV drugs. Bilirubin should be measured prior to starting atazanavir because that drug causes an increase in indirect bilirubin (see Atazanavir in Appendix A: Pediatric Antiretroviral Drug Information). For further details of adverse effects (AEs) associated with a particular ARV drug, see Tables 13a-13l in Management of Medication Toxicity or Intolerance.

**Clinical and Laboratory Monitoring After Initiation of Combination Antiretroviral Therapy (or After a Change in Antiretroviral Therapy)**

After initiation of ART, or after a change in ART regimen, children should be evaluated for clinical AEs and to support 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 who start ART or who change to a new regimen should be followed to assess effectiveness, tolerability, and AEs of the regimen and to evaluate medication adherence. Frequent patient visits and intensive follow-up during the initial months after a new ART regimen is started are necessary to support and educate the family. 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 AEs of medications, and both children and their caregivers need assistance to determine whether the effects are temporary and tolerable or 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, non-judgmental manner using layman’s terms. This promotes honest reporting and ensures dialogue between providers and both children and their caregiver(s), even when 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 to identify clinical AEs and to support 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**
While data are limited on which to base an exact recommendation about precise timing, most experts recommend laboratory testing at 2 to 4 weeks (and not more than 8 weeks) after initiation of ART to assess virologic response and laboratory toxicity. The selection of laboratory chemistry tests is regimen-specific (see above). Evaluation of hepatic transaminases is recommended at 2 weeks and 4 weeks for patients starting treatment that includes nevirapine (see Nevirapine in Appendix A: Pediatric Antiretroviral Drug Information). Plasma viral load monitoring is important as a marker of response to ART because a fall in viral load suggests medication adherence, administration of appropriate doses, and viral drug susceptibility. Some experts favor measuring viral load at 2 weeks to ensure that viral load is declining. A significant decrease in viral load in response to ART should be observed by 4 to 8 weeks of therapy.

**Children on ART should be monitored for therapy adherence, effectiveness (by CD4 cell count and plasma viral load), and toxicities (by history, physical, and selected laboratory tests) routinely every 3 to 4 months for the first 2 years (AII*).**

**CD4 count improvement is influenced by the baseline value at the time of initiation of ART; children with very low CD4 counts may take more than one year to achieve their highest values after viral load suppression.**

**Additional CD4 cell count and plasma viral load monitoring should be performed for evaluation of children with suspected clinical, immunologic, or virologic deterioration or to confirm an abnormal value (AIII).**

**Laboratory Monitoring of Patients Receiving Antiretroviral Therapy**
After the initial phase of ART initiation, regimen adherence, effectiveness (CD4 cell count and plasma viral load), and toxicities (history, physical, and laboratory testing as above) should be assessed every 3 to 4 months in children receiving ART. Children who develop symptoms of toxicity should have appropriate laboratory evaluations. 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.

CD4 cell count can be monitored less frequently (every 6–12 months) in children and youth who are adherent to therapy and have CD4 cell value well above the threshold for OI risk, sustained viral suppression, and stable clinical status for more than 2 to 3 years.

**Laboratory Monitoring of Patients Who Are Stable on Long-Term Antiretroviral Therapy**
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 in whom viral suppression has been sustained for at least a year.

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

- Consistent virus suppression for more than 2 years
- CD4 count consistently >300 cells/mm³
The Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV finds value in continuing viral load testing every 3 to 4 months to provide enhanced monitoring of adherence or disease progression among children and youth. Some experts monitor CD4 cell count less frequently (e.g., every 6 to 12 months) in children and youth who are adherent to therapy and have CD4 cell value well above the threshold for OI risk, sustained viral suppression, and stable clinical status for more than 2 to 3 years. Some clinicians find value in visits every 3 months even when lab testing is not performed in order to review adherence and update dosing for interim growth.

**Testing at the Time of Switching Antiretroviral Therapy**

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 antiretroviral agents and available resistance test results must be reviewed when making decisions regarding the choice of new agents (AII).

Viral coreceptor (tropism) assays should be used whenever the use of a CCR5 antagonist is being considered (A1*). Tropism assays should also be considered for patients who demonstrate virologic failure while receiving therapy that contains a CCR5 antagonist (A1*).

When a switch in regimen is made to simplify ART, labs appropriate to the toxicity profile of the new regimen should be measured at baseline, with follow up including plasma viral load at 4 weeks (and not more than 8 weeks) after the switch, to ensure efficacy of the new regimen. 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 to optimize the chance of identifying resistance mutations because resistant strains may revert to wild type within a few weeks of stopping ARV drugs (see Antiretroviral Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). Among children with prolonged or repeated periods of viral non-suppression in the face of serial ART regimens, phenotypic resistance testing, including co-receptor tropism testing, should be considered in addition to genotypic viral resistance testing.10

**Immunologic Monitoring in Children: General Considerations**

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).

Clinicians interpreting CD4 cell count and percentage in children must consider age as a factor. CD4 cell count and percentage values in healthy infants without HIV infection are considerably higher than values observed in adults without HIV infection (and 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 as well as in older children.11 The current pediatric HIV disease classification is based on absolute CD4 cell count, which is the preferred assay for monitoring and estimating risk for disease progression and OIs.12

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

While guidelines now recommend that children of all ages and adults receive ART regardless of CD4 count and clinical stage, CD4 count-associated risk profiles contribute to the level of urgency for recommendations on when to initiate therapy in a treatment-naive child with HIV infection (see When to Initiate). A website using the meta-analysis from the HPPM Collaborative Study is available 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-1 RNA viral load measurement (http://hppmcs.org).\textsuperscript{13}

Measurement of CD4 cell count and percentage can be associated with considerable intrapatient variation.\textsuperscript{14} Mild intercurrent illness, the receipt of vaccinations, or exercise can produce a transient decrease in CD4 cell count and percentage; thus, CD4 cell count/percentage are best measured when patients are clinically stable. No decision about therapy should be made in response to a change in CD4 cell count/percentage until the change has been substantiated by at least a second determination, with a minimum of 1 week between measurements.

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

Quantitative HIV-1 RNA assays measure the plasma concentration of HIV RNA as copies/mL, commonly referred to as the plasma viral load. During the period of primary infection in adults and adolescents, in the absence of therapy, plasma viral load initially rises to high peak levels and then declines by as much as 2 to 3 $\log_{10}$ copies to reach a stable lower level (the virologic set point) approximately 6 to 12 months after acute infection.\textsuperscript{15,16} In adults living with HIV, the stable lower level (or viral set point) correlates with the subsequent risk of disease progression or death in the absence of therapy.\textsuperscript{17}

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 load persists in untreated children for prolonged periods.\textsuperscript{18,19} In one prospective study of infants with perinatal infection born prior to ARV drug availability in 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 during the first year of life of 185,000 copies/mL.\textsuperscript{20} After the first year of life, plasma viral load slowly declined over the next few years.\textsuperscript{20-23} Viral load during the first 12 to 24 months after birth showed an average decline of approximately 0.6 $\log_{10}$ copies/mL per year, followed by an average decline of 0.3 $\log_{10}$ copies/mL per year until age 4 to 5 years. This pattern probably reflects the lower efficiency of an immature but developing immune system in containing viral replication and possibly the rapid expansion of HIV-susceptible cells that occurs with somatic growth.\textsuperscript{24}

Despite data indicating that high plasma viral load is associated with disease progression, the predictive value of specific HIV RNA concentrations for disease progression and death for an individual child is moderate.\textsuperscript{22} 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 in older children.\textsuperscript{19} In both children and adults living with HIV, CD4 cell count or percentage and plasma viral load are independent predictors of disease progression and mortality risk, and use of the two markers together more accurately defines prognosis.\textsuperscript{22,23,25,26}

**Methodological Considerations in Interpretation and Comparability of HIV RNA Assays**

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 twofold (0.3 $\log_{10}$ copies/mL) or more.\textsuperscript{27,28} If possible, because of the variability among assays in techniques and quantitative HIV RNA measurements, a single HIV RNA assay method should be used consistently to monitor an individual patient.\textsuperscript{29-31}

The predominant HIV-1 subtype in the United States is subtype B—the subtype for which all initial assays were targeted. Current kit configurations for all companies have been designed to detect and quantitate essentially all viral subtypes, with the exception of the uncommon O subtypes.\textsuperscript{32,33} 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 are infected with non-B viral subtypes.\textsuperscript{29,34-38} 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 measurement of plasma viral load using the same assay can vary by as much as threefold (0.5 $\log_{10}$ copies/mL) in either direction over the course of a day or on different days.\textsuperscript{25,28} This biologic variation may be greater in infants and...
young children with HIV infection. This inherent biologic variability must be considered when interpreting changes in plasma viral load in children. Thus, on repeated testing, only differences greater than fivefold ($0.7 \log_{10}$ copies/mL) in infants younger than 2 years and greater than threefold ($0.5 \log_{10}$ copies/mL) in children aged 2 years and older 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. Interpretation of plasma viral load for clinical decision making should be done by or in consultation with an expert in pediatric HIV infection because of the complexities of HIV RNA testing and the age-related changes in plasma viral load in children.

Based on accumulated experience with currently available assays, viral suppression is currently defined as a plasma viral load below the detection limit of the assay used (generally <20 to 75 copies/mL). This definition of suppression has been much more thoroughly investigated in adults than in children with HIV infection (see the Adult and Adolescent Antiretroviral Guidelines). Temporary viral load elevations (“blips”) between the level of detection and 500 copies/mL often are detected in adults and children on ART and should not be considered to represent virologic failure as long as the values return to below the level of detection at the time of repeat testing. 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 viral suppression and virologic failure are recommended for clinical use. Research protocols or surveillance programs may use different definitions.

Table 3. Sample Schedule for Clinical and Laboratory Monitoring of Children Before and After Initiation of Antiretroviral Therapy

<table>
<thead>
<tr>
<th>Entry Into Care¹</th>
<th>Pre-Therapy²</th>
<th>ART Initiation³</th>
<th>Weeks 1–2 on Therapy</th>
<th>Weeks 2–4 on Therapy</th>
<th>Every 3–4 Months⁴</th>
<th>Only Required Every 6–12 Months⁵</th>
<th>ARV Switch</th>
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<td>History and Physical</td>
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<tr>
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<td>Hepatitis B Screening⁶,⁷</td>
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</table>

¹ See text for details on recommended laboratory tests to obtain.

² Readiness for ARV adherence is assessed prior to starting ART. If abacavir is being considered as part of the regimen, send HLA-B*5701 testing prior to initiation of that ARV and choose an alternative ARV if HLA-B*5701 is positive (see Abacavir in Appendix A: Pediatric Antiretroviral Drug Information). Genotype resistance testing is recommended if not already performed (see Antiretroviral Drug-Resistance Testing in the Adult and Adolescent Antiretroviral Guidelines). Send tests appropriate to the toxicities expected from each patient’s ART regimen and history (see text).

³ If ART is initiated within 30 to 90 days of a pre-therapy lab result, repeat testing may not be necessary.

⁴ CD4 cell count can be monitored less frequently (every 6–12 months) in children and youth who are adherent to therapy and have CD4 cell value well above the threshold for opportunistic infection risk, sustained viral suppression, and stable clinical status for more than 2 to 3 years.

⁵ If lipids have been abnormal in the past, more frequent monitoring might be needed. For patients treated with TDF, more frequent urinalysis is considered.
Table 3. Sample Schedule for Clinical and Laboratory Monitoring of Children Before and After Initiation of Antiretroviral Therapy, continued

6 When considering starting ARV drugs with activity against hepatitis B, specifically lamivudine-, emtricitabine-, and tenofovir-containing regimens

7 Recommended only if individual previously demonstrated no immunity to hepatitis B

Key to Acronyms: ART = antiretroviral therapy, ARV = antiretroviral, CBC = complete blood count, CD4 = CD4 T lymphocyte

Table 4. Primary, FDA-Approved Assays to Monitor Viral Load

<table>
<thead>
<tr>
<th>Assay</th>
<th>Abbott Real Time</th>
<th>NucliSens EasyQ v 2.0</th>
<th>COBAS Ampliprep/TagMan v 2.0</th>
<th>Versant v 1.0</th>
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</thead>
<tbody>
<tr>
<td>Method</td>
<td>Real-time RT-PCR</td>
<td>Real-time NASBA</td>
<td>Real-time RT-PCR</td>
<td>Real-time RT-PCR</td>
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<tr>
<td>Dynamic Range (copies/mL)</td>
<td>40–10^7</td>
<td>25–10^7</td>
<td>20–10^7</td>
<td>37–11x10^7</td>
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<tr>
<td>Specimen volume*</td>
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<td>0.1–1 mL</td>
<td>1 mL</td>
<td>0.5 mL</td>
</tr>
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<td>Manufacturer</td>
<td>Abbott</td>
<td>bioMerieux</td>
<td>Roche</td>
<td>Siemens</td>
</tr>
</tbody>
</table>

* Note: Smaller volumes for children can be accommodated.

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

References


Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection


Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection


