RECOMMENDATIONS ON THE DIAGNOSIS OF HIV INFECTION

IN INFANTS AND CHILDREN

DRAFT FOR PUBLIC REVIEW (Ver 6)

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<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
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<tr>
<td>AFASS</td>
<td>accessible, feasible, affordable, safe, sustainable</td>
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<tr>
<td>ART</td>
<td>antiretroviral therapy</td>
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<tr>
<td>ARV</td>
<td>antiretroviral (drugs)</td>
</tr>
<tr>
<td>AZT</td>
<td>zidovudine</td>
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<tr>
<td>bDNA</td>
<td>branched chain DNA</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CPT</td>
<td>co-trimoxazole preventive therapy</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DNA</td>
<td>deoxyribose nucleic acid</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<tr>
<td>EIA</td>
<td>enzyme immunoassays (also known as enzyme linked immunosorbent assay)</td>
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<tr>
<td>[ELISA]</td>
<td>immune complex dissociated</td>
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<td>ICD</td>
<td>immune globulin G</td>
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<tr>
<td>IMCI</td>
<td>Integrated Management of Childhood Illness Strategy</td>
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<td>LIP</td>
<td>lymphocytic interstitial pneumonia</td>
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<tr>
<td>MEIA</td>
<td>microparticle enzyme immunoassay</td>
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<tr>
<td>MTCT</td>
<td>mother-to-child transmission</td>
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<tr>
<td>NASBA</td>
<td>nucleic acid sequence-based amplification</td>
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<tr>
<td>PA</td>
<td>particle agglutination</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PMTCT</td>
<td>prevention of mother-to-child transmission</td>
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<tr>
<td>p24Ag</td>
<td>p24 antigen test</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>RT PCR</td>
<td>reverse transcriptase PCR</td>
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<tr>
<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>WB</td>
<td>western blot</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>VCT</td>
<td>voluntary counselling and testing</td>
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<td>VL</td>
<td>viral load</td>
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INTRODUCTION

The majority of HIV infected children acquire the virus from their HIV infected mothers around the time of delivery or during breastfeeding. The most efficient and cost effective way to tackle paediatric HIV globally is, therefore, to reduce mother-to-child transmission (MTCT). However, every day there are nearly 1500 new infections in children under 15 years of age, more than 90% occurring in the developing world [1, 2]. HIV-infected infants frequently present with clinical symptoms in the first year of life, by one year of age an estimated one-third of infected infants will have died, and about half by 2 years of age [2, 3]. Early recognition of HIV exposure and diagnosis of HIV is crucial and can save lives by enabling early initiation of appropriate care including ART.

WHO recommends the routine offering of HIV antibody testing to all pregnant women as standard practice in antenatal care as a first step towards preventing HIV infection in the unborn infant. In addition, the knowledge of the mother’s HIV status provides an entry point for appropriate care for the mother, the child and other family members who may be infected with and affected by HIV. On the other hand, unknown maternal HIV status inhibits implementation of interventions that reduce the risk of MTCT of HIV and of interventions to ensure the health of the HIV-exposed infant, including co-trimoxazole preventive therapy (CPT) and early HIV diagnostic testing.

Early diagnosis of HIV infection in infants and young children is crucial for both individual and public health purposes as it facilitates medical management, reducing morbidity and mortality and improving quality of life. Knowledge of HIV infection status in an infant may also guide decisions related to feeding: an infant with confirmed HIV infection may accrue more benefit from continued breastfeeding than from stopping breastfeeding at an early age. On the other hand, in HIV-exposed infants found not to be HIV-infected, the risk of post-partum MTCT of HIV can be reduced if replacement feeding is accessible, feasible, affordable, safe and sustainable (AFASS) and the mother chooses not to breastfeed. Psychological support to the mother or caregiver will have a greater impact if it can be tailored according to the HIV infection status of the infant or child. In addition, early diagnosis offers societal benefits that extend beyond economic savings [4]. In many resource-limited countries however, the follow-up of infants exposed to HIV through maternal HIV infection and the early diagnosis of HIV thereafter in the exposed infant is neglected. Diagnostic protocols for HIV testing in infants are often considered too costly and complex for resource-limited settings. The higher cost and greater expertise required to perform tests that reliably identify infection in infants and children aged less than 18 months (i.e., virological testing methods) and the health system requirements for specimen collection and their transport to referral laboratories often deters programmes from establishing services to ensure early diagnosis of HIV infection.

The present publication is part of WHO’s commitment to achieve universal access to antiretroviral therapy (ART) by 2010. Related publications include the revised treatment guidelines for infants and children [5], revised treatment guidelines for adults (i.e. the 2006 revision), revised guidelines on ARV drugs for treating pregnant women and preventing HIV infection in infants, guidelines on the use of co-trimoxazole preventive therapy¹ and revised WHO clinical staging for adults and children [6].

OBJECTIVES OF THE GUIDELINES

¹These three documents are currently in preparation and are expected to be published by WHO in 2006.
The publication summarizes current knowledge on the diagnosis of HIV infection in infants and children and sets out recommendations for practice and policy. Recommendations are made for systematic early diagnosis of HIV in infants and children and reduced postnatal transmission of HIV, and for the purpose of improved clinical management of the HIV-exposed and infected child. Recommendations on HIV testing approaches are provided for prevention of mother to child transmission (PMTCT) follow-up settings and other health care services where infants and children may frequently present with or without signs and symptoms suggestive of HIV infection. The timing of diagnosis is also discussed considering breastfeeding, and the use of ARV drugs for MTCT prevention and treatment of the HIV infected mother.

WHO HIV case definitions for clinical and surveillance purposes have been revised in 2006 (REFERENCE) (Annex #); HIV cases diagnosed and not previously reported in a country should be reported according to a standard national case definition. Countries are therefore encouraged to develop and regularly review their testing algorithms based on the recommendations provided and according to the prevailing resources situation, for diagnostic and surveillance purposes.

This publication is primarily intended for use by national advisory boards, national reference laboratories, national AIDS programme managers and other senior policy-makers who are involved in the planning of national HIV preventions and care strategies for infants and children in resource-limited countries. They may also be used by child health care providers, professional bodies advising national programmes and developing treatment and care guidelines. Separate guidance on implementation for national programmes and laboratories is referenced.

DEVELOPMENT OF THE GUIDELINES

This publication is based on the work of an international group of experts who participated in several technical consultations on care, treatment and support for women living with HIV/AIDS and their children in resource-limited settings. In providing their advice, the experts considered best available scientific evidence: The recommendations were based on evidence from randomized controlled trials, high-quality scientific studies, or observational cohort data, or, if insufficient evidence was available, on expert opinion, and they have been identified as such. The strength of the recommendations has been indicated as a guide to the degree to which they should be considered by country programmes (Table #). Cost-effectiveness was not explicitly considered as part of these recommendations; the realities though with regard to laboratory infrastructure (i.e. availability of virological tests) as well as financial and human resource have been taken into account.

Following a consultation of the working group in Geneva on 19-20 April 2006 where draft guidelines were reviewed by a designated working group, the document was sent to institutional and organizational partners worldwide and made available on the WHO website for public consultation during the period ### 2006. All comments were considered and addressed, as appropriate, in the final document, which has been reviewed again by the group of experts. A list of experts who provided assistance in developing this publication is provided in Annex #.
Table #. Grading of recommendations and levels of evidence

<table>
<thead>
<tr>
<th>Strength of recommendation</th>
<th>Level of evidence to guide recommendation</th>
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<tbody>
<tr>
<td>A. Recommended – should be followed</td>
<td>I. At least one randomized controlled trial with clinical endpoints or several relevant(^a) high-quality descriptive or observational studies.</td>
</tr>
<tr>
<td>B. Consider – applicable in most situations</td>
<td>II. At least one randomized controlled trial with surrogate markers, at least one high-quality study or several adequate studies.</td>
</tr>
<tr>
<td>C. Optional</td>
<td>III. Observational cohort data, one or more case- controlled or analytical studies adequately conducted.</td>
</tr>
<tr>
<td></td>
<td>IV. Expert opinion based on evaluation of other site or programme experience.</td>
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</table>

\(^a\)Refers to studies that are representative of age, sex, genetic background and geography.

Source: Adapted from references [7, 8], and:
WHO Evidence Network, [http://www.euro.who.int/HEN/Syntheses/hepatitisC/20050408_5](http://www.euro.who.int/HEN/Syntheses/hepatitisC/20050408_5)
Part A. GENERAL CONSIDERATIONS ON HIV DIAGNOSTIC TESTING AND EARLY DIAGNOSIS OF HIV INFECTION IN INFANTS AND CHILDREN

Infants and children are primarily tested in provider-initiated or parent/caretaker-initiated diagnostic settings rather than through traditional, adult-focused client-initiated counselling and testing programmes (often called voluntary counselling and testing [VCT]). National policies need to be clear in their recommendations on how to provide HIV testing services to infants and children, including those children without a parent or legal guardian, and ensure national tools and resources provide clear specific guidance on who can perform HIV testing, how to elicit informed consent of and for the child, and on requirements for counselling and disclosure for HIV testing in infants and children.

If HIV infection is diagnosed in an infant or older child, usually the mother herself is also HIV infected and other siblings or family members may also be infected; HIV testing and counselling should be offered to other family members. The diagnosis of HIV infection in a child has thus major implications not just for the child but also - where existing - for the family. Legal and policy specification need to be in place to define a) how HIV testing is provided to children without legal guardians able to provide consent and b) ensure that consent is not a barrier to access to HIV care, or c) that HIV testing is used to deny access to care treatment or other social and welfare services including education.

It should be noted that in this publication, diagnosis of HIV is discussed in the context of clinical management, i.e. detecting HIV infection to facilitate and improve clinical management.

METHODS FOR EARLY DIAGNOSIS OF HIV INFECTION IN INFANTS AND CHILDREN

The definitive diagnosis of HIV infection at any age requires diagnostic testing that confirms the presence of human immunodeficiency virus. Antibody testing identifies HIV antibody generated as part of the immune response to HIV infection. In children older than 18 months of age, antibody testing should be used in the same manner as in adults (IA). However, as maternal HIV antibody transferred passively during pregnancy can persist for as long as 18 months in children born to HIV-infected mothers [9, 10], the interpretation of positive HIV antibody test results is more difficult in children below this age. In order to diagnose HIV infection definitely in children aged under 18 months, assays that detect the virus or its components (i.e. virological tests) are therefore required (IA). A range of laboratory based techniques are available, and these are discussed in more detailed in the following section.
LABORATORY BASED METHODS FOR EARLY DIAGNOSIS OF HIV INFECTION

VIROLOGICAL ASSAYS FOR THE EARLY DEFINITIVE DIAGNOSIS OF HIV INFECTION

GENERAL CONSIDERATIONS IN SELECTION OF VIROLOGICAL TESTS

HIV infection is diagnosed by detecting presence of HIV nucleic acid (i.e., viral RNA or viral DNA) often called nucleic acid tests (NAT) or viral components (p24); these are referred to as virological tests in the rest of the document.

Many methods for the detection of nucleic acids are commercially and non commercially available (see box ## below that describes nucleic acids). While some are standard in molecular biology laboratories, many are complex, technically demanding or inappropriate for non specialist diagnostic laboratories. Polymerase chain reaction (PCR) HIV DNA and HIV RNA assays [11-15] have become the most widely used assays, even in resource-limited settings. Newer assays, such as real time PCR and ultra-sensitive reverse transcriptase (RT) test [16-19], are valuable technologies to detect HIV DNA and/ or HIV RNA (See Box #). In settings with limited laboratory facilities, Ultrasensitive 24 antigen detection (Up24) may also be used as an less technical and somewhat lower cost alternative to PCR for diagnosis of HIV-1 in infants and children [18, 20-27]. HIV Culture is no longer a used or recommended method of diagnosing HIV infection due to the cost and complexity. (Box ##) briefly overviews methods of detection of HIV nucleic acids and table ## the major advantages and disadvantages thereof). The need for specific equipment, trained personnel, and consistent quality control of laboratory practice limit the availability of molecular-based diagnostic techniques for HIV DNA and RNA. In addition, the reagents and consumables required for performing the assays remain expensive.

BOX ###  Nucleic acids

Nucleic acids are complex, high-molecular-weight molecules made up of nucleotide chains. The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are found in all living cells and viruses.

Deoxyribonucleic acid (DNA) is a nucleic acid; each strand of DNA is a chain of nucleotides, usually organized as two complementary strands. In most living organisms (except for viruses), genetic information is stored in DNA. In cells such as those from plants, animals, fungi and protozoa, most of the DNA is located in the cell nucleus.

Ribonucleic acid (RNA) is another nucleic acid closely related to DNA. In some organisms such as viruses RNA is the primary carrier of genetic information, and is also important in the production of proteins. RNA serves as a genetic messenger, relaying the information stored in the cell's DNA out from the nucleus to other parts of the cell where it is used to help make proteins. Unlike DNA, RNA is almost always a single-stranded molecule and has a much shorter chain of nucleotides and is not always found in the cell. It is transcribed from DNA by enzymes.

The HIV virus is a retrovirus that uses RNA as its genetic material. It relies on the enzyme reverse transcriptase to perform the reverse transcription of its genome from RNA into DNA.
Box # HIV NUCLEIC ACID DETECTION 
*(PCR, and real time PCR)*

Newer techniques allow characterization, isolation, measurement and manipulation of DNA and RNA. Several nucleic acid amplification techniques exist. Polymerase chain reaction (PCR), nucleic acid sequence-based amplification (NASBA), and transcription-mediated amplification are all amplification techniques that use different approaches to achieve the in vitro amplification of HIV nucleic acids.

The *polymerase chain reaction* (PCR) is an extremely versatile technique for copying both DNA and RNA allowing a single sequence to be copied millions of times. PCR uses DNA sequences as targets and so for detection of RNA viruses, an initial reverse transcription step which converts RNA to DNA is used. PCR involves a series of steps performed using specialized equipment that is conducted in tertiary laboratory facilities and requires trained staff. Its use is not restricted to detection of HIV nucleic acid.

**Reverse Transcription PCR( RT-PCR)** is a method used to amplify, isolate or identify a known sequence from RNA. Transcription by Reverse transcriptase (to convert the RNA to DNA) is followed by PCR amplification.

**Quantification of HIV RNA** is useful in children and adults for monitoring the progression of HIV disease and response to ART. It is not however a prerequisite for initiating or continuation of ART.

**Real time PCR** quantifies HIV nucleic acid specifically, sensitively and reproducibly; it is cheaper, easier to standardize, reliable, rapid, and adaptable to the different sub-types of HIV-1 (including Group O) and HIV-2 than earlier methods that detect the amount of final amplified HIV nucleic acid. As opposed to conventional PCR methods, in real time PCR product accumulation is measured using a closed-tube detection system. While this requires specialized equipment, due to the closed nature of the system it is less likely to produce false positive signal generation.

General factors that need to be considered in selecting methods and equipment for performing virological testing for diagnosis of HIV infection in infants include:

- equipment required
- commercial availability of equipment and reagents
- training and availability of laboratory staff
- cost of equipment and reagents
- volume of sample specimens required
- number of samples required to be processed (sample throughput)
- specimen storage and transport
- level of laboratory capacity
- ongoing laboratory quality assurance
- availability of maintenance and service of equipment
- numbers of tests required from what kind of geographical area
- viral types and subtypes
- sample collection and processing (including DBS specimen to machine)
- use for other purposes

The major advantages, disadvantages and characteristics of available virological testing modalities are summarized in *table XX*

**Specimens required for virological tests**

Phlebotomy
Depending on the type and volume of specimen required blood needs to be taken from the infant or young child for all methods of virological testing. Taking specimens of blood from infants and children is more difficult than taking blood from adults. This should only be conducted by persons trained in the techniques for blood collection from the paediatric patient.

Volume and type of specimens required

In general many of the virological tests require at least  of blood or serum to enable the test to be performed

Dried blood spots (also see section XX)

Blood sampling on filter paper has many advantages for the detection of HIV infection by all virological methods. Dried blood spots can be obtained by finger stick with a sterile lancet from older children or by heel stick with a sterile lancet from infants. This is then dropped onto absorbent specimen collection (filter) paper. The procedure is less traumatic than venepuncture, uses only a small volume of blood and is reliable for safe specimen collection and delivery at a significantly lowered cost. Training is still required to adequately perform specimen collection. Specimens require to be thoroughly is air dried for a minimum of 3 hours prior to packing or sending for testing, and the paper can be stored at room temperature, once dried, the blood samples are not infectious. Dried blood spots can also be used for serological testing [28-32]

Interpreting virological tests in infants

HIV Type and Subtype
The diversity of the HIV virus means that molecular laboratory techniques for identification of HIV DNA and RNA require constant monitoring and assessment. Virological tests for HIV nucleic acids also need to be able to detect the predominant subtypes found within the local target population. Children infected with HIV either during pregnancy, at the time of labor/delivery or through breastfeeding may acquire a variety of HIV subtypes and heterogeneity, coinfection, and recombinants are not uncommon.

Box ## Diversity of the HIV virus

HIV is a virus that replicates rapidly in the human host and due to high error rates of reverse transcriptase (RT) and frequent recombination, a characteristic of retroviruses, diversity of HIV genetic material easily results. Many of these new hybrid virus strains created by recombination or mutation do not survive, but those that infect more than one person are known as "circulating recombinant forms" or CRFs. Strains are classified into types, groups and subtypes based upon genetic similarities. The classification of HIV strains into subtypes (or clades) and (CRFs) is a complex issue and subject to change.

HIV 1
The strains of HIV-1 can be classified into three groups: group M (99% of infections
globally) group O and group N. Group O appears to be restricted to west-central Africa and group N -is extremely rare. Over 90% of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least ten genetically distinct subtypes (or clades) of HIV-1 (clades A, B, C, D, E, F, G, K and O). Subtypes C accounts for over 50% of HIV-1 infections globally followed by subtype A and B. Subtype B is dominant in Europe, the Americas and Australia. Subtype C is common in South Africa and India. Sub-types A and D are found in central and eastern Africa; and A/G in W. Africa. In China, inter-subtype recombinants between subtypes C and B are becoming common; whereas in Thailand, subtypes E and B and recombinants are seen. Geographical restrictions of HIV-1 are increasingly breaking down.

[33, 34]

**HIV-2**

HIV–2 is highly concentrated in West African countries. A number of strains of HIV-2 have been identified, classified into four subtypes (or clades A, B, C, D) which are not closely related to each other. Immunodeficiency caused by HIV-2 takes longer to develop, and HIV 2 is less frequently transmitted to children through MTCT.

Not all virological tests detect the subtypes as reliably. Further details on the performance of different virological tests with different HIV subtypes or strains are provided in annex ###

Currently available virological tests are not reliably able to diagnose HIV 2 infection and so serological testing is recommended to diagnose HIV 2 and can be used before 18 months in the same way that HIV-1 antibody testing is used. This should be confirmed after 18 months age. Vertical transmission of HIV 2 is unusual Further information is in section ###

**Timing of the virological testing**

Infants and children are infected by the HIV virus during pregnancy, during delivery and throughout breastfeeding. Infants infected in the pregnancy usually have detectable HIV virus at birth , and go on to progress more rapidly. Infants infected at the time of birth may take a short time to have detectable virus or its components. The sensitivity of all the methods of virological testing to detect HIV infection are therefore lower at birth. In infants with in-utero HIV-infection, HIV DNA and RNA can be detected in peripheral blood specimens obtained within 48 hours of birth. However, HIV DNA and RNA is not detected in early peripheral blood samples but becomes detectable at or after 1 - 2 weeks of age in infants with peri-partum acquisition of HIV. Therefore, the sensitivity of virological testing depends on the timing of performing the test[41]. Table # and Table ### provide data on sensitivity of HIV DNA HIV RNA and U p24Ag at various infant ages, in different settings. By six weeks of age almost all children infected prior to, at or around birth can be identified by virological testing.

Infants and children who continue to breastfeed are at ongoing risk of acquiring HIV infection and so virological testing where negative cannot reliably exclude HIV infection. Once breastfeeding is completely discontinued it is considered that virological tests conducted at least 6 weeks after discontinuation of breastfeeding can be relied upon i.e. the window period for virological testing after stopping breastfeeding is any time from 6 weeks . This is the same for all currently available methods of virological detection[42].

Commercially versus non commercially available tests
Commercially available reagents including primers are standardized but not always purchased with the equipment, and may not be suitable for subtypes or strains of HIV found locally. Locally produced reagents that have not been standardized may not be reliable and may compromise the reliability of the performance of the test. Non commercial/in house assays are not generally recommended for wide spread use by national programmes, but may be used where external ongoing laboratory quality assurance is in place and they are standardized.

Reliability of Virological tests in the presence of ARV exposure

There are theoretical concerns about the use of RNA or p24 antigen virological testing in infants who have been administered or are taking ARV prophylaxis for MTCT prevention or are breastfeeding where the mother is taking ART. The theoretical or potential problem arises because detection of viral RNA and p24 require viral replication, and ARV drugs inhibit viral replication. Administration of more potent antiretroviral drugs or combinations temporarily suppresses HIV RNA levels to low or undetectable levels [13]. However there is currently no data available to suggest that existing recommendations need to be revised for these different circumstances as virological testing should be conducted after ARV prophylaxis has been discontinued.[34, 36]

Refer to table ## & ## & ## for details of timing and regimens for virological tests

In order to build laboratory capacity, national HIV AIDS programmes should invest in quality assurance of all virological laboratories, and should use existing available services provided by WHO and others including CDC to support external quality investment schemes. Additional references and links are included in the annexes.

OVERVIEW OF VIROLOGICAL TESTS

HIV DNA testing

Qualitative HIV DNA PCR is currently widely used as the standard reference method for diagnosis of HIV infection in infants and is the test against which other tests are usually compared in research settings. [11, 40, 43]. Some of the main characteristics of this test pertaining to infant diagnosis can be summarized as follows:

DNA is found in the cell, and so detection of cell associated HIV DNA within peripheral blood mononuclear cells by PCR is one of the most sensitive non-serologic methods for establishing HIV infection in children less than 18 months of age. (see also Section # in annex ).

Table # in annex provides data on sensitivity of DNA PCR at various infant ages, in different settings.

HIV DNA tests are reliable in the presence of ARV exposure for MTCT prevention or maternal ART. HIV-1 DNA remains detectable in the peripheral blood mononuclear cells and lymphoid tissue of HIV-infected children who have received ART for several years and have undetectable viral replication as measured by HIV RNA assays [44].
The current commercially available HIV DNA PCR (ROCHE amplicor V 1.5) assay has acceptable sensitivity for detection of most common HIV-1 subgroups. Newer versions of Roche Amplicor have improved detection capacity for HIV subgroups A, B, C, D, E and G, although problems still exist in particular for HIV subgroups A and C. Further details regarding commercially available assays are provided in Annex #.

DNA PCR can be performed using dried blood spots (DBS) using whole blood on filter paper which facilitates collection, transport and storage of blood samples for laboratory use (see Section #) [11, 45, 46].

**HIV RNA Assays**

HIV RNA assays detect viral RNA from non-cell associated (free) virus particles in blood plasma and serum using a variety of methodologies. These include RT PCR, *in vitro* signal amplification nucleic acid probes (branched chain DNA [bDNA]), and nucleic acid sequence-based amplification (NASBA). Because of the possibility to quantify HIV RNA, these assays provide additional (quantitative) information on the child's virological status, and they are useful to follow HIV progression and response to ART. [12, 14, 34, 36, 37, 40, 47-49]. As RNA is found in free in the serum, specimen collection and handling is important [50].

- RNA detection methods are able to detect a wide range of viral subtypes [51] and more recent commercially available platforms have improved quantification of HIV-1 RNA regardless of the subtype [52].
- RNA detection methods allow quantification of HIV viral load and therefore provide additional information on status of the HIV infected child.
- RNA detection methods appear as or more sensitive than DNA PCR in the first few weeks of life [34, 40, 53, 54].
- Administration of maternal and infant ARV (zidovudine [AZT] or nevirapine) for PMTCT is associated with reduced replication of HIV however studies in general have shown no loss of sensitivity of regardless of infant or maternal ARV prophylaxis, [12, 14, 34].

Because low viral loads (<5,000-10,000 copies/ml) during the first year of life are very rare in HIV-infected infants not receiving ART [14], where capacity exists additional diagnostic testing using a repeat or different virological assay is recommended for an HIV-exposed infant with a low-level positive HIV RNA test (i.e., <1,000 copies/ml) to determine whether this is a true positive test result even with commercial assays.

**Ultra sensitive p24 antigen based testing**

p24 antigen assays measure the HIV viral protein p24 in blood. P24 antigen is found in serum in either free form or bound by anti-p24 antibody. When antibodies to HIV become detectable, p24 antigen is often no longer demonstrable, probably due development of antigen-antibody complex in the blood. Assays that use ultra-sensitive methods, including signal-amplification for p24 antigen allows smaller quantities of p24 antigen to be detected, and have improved the sensitivity of p24 antigen testing. The technical improvements have enabled the ultra-sensitive p24 antigen test to perform almost as well as PCR for diagnosis of HIV in infants. Several p24 antigen tests have been evaluated in clinical studies and compared with the most sensitive PCR methods available (summarized in Table #). [25]. Ultra-sensitive p24Ag assay has been validated in countries where HIV subtypes A, B, C, D, C and F predominate [26, 55]. When detected, p24 antigen is highly specific for HIV infection but their sensitivity depends on the type of test used (see...
WHOreroidiation in infants and children

Annex [38]). False positive results do occur, and it is usually considered necessary to confirm positive results. Only the newer Immune complex dissociated ultrasensitive detection methods offer sensitivity and specificity that approaches DNA and RNA methods.

- Advantages of p24 antigen based tests include that it is less complex, is in some cases less costly and can be done in lab settings than can perform EIA testing.
- Testing can be performed on, serum and cerebrospinal fluid (CSF). There is no need for nucleic acid extraction and samples are generally stable. A minimum requirement for laboratories would be the capacity to perform EIA.
- Current data suggest Up24 can be used up to the age of 18 months, although there is theoretical concern that the sensitivity may decline with increasing age.
- There are theoretical concerns about the use of Up24 antigen detection assays when maternal or infant ARV/ or ART is given to reduce MTCT. It is therefore suggested that Up24 can be only be used for detection if ARV prophylaxis has been discontinued.
- Optimisation of the standard assay with a specific buffer also improves assay performance, currently security and reliability of supplies of buffer and standard equipment is not sufficient to be able to recommend national programme use.

Considerations on the use of dried blood spots (DBS)

The time between sample collection and testing can be important as viral nucleic acids may degrade over time, particularly if stored at high ambient temperature (e.g. during transportation to the laboratory) for extended periods. It is thought that nucleic acid in cells (i.e. DNA) is more stable than cell free nucleic acid (i.e. RNA). Samples can be protected if transported rapidly at a cool temperature (2-10°C). However, transport and refrigeration are often problematic, in particular in remote or rural areas of resource-limited settings.

The use of Dried blood spots (DBS) or filter paper-based HIV- DNA or HIV RNA [56] testing and p24 antigen assays overcomes blood sampling and logistical obstacles that limit access to early HIV diagnosis in children in resource limited settings. DBS have been used successfully in resource-constrained settings to ease collection and transport of specimens. DBS require only a few droplets of blood (i.e., 50-100 µl, obtained from finger or heel stick) to be collected directly on filter paper [11]. In addition, DBS carry less of a biohazard risk than liquid samples, can be stored at room temperature for prolonged periods, and are easy to ship to central laboratory testing facilities [57].

Nucleic acids do degrade with time, and as results are usually urgently required for clinical purposes it is currently recommended that RNA virological testing on dried blood spots should be conducted as soon as possible. If required specimens can be stored once dried over night and specimens appear to be preserved for up to one year at room temperature [58, 59]). The stability to heat, humidity, and prolonged storage of DBS for HIV DNA detection is good, [58] and DNA specimens last at least up to 19 months. Up24 antigen appears to last for at least 15 months [60].

Use of DBS should be more widely implemented to improve access to virological testing in a range of resource-limited settings. Some national programmes have had good experience with implementing DBS HIV DNA in selected areas (South Africa and Botswana) and studies are on going, and DBS HIV-RNA has been successfully utilized by Cote D'Ivoire within the national programme. Standardization of materials, collection protocols, and assays, are required prior to recommending widespread implementation in clinical settings [56]. For example, if DBS are collected and transported to the diagnostic laboratory by mail, the
promptness and reliability of the system should be evaluated at each point in the chain of events (collection, mailing in, testing, reporting back, etc.).

Table # in the annex summarizes key characteristics of tests (RNA and DNA PCR and p24 antigen) in DBS samples.
HIV ANTIBODY TESTING (SEROLOGICAL TESTING)

Antibodies to HIV can be measured using a range of tests, although these do not detect the HIV virus itself, but detect the immune response to the virus, and therefore take some time to become positive (or reactive) after HIV infection has been acquired. Antibodies to HIV-1 and HIV-2 are detected by enzyme immunoassay ([EIA], also known as enzyme-linked immunosorbent assay [ELISA]). However because of the passive transfer of maternal HIV antibodies (IgG) across the placenta, HIV antibody tests are difficult to interpret in infants and children aged under 18 months; hence infants born to HIV infected women will initially test antibody positive, irrespective of their own infection status.

The three main types of antibody tests include:
- Rapid HIV EIA tests;
- Laboratory based EIA ; and
- Western Blot tests.

EIA was the first test developed for the detection of HIV antibodies. Most EIA tests currently available have high sensitivity and specificity, and are able to detect HIV-1/HIV-2 and HIV variants. A wide range of HIV serological antibody tests are available, and it is therefore important to identify the most suitable assays for a given set of programme circumstances.

General factors that need to be considered in selecting methods and equipment for performing serological testing for diagnosis of HIV infection in infants are similar to those for virological testing and include:
- commercial availability of reagents and equipment
- cost of tests
- training and availability of staff
- volume and type of sample specimens required
- number of samples required to be processed (sample throughput)
- ongoing laboratory quality assurance (to assess both rapid and EIA test kit performance )
- numbers of tests required
- need to detect HIV 1 and 2
- location of testing site
- sample collection and processing

These major advantages, disadvantages and characteristics of available serological testing are summarized in (table XX. Comparison of HIV antibody testing technologies: EIA and rapid tests) and further technical and operational characteristics are provided in provided in WHO Reference XXX.

WHO describes testing strategies based upon the purpose of the HIV testing and has developed standardized testing algorithms. These outline combinations of two or three antibody tests (EIAs and/or rapid tests or any combination of these) used to confirm HIV test results (Section #, Figure #). The first positive test should be confirmed with a second test, using a different test kit on the same sample. A well-selected combination of two different rapid tests has positive and negative predictive values comparable to EIA tests. Additional information can be found in other WHO publications.
WHO HIV diagnosis in infants and children

Box ##

**Diagnosis**
Serial Testing
Strategy (2 tests)
Prevalence \( \geq 5\% \)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Assay 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Result</td>
<td>Non-reactive Result</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Indeterminate</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Indeterminate</td>
</tr>
<tr>
<td>Non-reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Non-reactive Result</td>
<td>Report Negative</td>
</tr>
</tbody>
</table>

Refer clients for clinical follow-up and care.

**Use of 2 test recommended for sites with:**
- Limited capacity
- Low volume testing

**The majority of indeterminates are negative, however, some will be positive. Repeat test after 14 days or refer for further testing.**

Refer clients for further testing.

Box##

**Diagnosis**
Serial Testing
Strategy (3 tests)
Prevalence \(<5\%\) 

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Assay 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Result</td>
<td>Non-reactive Result</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Presumptive Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Presumptive Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Presumptive Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Presumptive Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Presumptive Positive</td>
</tr>
<tr>
<td>Reactive Result</td>
<td>Report Negative</td>
</tr>
</tbody>
</table>

Use of a 3rd test as tie-breaker recommended when feasible to resolve HIV status. Considerations include:
- High prevalence
- High volume testing
- On-site capacity/ ease of referral

Presumptive positive will include some false positives.
Serological testing may be done serially or in parallel. Serial testing refers to the performance of the second, confirmatory test after an initially positive result. In parallel testing, the two separate tests are performed at the same time. WHO recommends that serial testing be used except in settings where a very rapid result is urgently required (e.g. on the labour ward) where parallel testing may be preferred.

Selection of the serological test

Specimens required for serological tests

Serological tests on whole blood or oral fluid specimens have now been developed and make point-of-care (POC) HIV testing feasible. In most cases, a blood sample is tested, but other types of EIAs that use saliva and urine have also been developed. Originally HIV serological testing was designed for performance on serum or plasma which requires preparation of the specimen and use of reagents that often require refrigeration. Using whole blood the specimen requires no processing, and so the need for equipment (such as a centrifuge) has been avoided.

Other specimens may be used, but are not yet generally recommended for routine use in diagnosis of HIV infection within national programmes (AI). A report comparing oral fluid HIV tests with serum testing results suggests that use of the former reduces the percentage of infants and children requiring repeat HIV tests from 45% to 8-12%. The ability of oral fluid and serum to predict an HIV-uninfected status were comparable with negative predictive values of over 99 per cent [61].

Interpreting serological tests in infants
HIV diversity

Some serological tests can only detect antigens or antibodies to HIV-1 and others reliably detect HIV-2 antibodies, and some differentiate HIV-1 from HIV-2. Despite the variation of HIV subtypes commercial EIA serological tests based on HIV-1 subtype B proteins appear to detected all HIV 1 infections [62, 63]. Newer rapid tests commercially available that reliably detect HIV 1 and 2 appear to offer reliable detection of both HIV 1 and HIV 2 compared to EIA assays [64].

Timing of the serological testing

The performance of most EIA and rapid tests are reliable within 7-21 days of acquiring infection in adults. The most recent advances in EIA technology have produced "combination assays" which combine p24 antigen EIAs with traditional antibody EIAs, allowing for the simultaneous detection of HIV antigen and antibodies using a single test. This approach has further shortened the window period, i.e. the interval between HIV infection and detectable HIV antigen/antibodies. Data from seroconversion panels demonstrate the analytic sensitivity of many newer rapid assays to be comparable to that of the EIAs [65, 66]. It is estimated that HIV antibody is usually detectable usually within 14 - 21 days of acquiring HIV infection, although this does depends on the assay used. Rapid tests appear to offer similar performance characteristics but they detect antibody 2-8 days later than third-generation EIAs [67].

All children infected born to HIV infected mothers carry detectable maternal HIV antibody and this declines slowly over the first few months of life. The rate of decay of maternal antibody has been ascertained largely by analysis of studies to detect HIV antibody in children who have not been breastfed. The mean and/or median age at the time of seroconversion ranges between 9 months to 16 months of age in studies from both Western and resource-limited countries [9, 68-73]. These data indicate that maternal antibody levels of HIV antibody remain detectable through the first 6 months of life but decay significantly by 9-12 months of age, and most HIV uninfected children do not have detectable antibody by one year of life; HIV antibody-positive testing in HIV-exposed asymptomatic children at this age can be considered indicative of HIV infection (i.e. 94.5% seroconversion at the age of 12 months) [74]. Similar findings have been observed in studies assessing decay of passively transferred maternal antibody for measles, and hepatitis A, although the decay of passively acquired antibodies occurred even more rapidly in Nigerian children than German children, resulting in susceptibility to measles in most of the Nigerian infants by 4-month-of age. While the majority of uninfected non-breastfed children will have cleared maternal antibody by age 12 months, a small percentage of children do not seroconvert until age 18 months [9, 69] and in rare instances even beyond [68]. Further analysis and studies are required to determine the rate of decay of maternal HIV antibody in HIV exposed infants, particularly in African, Latin American and Asian children.

Table # lists timing of antibody seroconversion as provided by different reports.

HIV Antibody testing can therefore be useful in younger infants to indicate HIV exposure in children where maternal HIV status is not known. When HIV antibody is detected in older infants from the age of 9-12 months depending on the results the following interpretations can be made:

- If negative and no longer HIV exposed through breastfeeding (i.e. => 6 wks post weaning)- HIV infection in unlikely.
WHO HIV diagnosis in infants and children

- If positive; regardless of current breastfeeding children are likely to be HIV infected and virological testing should be used to definitively confirm HIV infection status.

These are summarized in the algorithms in annex ###:

For the purposes of testing in children in relation to breastfeeding, the window period before antibody testing can be performed after cessation of breastfeeding using rapid or laboratory based EIA is recommended to be six weeks (A IV)

Isolated unpublished reports of negative rapid HIV tests in sick children in children who then go onto be HIV infected need to be investigated. Little data are available on antibody response in HIV infected young infants, in older children antibody levels are raised[75] [76]

Reliability of serological tests in the presence of ARV exposure

The use of serological tests to diagnose infection in infants is not affected by the use of ARV drugs for MTCT prophylaxis. Because antibody testing is qualitative (i.e. it identifies whether or not antibodies to HIV exist), the test results should not be influenced by prior exposure of the infant or child to ARVs. There are reports of infants infected at the time of delivery commencing ART very early and becoming HIV seronegative by age XX.

Commercially versus non commercially available tests

The sensitivity and specificity of different tests varies, and test devices or equipment can be made by a company but distributed and sold under several brand names. WHO, through its Department of Essential health Technologies (EHT) periodically evaluates EIAs and rapid tests that are available for bulk purchase. The evaluations are done voluntarily, usually at the request of the manufacturer, and results of these evaluations are made available via the internet. (Ref http://www.who.int/eht/en/ )Operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human sera. Geneva, Switzerland: United Nations Programme on AIDS and World Health Organization). It is not recommended that rapid or EIA serological tests not evaluated by nationally or internationally recognized laboratories be used . See above for further recommendations on selection of test kits.

OVERVIEW OF SEROLOGICAL TESTS

Rapid HIV EIA tests

Several rapid screening tests with diagnostic performance comparable to that of traditional lab based EIA (i.e., sensitivity > 99% and specificity >99%) have become available and may be particularly appropriate for use in resource-constrained settings since they can be performed in clinic or community settings and little laboratory equipment is required. Only rapid tests
recognized and validated in national reference or international reference laboratories should be used.

Rapid HIV assays can be based on several test formats. These tests are designed for use with individual, specimens are quick and easy to perform (less than 30 minutes, little or no additional equipment), making them more efficient in low throughput laboratories than EIA tests.

Most rapid tests are presented in a kit form incorporating the reagents and not requiring additional equipment. As the procedures are very easy, involve a limited number of steps, and do not require high precision, there is less chance of error and they can be carried out by any health care worker who has received appropriate training. Test results become available within 10-30 minutes, their interpretation is in general straightforward and many rapid tests include an internal control which validates the test result. Rapid tests are designed either as single tests or in a multiple format suitable for a limited number of specimens, which allows for flexibility in the number of tests to be performed at a time. Most rapid HIV test kits can be stored at room temperatures (2–30ºC, consult test kit insert).

**Laboratory based EIA**

EIA was the first test developed for the detection of HIV antibodies. Most EIA tests currently available have high sensitivity and specificity, and are able to detect HIV-1/HIV-2 and HIV variants. The most recent advances in EIA technology have produced "combination assays" which combine p24 antigen EIAs with traditional antibody EIAs, allowing for the simultaneous detection of HIV antigen and antibodies using a single test. This approach has further shortened the window period, i.e. the interval between HIV infection and detectable HIV antigen/antibodies.

In most cases, a blood sample is tested, but other types of EIAs that use saliva and urine have also been developed.

EIAs are designed specifically for screening large numbers of specimens (usually 80 or more) for batch testing, making them particularly cost-effective for use in surveillance and centralized blood transfusion services and less suitable for smaller numbers of samples. An EIA procedure takes usually 2 hours, hence high volume laboratories are able to report on the EIA results the same or next day. However, laboratories with limited numbers of samples need to wait until they have at least 40 samples to test (½ EIA plate + controls), often causing delays of several days to 2 weeks in availability of results.

EIAs require sophisticated equipment, and are technically demanding; automatic pipettes, incubators, washers, readers and a constant electricity supply must be available. The equipment needs to be regularly maintained and adjusted to ensure accuracy of test results. The validity of the test results depends on skilled technicians who are able to prepare correctly the necessary reagents, pipette with accuracy and operate the equipment. The requirements are however less than for common methods of DNA or RNA detection, for which higher capacity of laboratory facilities are needed.

The performance characteristics of commonly used antibody tests have been previously summarized [77], and many have had performance assessed by WHO; details are available on http://www.who.int/eht/en/. Table # summarizes the general and operational characteristics of EIA and rapid tests.
Serological HIV antibody testing using commercially available laboratory based EIA or rapid tests can be used to:

- Diagnose HIV infection in children from the age of 18 months (A1), and;
- in non-breastfeeding infants and children under the age of 18 months detect children who are not likely to be HIV infected (A1)
- identify HIV exposed children remaining HIV seropositive aged 9 - 18 months and who are likely to be HIV infected and need virological testing.
- in areas of high HIV prevalence, where PMTCT coverage is still low and children with HIV infection commonly present in child health clinics and in settings such as malnutrition or tuberculosis clinics, can be used to identify sick infants who are HIV exposed and or infected (A IV);
- can be used to recognize HIV exposure and/or diagnose HIV infection in children who have never breastfed (A I)
- can be used to recognize HIV exposure and/or diagnose HIV infection in children who ceased breastfeeding completely for 6 weeks or more (A IV.).

Western Blot assay

The Western Blot (WB) is only used as a confirmatory test of another positive antibody test (usually EIA). The test consists of a multi-layer process similar to that of the EIA test. HIV antigens are laid out - from largest to smallest - on a strip of film. When patients serum is added, any existing HIV antibodies bind to these HIV antigens. Addition of enzyme leads to antibody-enzyme complex. In a final step, a chemical is added that changes color when it comes into contact with the protein-antibody-enzyme layers.

The WB can be positive, negative, or indeterminate. Indeterminate tests are neither positive nor negative, usually reflecting the beginning of seroconversion at the time of the test. In these rare situations, retesting should be performed, usually about one month later. Western blot testing requires sophisticated equipment, is technically demanding; and with the improvement in performance characteristics of antibody and virological testing is no longer recommended as a required confirmatory HIV test for adults or children.

LABORATORY QUALITY ASSURANCE

Having highly accurate tests does not necessarily guarantee reliable laboratory results. Many processes are involved from the time the specimen is taken, arrives in the laboratory and until results are recorded, during which errors can occur. Therefore, the ongoing quality assurance
of the laboratory system, both internally and externally, is essential. Laboratory
reliability becomes even more important in light of the simplified testing recommendations
(i.e. reliance on one positive virological test result only) within a public health approach, as
outlined in Section # in this publication. More details on national quality assurance and
laboratory quality assurance are provided in additional reference materials.
Clinicians and staff providing laboratory services need regular communication about the
performance of tests to improve and ensure appropriate performance. Well defined Standard
operating procedures (SOP), following nationally defined and assessed algorithms are
essential for optimal use of rapid or lab based serological testing for HIV.

Example SOPs are included in the resource list in annex X.
SUMMARY RECOMMENDATIONS

Table###
SUMMARY OF LABORATORY BASED HIV DIAGNOSTIC METHODS
Table # below summarizes the laboratory based methods for diagnosis of HIV described in the respective sections above

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV RNA Assays</td>
<td>Can be used for diagnosis in infant &lt;18 months of age. As sensitive and specific as DNA PCR, in the first weeks of life in infants Equipment is increasingly commercially available Good detection of most viral subtypes RT RNA PCR is becoming cheaper, is easy to standardize If the child is HIV-infected, quantitative RNA results can provide information about the risk of disease progression and assist in decisions about initiating antiretroviral therapy Can be performed on small volume about 500 - 700ul of plasma/whole blood Can be performed on DBS Equipment can be used for other diagnostic or monitoring purposes Used and evaluated in resource limited settings</td>
<td>Complex technology, need for dedicated equipment, space and skilled technicians Expensive False positive at low levels of viraemia (&lt;5000 copies/mL) may be possible and can be due to laboratory error Not all primers and reagents standardized Theoretical concerns about reduced sensitivity if mother is on ART and breastfeeding Technology only licensed or approved for monitoring purposes</td>
</tr>
<tr>
<td>HIV DNA PCR</td>
<td>Can be used for diagnosis in infants &lt;18 months of age High sensitivity and specificity (100% of infected children identified by age 6-weeks, slightly less if performed in children &lt;1 month) DNA PCR remain positive in infected child even if they are receiving antiretroviral therapy Can be done on small volume of plasma/whole blood (approx 100ul whole blood) Can be performed on DBS Standardized equipment commercially available Used and evaluated in resource limited settings</td>
<td>Complex technology, need for dedicated equipment, space, skilled technicians Few commercial assays available HIV-1 subgroup diversity can affect sensitivity of assay in different settings ? False positive results may result from laboratory error Technology and commercially available equipment only approved for use in diagnosis for research purposes.</td>
</tr>
<tr>
<td>Ultrasensitive ICD p24 antigen</td>
<td>Can be used for diagnosis in infants &lt;18 months of age Easier to perform than other virological assays Costs are similar to those of last generation PCR tests, primarily reagents and plate reader May be feasible using filter paper technology for plasma, not yet for whole blood Can be done on small volume of plasma</td>
<td>High specificity similar sensitivity as HIV DNA PCR or HIV RNA assay; negative result does not exclude infant HIV infection Sensitivity lower for children less than one month or younger Theoretical concerns about reduced sensitivity if mother is on ART and breastfeeding or infant is on ARV prophylaxis</td>
</tr>
<tr>
<td>HIV Antibody Test</td>
<td>Inexpensive, increasingly available does not require sophisticated laboratory technology In infants between 9-18 months of age, negative results (in non breastfed children) can be used to suggest absence of HIV infection in an infant born to an HIV-infected woman (or an HIV-exposed infant) Positive results in infants 9-12 months or more usually indicate HIV infection Confirmatory in children older than 18 months</td>
<td>Interpretation of positive results difficult in younger infant due to persistence of maternal antibody Cannot be used for confirmation of HIV infection in infants &lt;18 months of age due to persistence of maternal HIV antibody May be absent in very sick HIV infected infants</td>
</tr>
</tbody>
</table>
SUMMARY OF RECOMMENDATIONS ON LABORATORY BASED HIV DIAGNOSTIC METHODS FOR EARLY INFANT DIAGNOSIS OF HIV INFECTION IN INFANTS AMONG INFANTS <18 MONTHS

The following is a summary of recommendations made for national programmes to ensure early detection of HIV infection, facilitate early access to HIV treatment and reduce the risk of early mortality:

Recommendations for virologic testing for early infant HIV diagnosis

1. Given the high risk of death by 2 years for infected infants and the increasing availability of pediatric antiretroviral treatment in many resource limited settings countries should move as rapidly as possible to set up programs that ensure access to early infant virological testing for HIV be made available nation wide. (A I)

2. Currently available assays that should be considered by programs for early infant HIV diagnosis include appropriately ongoing externally validated commercially and non commercially available:
   - HIV PCR DNA (A I)
   - HIV RNA (AI)
   - Up24 (B III)

3. Laboratory capacity to perform virological testing using HIV-DNA and/or HIV-RNA should be made available at least at tertiary level and organized to facilitate national coverage by referral (A I).

4. National programmes should ensure a system is established to collect and transport pediatric specimens for early virological testing (at all levels of the health care system) and return results in a timely manner (A I).

5. Dried blood spots (DBS) can facilitate decentralization of access to virological diagnostic testing. Currently available methods for DBS testing using the following can be used:
   - HIV DNA (A I)
   - HIV RNA (AII)
   - HIV UP24 (C IV)

6. More evidence to support these recommendations should become available and published shortly.

Recommendations for use of serological tests to support for early infant diagnosis of HIV infection.

7. Antibody tests are readily available and if positive at any age < 18 months can identify those HIV exposed infants who need follow up virological testing (i.e. child is HIV exposed and or HIV infected)

8. Antibody tests if negative in a child < 18 months who is no longer breastfeeding and has not breastfed in the last 6 weeks mean the child is presumed to be uninfected, and virological testing is only indicated if clinical signs or subsequent events suggest HIV infection.
9. In all HIV exposed children confirmatory antibody testing at 18 months is recommended. However it is recognized that follow up testing of HIV exposed infants may be difficult to operationalize in many resource limited settings.

10. **Recommendations for Serologic HIV Antibody Testing for children over 18 months and general recommendations for pediatric HIV testing**

11. EIA Serological testing and Up24 Ag testing and can be performed at non tertiary levels of laboratory capacity.

12. Serological testing using rapid tests are more widely available, less costly than EIA and can be performed at all levels of service delivery in resource limited settings (A1)

13. Antibody tests (rapid or lab based EIA) are the preferred method of diagnosis for HIV infection, for in children from the age of 18 months, (A1) and older.

14. In areas of high HIV burden in paediatric populations rapid tests should be made widely available and used at POC for diagnosis of HIV infection in older sick children (A1) (Section #).

15. HIV antibody tests that detect both HIV 1 and 2 should be used.

16. In areas of high HIV prevalence, children with HIV infection may commonly present in settings such as hospital wards, malnutrition or tuberculosis clinics; routine diagnostic screening for HIV infection should be the standard of care in order to rapidly identify those who are HIV infected and enable early comprehensive HIV treatment and care.

17. National policies should address the most appropriate settings (i.e. in the health care system or settings which provide entry into health care) for such screening for HIV among children and consider the use of rapid tests for that purpose.

18. In generalized HIV epidemics national programmes may choose to ensure mothers attending child health immunisations clinics are routinely offered HIV testing for themselves and/or their infants at the 6 week immunization visit if their HIV status is unknown.

Some operational guidance to develop systems and support programme implementation is already available, more is required see the resource list provide at the end of the document.
Table ###. Summary of recommendations on use of HIV tests in infants and children

<table>
<thead>
<tr>
<th>Method of diagnosis</th>
<th>Recommendations for use</th>
<th>Strength of recommendation/ level of evidence</th>
</tr>
</thead>
</table>
| Virological methods | To diagnose infection in infants & children aged under 18 months; initial testing is recommended from 6 weeks of age | HIV DNA [A(I)]
|                     |                                                                                        | HIV RNA [A(I)]
|                     |                                                                                        | U p24 ag [CII] |
| HIV antibody testing| To diagnose HIV infection in mother or identify HIV exposure of infant                   | A(I) |
|                     | To diagnose HIV infection in children aged 18 months or more                            | A(I) |
|                     | To identify HIV-antibody positive children aged under 18 months and support a presumptive clinical diagnosis of severe HIV disease to allow initiation of ART | A(IV)\(^a\) |
|                     | To exclude HIV infection where HIV antibody negative in children aged under 18 months who are HIV exposed and never breastfed | A(I) |
|                     | To exclude HIV infection where HIV antibody negative in children aged under 18 months who are HIV exposed and discontinued breastfeeding for more than 6 weeks | A (IV) |

\(^a\)Children aged under 18 months who have positive HIV antibody tests include those who are truly HIV-infected and those who have persisting maternal antibody but are uninfected. By the age of 12 months most uninfected children have lost maternal antibody and positive antibody testing at this time usually indicates HIV infection, although confirmatory testing at 18 months is recommended.
Part B. SERVICE DELIVERY

ENTRY OF HIV EXPOSED INFANTS AND HIV INFECTED CHILDREN INTO DIAGNOSTIC PATHWAYS AND HIV CARE

Most infants with HIV infection are asymptomatic at birth. The identification and follow-up of infants born to HIV-infected women are a necessary first step in infant diagnosis. It needs to be emphasized that all children under 18 months of age who are known or suspected to have been exposed to HIV should be closely monitored and should benefit early in life from interventions such as CPT, even in situations where virological testing is not available for the definitive early diagnosis of HIV infection. Since close to half of HIV infected infants will die before their 2nd birthday without antiretroviral treatment, and pediatric treatment is becoming more widely available in resource limited it is imperative that programme efforts focus on introducing early infant diagnosis in resource limited settings.

In this section, recommendations for programmatic approaches to the delivery of diagnostic services are discussed.

Regardless of existing capacity, National Programme managers are encouraged to urgently establish policies on early HIV testing approaches in infants and children that are appropriate for the prevailing national epidemic and resource situation. These need to address:

- routine HIV diagnostic testing of HIV-exposed infants within PMTCT follow up services and the optimal care and follow up of HIV-infected pregnant women to prevent new infections in infants and children.
- HIV diagnostic testing of children in acute and chronic medical services and other settings where sick children come into contact with health services.
- define age-appropriate testing algorithms especially in relation to national breastfeeding practices and recommendations;
- promote the increased use of antibody testing in children;
- ensure existence of reliable laboratories (as per frequent standard quality assessments) with capacity to perform virological testing in infants and children age under 18 months;
- articulate requirements for consent, confidentiality and best interests of children.

Linkages of policies with operational guidance for implementation and to the existing national monitoring and evaluation system should be ensured.

Infants and children need HIV diagnostic services to be provided and will enter into health services either well or sick with maternal status known, or well or sick with maternal HIV status unknown.
Services need to be configured to optimize infants and children access to diagnostic testing for HIV.

### Key Testing considerations

<table>
<thead>
<tr>
<th>Children known to be HIV exposed</th>
<th>Sick Children - where maternal HIV status is unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV testing usually desired</td>
<td>HIV not anticipated</td>
</tr>
<tr>
<td>Often assumed to be infected</td>
<td>May be index HIV infection within family</td>
</tr>
<tr>
<td>Earlier diagnosis facilitates optimal early HIV care</td>
<td>Often associated with severe or advanced disease - HIV care urgently required</td>
</tr>
<tr>
<td>Systematic testing recommended</td>
<td>Usually initiated by the health care provider</td>
</tr>
<tr>
<td>May be initiated by Health care provider or family</td>
<td>Less time to adjust or accept HIV testing by child, parents and other family members</td>
</tr>
<tr>
<td>May be used to guide infant feeding decision making</td>
<td>Difficulties and lack of time for informing and disclosing.</td>
</tr>
<tr>
<td>Rate of positive results depend on intra and peripartum MTCT interventions and infant feeding practices.</td>
<td>Likelihood of detecting HIV depends on seroprevalence and coverage of MTCT</td>
</tr>
</tbody>
</table>

### KNOWN HIV INFECTED MOTHER

- Babies born to known HIV positive mothers should all be offered early diagnostic testing using virologic testing, with the first test being performed from 6 weeks of age. Follow up for the HIV exposed infant needs to be continued until HIV infection status is confirmed and the child is discharged to well baby or ART HIV care.
- Serological testing from 9-12 months will be useful to identify HIV exposed children who are not HIV infected and can reduce the proportion of children requiring virological testing.
- Recording mothers HIV testing and or serological status on child health card is recommended to help identify children who require diagnostic testing.
- Age appropriate diagnostic screening should be provided for all siblings.
- Symptomatic children under 9 months of age require virological testing regardless of breastfeeding status.

See annex XX for detailed algorithm and principles.
Testing algorithm and type of test required depends on age of child at time of initial evaluation:

Maternal HIV Status Known:

Detailed algorithm is in annex ### Can remove this detail to annex too

**Infant aged 6 weeks to 9 months:**
Perform a **HIV viral test**.
- If **viral test** is *positive*, infant is infected.
- If **viral test** is *negative*, consider if infant is breastfeeding (current or within past 6 weeks)
  - If infant is not breastfeeding, infant is uninfected
- If **infant is breastfeeding**, repeat testing 6 weeks or more after the infant has completely ceased breastfeeding; type of test depends on age of child
  - If **infant is <9 months of age at time stopped breastfeeding**, do **HIV viral test**
    - If **viral test** is *positive*, infant is infected
    - If **viral test** is *negative*, infant is uninfected
  - If **infant is >9 months of age at time stopped breastfeeding**, do **HIV antibody test**
    - If **antibody test** is *negative*, infant is uninfected
    - If **antibody test** is *positive*, interpretation and further testing depends on **age at time of antibody test**
      - If child is >18 months of age at time stopped breastfeeding, child is infected
      - If child is <18 months of age at time stopped breastfeeding, do **HIV viral test**
        - If **viral test** is *positive*, child is infected
        - If **viral test** is *negative*, child is uninfected

**Child aged 9-18 months:**
- Perform a **HIV antibody test**.
  - If **antibody test** is *positive*, perform a **HIV virological test**
    - If **viral test** is *positive*, child is infected
    - If **viral test** is *negative*, consider if infant is breastfeeding (current or within past 6 weeks)
      - If child is not breastfeeding, child is uninfected
      - If child is breastfeeding, follow below algorithm for breastfeeding testing
  - If **antibody test** is negative, consider if infant is breastfeeding (current or within past 6 weeks)
    - If child is not breastfeeding, child is uninfected
If child is breastfeeding, follow below algorithm for breastfeeding testing

- If child is breastfeeding, 6 weeks or more after the infant has completely ceased breastfeeding do HIV antibody test
  - If antibody test is negative, child is uninfected.
  - If antibody test is positive, interpretation and further testing depends on age at time of antibody test
    - If infant is >18 months of age at time stopped breastfeeding, child is infected
    - If infant is 9-18 months of age at time stopped breastfeeding, do HIV viral test.
      - If viral test is positive, child is infected
      - If viral test is negative, child is uninfected

Child aged >18 months:
- Perform a HIV antibody test.
  - If antibody test is positive, child is infected
    - If antibody test is negative, consider if infant is breastfeeding (current or within past 6 weeks)
      - If child is not breastfeeding, child is uninfected
    - If child is breastfeeding, follow below algorithm for breastfeeding testing
      - If antibody test is negative, consider if infant is breastfeeding (current or within past 6 weeks)
        - If child is not breastfeeding, child is uninfected
      - If child is breastfeeding, 6 weeks or more after the infant has completely ceased breastfeeding do HIV antibody test
        - If antibody test is negative, child is uninfected
        - If antibody test is positive, child is infected

Questions and Principles:

1. Why 3 age groupings?
   a. Test availability and cost effectiveness
      i. Because most HIV-exposed infants serovert to negative by age 9-12 months, screening of infants between 9 months to 18 months with HIV antibody test first is practicable (test more available – rapid test can be used)
      ii. If HIV antibody negative, do not need to proceed to viral test (reduces costs and complexity of service provision)
      iii. If HIV antibody test is positive, ideally must confirm with HIV viral test

2. What is age of child?
   a. Determines type of first test performed on infant
      i. <9 months, do HIV viral test
      ii. 9-18 months, do HIV antibody test (confirm positive results with HIV viral test)
      iii. >18 months, do HIV antibody test
   b. Positive HIV viral test: always indicates child is infected; initial testing recommended at age 6 weeks to maximize sensitivity of test
   c. Positive HIV antibody test: interpretation depends on age of child
3. What is breastfeeding status?
   a. If initial test is negative and child is breastfeeding, child must be re-tested 6 weeks after complete cessation of breastfeeding, risk of transmission continues throughout entire period breastfeeding.
   b. Caveat is that if child gets sick with possible HIV symptoms, immediately test again (do not wait until weaning) (see #5).

4. If breastfeeding, what is age of complete cessation of breastfeeding?
   a. Determines the type of test performed, all can 6 weeks or more after stops breastfeeding.
      i. If <9 months at time stops breastfeeding, do HIV viral test.
      ii. If 9-18 months at time stops breastfeeding, do HIV antibody test (must confirm positive with HIV viral test).
      iii. If >18 months at time stops breastfeeding, do HIV antibody test.

5. Is the child well or sick (sick = HIV-related symptoms, such as IMCI algorithm)?
   a. If child was initial age-related test negative and gets sick, repeat age-appropriate test even if child breastfeeding and not yet weaned.

6. Confirmatory testing?
   a. Positive HIV antibody test should have confirmatory test either in parallel testing of same sample or serial testing of separate sample.
   b. Positive HIV viral test should have confirmatory test on separate sample when possible.

Where mothers HIV status in not known

Well Infant

These children will be seen in child health clinics (MCH, EPI or other child health services)
- Routine HIV serological screening of well infants is not recommended.
- While PMTCT coverage is still low and in settings where HIV epidemic is generalized (i.e. > 5% prevalence in the ANC population) and the mothers HIV status is not known or not recorded, national MCH programmes may wish to consider routinely offering antibody testing to mothers or to their infants to identify HIV exposed infants at or around the first immunization clinic visit. This strategy provides a repeat chance to ascertain HIV exposure if women were not tested during the antenatal period.

Sick child
HIV-infected infants frequently present with clinical symptoms in the first year of life, and by one year of age an estimated one-third of infected infants will have died, and about half by 2 years of age [3]. Early diagnosis of HIV by virologic testing during infancy to enable initiation of appropriate care including ART is therefore essential. In resource-limited settings, where access to virological testing is still limited, clinical signs and conditions sometimes have to be relied upon to guide these decisions because interpretation of HIV antibody testing,

7/31/2006
which is usually the only diagnostic test available, is difficult in infants and children aged under 18 months.

Certain signs and conditions are common to both HIV-infected and uninfected children, while other signs and conditions may be more specific or suggestive of HIV infection. Where HIV prevalence is high, hospital admissions or outpatient attendances of infants and children – especially in child health, malnutrition, or tuberculosis clinics - may frequently be related to HIV infection. When children present with signs and symptoms suggestive of HIV, clinical care providers need to consider HIV infection in the differential diagnosis of a sick infant or child, and should routinely offer age appropriate diagnostic testing. This is particularly important in children known to be HIV exposed but without established diagnosis of HIV infection status. If the constellation of symptoms or conditions is suggestive of HIV infection, the child should be tested as soon as possible for HIV infection with the best laboratory test available for that age group.

The provision of HIV testing in infants and children differs from standard testing and counselling services available for adults and must balance increasing access, protection of the child’s right and the child’s best interest. Consent, counselling and confidentiality, i.e. the three ‘Cs’, must be ensured for children of any age and regardless of whether they have a parent or legal guardian. Policy and operational guidance developed at the national level needs to explicitly state the options and standards required for HIV testing and counselling services provided to children.

**DIAGNOSTIC TESTING IN SICK CHILDREN OF UNKNOWN HIV EXPOSURE STATUS (see algorithms on page XX)**

- Infants and children being seen in acute health care facilities, with signs and symptoms suggestive of HIV and where maternal HIV status is unknown, and cannot be determined need to have HIV antibody testing performed to detect HIV antibody.
- Infants and children under 9 months of age at this time if not breastfeeding and found to be HIV negative can be assumed to be HIV unexposed and HIV uninfected.
- Infants and children who are aged 9-18 months and have positive HIV antibody tests and assumed to be unwell related to the HIV should have Virological testing performed wherever possible.
- Infants with positive virological testing can be assumed to be HIV infected and should be managed as such.
- If virological testing is not available then HIV seropositive infants unwell with signs and symptoms suggestive of HIV( see section XX)) need to be managed as if HIV infection may be the cause. CD4 testing should be performed to assess any immunodeficiency. Presumptive severe HIV disease may be diagnosed. Age appropriate diagnostic screening should be provided for all siblings.

All children undergo HIV antibody testing; testing algorithm and interpretation depends on age of child at time of initial evaluation:

**Infant aged 6 weeks to 9 months:**
- Perform HIV antibody test
  - If antibody test is negative, infant is not HIV-exposed
WHO HIV diagnosis in infants and children

- If antibody test is positive, infant is HIV-exposed, and further testing is warranted

- Perform a HIV viral test.
  - If viral test is positive, infant is infected.
  - If viral test is negative, consider if infant is breastfeeding (current or within past 6 weeks)
    - If infant is not breastfeeding, infant is uninfected

- If infant is breastfeeding, repeat testing 6 weeks or more after the infant has completely ceased breastfeeding; type of test depends on age of child
  - If infant is <9 months of age at time stopped breastfeeding, do HIV viral test
    - If viral test is positive, infant is infected
    - If viral test is negative, infant is uninfected
  - If infant is >9 months of age at time stopped breastfeeding, do HIV antibody test
    - If antibody test is negative, infant is uninfected
    - If antibody test is positive, interpretation and further testing depends on age at time of antibody test
      - If child is >18 months of age at time stopped breastfeeding, child is infected
      - If child is <18 months of age at time stopped breastfeeding, do HIV viral test
        - If viral test is positive, child is infected
        - If viral test is negative, child is uninfected

Child aged 9-18 months:
- Perform a HIV antibody test.
  - If antibody test is negative, infant is not HIV-exposed
  - If antibody test is positive, infant is HIV-exposed and further testing is warranted

- Perform a HIV virologic test
  - If viral test is positive, child is infected
  - If viral test is negative, consider if infant is breastfeeding (current or within past 6 weeks)
    - If infant is not breastfeeding, child is uninfected
  - If child is breastfeeding, 6 weeks or more after the infant has completely ceased breastfeeding do HIV antibody test
    - If antibody test is negative, child is uninfected.
    - If antibody test is positive, interpretation and further testing depends on age at time of antibody test
      - If infant is >18 months of age at time stopped breastfeeding, child is infected
      - If infant is 9-18 months of age at time stopped breastfeeding, do HIV viral test.
        - If viral test is positive, child is infected
        - If viral test is negative, child is uninfected

Child aged >18 months:
- Perform a HIV antibody test.
  - If antibody test is negative, child is not HIV-exposed
  - If antibody test is positive, child is infected
Maternal HIV Status Unknown: Questions/Principles

1. If mother’s HIV status is unknown, HIV antibody testing of the child is done first to screen and ascertain whether the child is HIV-exposed or not. The mother should be offered HIV testing to ensure she is aware of her HIV status (either by referral to VCT or on site)
   c. If child is HIV antibody negative, assume mother not HIV-infected and child not HIV-exposed (unless there is some reason to suspect acute maternal infection) and no further testing is needed
   d. If child is HIV antibody positive, assume mother is HIV-infected, child is HIV-exposed, and therefore further evaluation is warranted

2. What is age of child?
   e. Determines whether further testing is needed and which test to do
      i. <9 months, do HIV viral test
      ii. 9-18 months, do HIV viral test
      iii. >18 months, child has positive HIV antibody test, and hence is identified as infected and no further diagnostic testing is needed

3. What is breastfeeding status?
   f. If HIV viral test in child <18 months of age is negative and child is breastfeeding, child must be re-tested 6 weeks after complete cessation of breastfeeding due to continued exposure through breastfeeding
   g. Caveat is that if child get sick with possible HIV symptoms, immediately test again (do not wait until weaning) (see #5)

4. If breastfeeding, what is age of complete cessation of breastfeeding?
   h. Determines the type of test performed 6 weeks or more after stops breastfeeding
      i. If <9 months at time stops breastfeeding, do HIV viral test
      ii. If 9-18 months at time stops breastfeeding, do HIV antibody test (must confirm positive with HIV viral test)
         1. Because most HIV-exposed infant serorevert to negative by age 9-12 months, can screen infants between 9 months to 18 months first with HIV antibody test (test more available and less expensive – rapid test can be used)
      iii. If >18 months at time stops breastfeeding, do HIV antibody test

5. Is the child well or sick (sick =HIV-related symptoms, identified using IMCI or other clinical algorithms (see following section)?
   i. If child was initial age-related test negative and gets sick, repeat age-appropriate test even if child breastfeeding and not yet weaned

6. Confirmatory testing?
   j. Positive HIV antibody test should have confirmatory test either in parallel testing of same sample or serial testing of separate sample
   k. Positive HIV viral test should have confirmatory test on separate sample when possible.
SIGNS AND SYMPTOMS ASSOCIATED WITH HIV INFECTION

5.1.1. NON-SPECIFIC EARLY CLINICAL SIGNS OR CONDITIONS SEEN IN BOTH HIV-INFECTED AND UNINFECTED CHILDREN

Most infants with HIV infection are asymptomatic at birth. Non-specific symptoms of HIV infection may be seen as early as at age 2-3 months in untreated HIV-infected children, and the clinical presentations of HIV usually resembles that of other common illnesses in children. While the early HIV-related symptoms are not specific for HIV, they may occur earlier, more frequently, and be more severe among HIV-infected than uninfected children.

Annex Table # provides more details on these and other non-specific clinical signs and conditions that have been described in several reports. Table # lists the three most common early non-specific signs and symptoms.

SIGNS OR CONDITIONS SUGGESTIVE OF HIV INFECTION

While the early clinical expression of HIV in children is variable and nonspecific (see above), certain signs or conditions are common in HIV-infected children and uncommon in HIV-uninfected children. Observation of these signs or conditions should lead to an increased suspicion of HIV infection in a presenting sick child, in particular in an HIV-exposed child, and should prompt HIV testing; or re-testing if the initial HIV test was negative, if there is a continued risk for HIV infection, such as continued breastfeeding, sexual abuse, or sexual activity in adolescents.

Annex Table # summarizes reports describing signs or conditions suggestive of HIV infection. Table # lists the most frequently observed signs and conditions suggestive of HIV infection.

SIGNS OR CONDITIONS MORE SPECIFIC FOR HIV INFECTION

Some signs or conditions are very specific for HIV-infection and they should prompt HIV testing with the best test for age available (Table #).
Table # summarizes the clinical signs or conditions associated with HIV infection.

<table>
<thead>
<tr>
<th>Specificity for HIV infection</th>
<th>Signs/Conditions</th>
</tr>
</thead>
</table>
| Common in HIV-infected children but also common in sick, HIV-uninfected children | Chronic or recurrent otitis with ear discharge  
Persistent or recurrent diarrhea  
Growth faltering or failure to thrive  
(see and refer to IMCI) |
| Common in HIV-infected children and uncommon in HIV-uninfected children | Severe bacterial infections, particularly if recurrent  
Persistent or recurrent oral thrush  
Chronic parotitis  
Generalized persistent lymphadenopathy  
Hepatosplenomegaly  
Persistent and/or recurrent fever  
Neurologic dysfunction  
Herpes zoster (shingles), single dermatome  
Persistent generalized dermatitis unresponsive to treatment  
TB |
| Very specific to HIV infection | Pneumocystis pneumonia  
Oesophageal candidiasis  
Lymphoid interstitial pneumonitis  
Herpes zoster (shingles) with multi-dermatomal involvement  
Kaposi’s sarcoma  
Cryptococcal meningitis |

*Children with LIP present with a diffuse interstitial micronodular pattern on chest X-ray. LIP is more common in HIV-infected children aged 12 months and more and is often associated with lymphadenopathy and chronic parotitis.

To increase detection of HIV infection and increase access to prevention care and treatment, routine HIV diagnostic testing for infants and children should be performed in clinical care settings where HIV infected children are more likely to be seen including malnutrition and tuberculosis clinics, hospitals, ART clinics and orphanages. Move to section on sick child

**PRESUMPTIVE CLINICAL DIAGNOSIS OF SEVERE HIV DISEASE IN CHILDREN AGED UNDER 18 MONTHS WHERE LABORATORY BASED METHODS ARE NOT AVAILABLE IN ORDER TO START HIV TREATMENT**

For infants and children aged under 18 months where access to virological testing is not yet available but a child has symptoms that are suggestive of HIV infection, a presumptive clinical diagnosis of severe HIV infection may need to be made.

No single clinical diagnostic algorithm has proved to be highly sensitive or specific for diagnosis of HIV infection. Clinical algorithms are rarely more than 70% sensitive for accurate diagnosis of infection [79] and vary considerably with age; they are less reliable in particular in children aged under 12 months [70]. HIV antibody testing, particularly rapid
testing, and increased access to early virological testing must be made available to help clinicians implement improved diagnostic algorithms. However, there are situations where the use of a clinical algorithm may be required to initiate appropriate, life-saving treatment of a seriously ill child under age 18 months. There are currently insufficient data available to make firm recommendations of the use of clinical algorithms combined with measurement of CD4 or other parameters to establish HIV infection.

WHO, guided by expert opinion, has developed clinical criteria to diagnose presumptively severe HIV disease in a child less than 18 months of age to allow appropriate management of the potentially HIV-infected child (Box #). Presumptive clinical diagnosis of severe HIV-related disease warrants the appropriate management of the presenting acute illnesses first and institution of, or referral for, management of presumed HIV infection, which may include initiation of ART. Use of a presumptive clinical diagnosis of infection in a child aged under 18 months for initiation of ART should be accompanied by immediate efforts to establish the HIV diagnosis with the best nationally or locally available test for age but at the latest with HIV antibody testing at 18 months of age. Decisions on further treatment should be adjusted at that time by the results.

Initiation of ART based on presumptive clinical diagnosis of severe HIV disease is not recommended for use by clinical care providers who are not appropriately trained in HIV care or administration of ART.

**Box 1. Clinical criteria for presumptive diagnosis of severe HIV disease in infants and children aged under 18 months requiring ART in situations where virological testing is not available**

A presumptive diagnosis of severe HIV disease should be made if:

- The infant is confirmed HIV antibody positive;
  
  and

- Diagnosis of any AIDS-indicator condition(s)\(^a\) can be made;
  
  or

- The infant is symptomatic with two or more of the following:
  - Oral thrush;
  - Severe pneumonia;
  - Severe sepsis.

Other factors that support the diagnosis of severe HIV disease in an HIV seropositive infant include:

- Recent HIV-related maternal death; or advanced HIV disease in the mother;

- CD4 < 20%\(^b\).

Confirmation of the diagnosis of HIV infection should be sought as soon as possible.

Notes:

\(^a\) AIDS indicator conditions include some but not all HIV Paediatric Clinical Stage 4 conditions such as Kaposi sarcoma, pneumocystis pneumonia, cryptococcal meningitis, HIV wasting.

\(^b\) It is unclear how often CD4 is lowered in the above conditions in HIV-uninfected children.
WHO encourages researchers and national programmes to validate approaches to presumptive clinical diagnosis in children under 18 months of age, including studies to determine if CD4 combined with clinical signs and symptoms improves the diagnosis of HIV infection. WHO urges National Programmes to increase access to diagnostic testing for HIV infection for all children born to HIV infected women. The development of tests applicable to resource-limited settings which would allow early diagnosis of HIV infection in infants is critical to the implementation of recommendations for the initiation of appropriate care, including ART, in children aged less than 18 months.

For children aged 18 months and more with signs and symptoms suggestive of HIV (see Section #), WHO strongly recommends the use of antibody testing following national protocols to diagnose HIV infection (Section #, Figure #). presumptive clinical diagnosis of severe HIV disease is therefore not indicated in this age group because standard HIV antibody testing is diagnostic of HIV infection at this age. Some clinical conditions are very unusual without HIV infection (see Section #), and the diagnosis of these conditions thus suggests HIV infection and indicates the need to perform HIV antibody testing.

Screening for HIV infection at lower levels of the health care system

USE OF A CLINICAL ALGORITHM TO SCREEN FOR SYMPTOMATIC HIV INFECTION IN PRIMARY HEALTH CARE SETTINGS

The Integrated Management of Childhood Illness (IMCI) strategy was developed to improve the management and survival of children; it uses an algorithmic approach with a combination of symptoms to inform management of common illnesses [80]. A clinical algorithm has been developed, field-tested and revised to identify children who may have symptoms attributable to HIV infection and enable recognition of the possible HIV infected childr and referral for diagnostic testing and appropriate management. The suspected HIV-symptomatic child needs to be referred for HIV testing and consideration of ART. Where no HIV testing is available referral for care at the next health system level is warranted. (The generic screening flow chart is referenced in Box #) [79]. This algorithm can be used to screen children under the age of five years for suspected symptomatic HIV infection and guide subsequent initiation of appropriate clinical management and care, including referral for HIV testing. Adapted tools, modules and information on including HIV into IMCI are available.

5.3. Additional support and tools

The WHO web site and online toolkit on HIV testing and counselling include a section of resources on HIV testing and counselling in children; it is available at: http://who.arvkit.net/tc/en/index.jsp.

5.4 Monitoring

Add links to revised national programme indicators.
5.6 Key supporting programme activities:

- Systems established to ensure tracking and follow up of children who are seen in child health services from any PMTCT/MTCT service.
- Adaptation of child and maternal held records including hand held health cards to ensure it captures maternal HIV status and infant HIV exposure and test requirements.
- Inclusion of maternal and child health staff in human capacity building for HIV.
- Providers who deliver health care services for children and infants should be trained in basics of diagnosing HIV infection in children.
ANNEXES

ANNEX #. LIST OF EXPERTS

Working group members

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ANNEX #. REVISED HIV/AIDS CASE DEFINITIONS FOR SURVEILLANCE
ANNEX #. OVERVIEW OF AVAILABLE COMMERCIAL HIV DIAGNOSTIC TESTS

This is regularly updated and is available on line at:
Annex ### DNA virological testing

Table # lists the sensitivity and specificity of HIV DNA tests as reported by different studies.

Real time DNA PCR (Box #) has been shown to be sensitive (i.e., overall of 100%) and is less time consuming and less costly, and does not require dedicated laboratory space needed than standard PCR techniques. It operates using a closed system and therefore requires less complex system and less space - not yet commercially available is under evaluation. [46].

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study (test used)</th>
<th>Age of infant</th>
<th>Diagnostic sensitivity</th>
<th>Diagnostic specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bremer et al., 1996 [35]</td>
<td>Longitudinal, multicenter study (Roche Amplicor DNA PCR testing kit)</td>
<td>0-7 days 1 month 2 months 4 months 6 months 9-12 months 15-36 months 1-36 months (overall)</td>
<td>29% 92% 90% 93% 100% 96% 97% 95%</td>
<td>100% 100% 98% 96% 95% 97% 95% 97%</td>
</tr>
<tr>
<td>Dunn et al., 1995 [43]</td>
<td>Meta-analysis (DNA PCR test not specified)</td>
<td>0-48hrs Day 14 Day 28</td>
<td>38% 93% 96%</td>
<td>-</td>
</tr>
<tr>
<td>Kline et al., 1994 [38]</td>
<td>DNA PCR</td>
<td>Birth 1 month 2 months 3 months 4-6 months Overall (0-6 months)</td>
<td>100% 100% 100% 100% 100%</td>
<td>100% 100% 100% 100% 97%</td>
</tr>
<tr>
<td>Kovacs et al., 1995 [39]</td>
<td>Prospective study (Roche Amplicor HIV-1 DNA PCR)</td>
<td>Cord blood 0-2 days 3-14 days 15-30 days 31 days-2 month 2-6 months &gt; 6 months</td>
<td>60% 40% 67% 80% 100% 95% 97%</td>
<td>94% 100% 100% 99% 100%</td>
</tr>
<tr>
<td>Lambert et al., 2003 [40]</td>
<td>Multicenter randomized controlled trial (commercial DNA PCR assay)</td>
<td>Birth 6 weeks 24 weeks</td>
<td>11% 83% 67%</td>
<td>100% 99% 100%</td>
</tr>
<tr>
<td>Nelson et al., 1996 [81]</td>
<td>Prospective, blinded study (commercially available DNA PCR test; not specified)</td>
<td>8 days-6 months all ages (overall)</td>
<td>95% 91%</td>
<td>- 99.4%</td>
</tr>
<tr>
<td>Owens et al., 1996 [82]</td>
<td>Meta-analysis (DNA PCR test not specified)</td>
<td>&lt; 30 days &gt; 30 days</td>
<td>93% 98%</td>
<td>-</td>
</tr>
<tr>
<td>Sherman, Cooper et al., 2005 [83]</td>
<td>Retrospective review (Roche Amplicor HIV-1 DNA PCR version)</td>
<td>6 weeks all ages (overall)</td>
<td>98.8% 99.3% 99.4% 99.5%</td>
<td>99.4% 99.5%</td>
</tr>
<tr>
<td>Sherman, Stevens et al., 2005 [57]</td>
<td>Cohort study (Roche Amplicor HIV-1 DNA PCR version 1.5 assay on DBS)</td>
<td>6 weeks</td>
<td>100%</td>
<td>99.6%</td>
</tr>
</tbody>
</table>
Annex ### RNA virological testing

In **RT RNA PCR**, RNA is converted into DNA by reverse transcriptase and then amplified by PCR (Box #). A RT activity assay reliably quantified HIV-2 and HIV-1-group O infected patient samples [16]. An ultrasensitive RT assay (i.e., Amp-RT) was found to be more sensitive in the first 12 days of life of HIV-infected infants than either NASBA or DNA PCR in detecting HIV infection, although this difference did not reach statistical significance [53]. **Real-time RT PCR** has shown a high level of sensitivity with similar low detection limits of 50 copies per ml [49] as ultra-sensitive RT PCR [84]. Experiences, even in resource-limited settings, suggest that real time RNA PCR is feasible, cheap, simple, easy to standardize, reliable and comparable with commercially available kits [13, 85]. Commercial tests used to quantify HIV-1 subgroup M plasma load are not suitable for HIV-1 subgroup O; a real-time PCR assay based on Light Cycler technology was reported to be suitable to quantify HIV-1 subgroup O RNA [86]. Use of real-time RT PCR assay in Cote d’Ivoire for HIV diagnosis in infant has shown both sensitivity and specificity of 100% (95% confidence intervals, 93.7 to 100 and 98.3 to 100, respectively), making them comparable to results obtained from commercially available HIV-1 RNA viral load test kits. At approximately USD 12 per test, the real-time RT RNA test is 5 to 10 times less costly than commercial HIV RNA viral load tests1. [19].

The **bDNA** is considered widely as reference method for HIV-1 RNA viral load measurement [49]. It sets off a light-emitting chemical reaction with HIV RNA in which the amount of light produced is direct proportional to the amount of RNA in the sample. More advanced (2nd generation) bDNA tests can identify as few as 50 copies of HIV RNA. bDNA can accurately detect non-B HIV-1 subgroups and provides results concordant with HIV-1 DNA PCR [19]. **NASBA** is an isothermal method of nucleic acid amplification (i.e., RNA). Amplification by NASBA involves three enzymes, AMV Reverse Transcriptase, RNase H, and T7 RNA Polymerase. Quantitative detection is achieved by way of internal calibrators, added at nucleic acid isolation, which are co-amplified and subsequently identified along with the wild type of RNA using electrochemiluminescence. The quantitative HIV-1 RNA NASBA assay is frequently used in Western countries to determine viral load in HIV-1 infected patients.

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1 The cost of p24 antigen assay – which is considered a cheap test alternative - is USD 21 per test
Table # lists sensibility and specificity for different RNA tests as reported by studies.

Table #. Early diagnostic sensitivity and specificity of RNA PCR

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of test used</th>
<th>Age of infant</th>
<th>Diagnostic sensitivity</th>
<th>Diagnostic specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delamare et al., 1997 [48]</td>
<td>NASBA</td>
<td>0 - 10 days</td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 days – 3 months</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td>Lambert et al., 2003 [40]</td>
<td>NASBA</td>
<td>Birth</td>
<td>26.7%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 weeks</td>
<td>94.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 weeks</td>
<td>85.7%</td>
<td></td>
</tr>
<tr>
<td>Nesheim et al., 2003 [12]</td>
<td>NASBA and RNA PCR</td>
<td>0-7 days</td>
<td>29%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-28 days</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>29-60 days</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>61-120 days</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120-180 days</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Pineau et al., 2004 [13]</td>
<td>Real-time RT PCR (TaqMan)</td>
<td>Overall (1 month, 3 months, 2 months after weaning)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Rouet et al., 2005 [19]</td>
<td>Real-time RT PCR (automated TaqMan)</td>
<td>At all ages (day 2, 4-6 weeks, and 3-6 months)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Young et al., 2000 [34]</td>
<td>Quantitative RNA PCR (Roche Amplicor)</td>
<td>Birth</td>
<td>47%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 months</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 months</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

*Overall data (i.e. for infants with and without ARV (zidovudine) prophylaxis*
 Annex ### U P24 antigen testing

p24 antigen can be measured using EIA technology with modifications to detect antigen, not antibody. To improve sensitivity of the p24 antigen assay, an **Immune Complex Dissociation (ICD)** procedure using acid base or to dissociate p24 antigen/anti-p24antibody complexes before performing the antigen assay has been introduced, but has been shown to not be sensitive enough. ANNEX ### P24 antigen testing

<table>
<thead>
<tr>
<th>Study</th>
<th>Test used</th>
<th>Diagnostic sensitivity/age</th>
<th>Diagnostic specificity/age</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Baets et al, 2005 [21]</td>
<td>US p24 Ag (paediatric)</td>
<td>100%</td>
<td>100%</td>
<td>Can be performed on capillary plasma stored on filter paper</td>
</tr>
<tr>
<td>Lyamuya et al., 1996 [87]</td>
<td>ICD amplified p24 antigen</td>
<td>1-8 weeks: 100% 9-26 weeks: 97% 27-52 weeks: 100% &gt;52 weeks: 97%</td>
<td>100% for all samples</td>
<td>Using heated plasma or serum increases the sensitivity of the p24 Ag assay significantly</td>
</tr>
<tr>
<td>Nadal et al, 1999 [22]</td>
<td>Heat-mediated ICD, signal amplified boosted HIV-1 p24 Ag EIA</td>
<td>100% sensitivity of all methods after 10 days of age</td>
<td>99.2% diagnostic specificity of p24 after neutralization (for comparison: RNA specificity 98.6%)</td>
<td>Quantification of p24 Ag is as precise and sensitive as quantification of HIV RNA by commercial PCR kit (paediatric study). Diagnostic sensitivity same as PCR detection of DNA or RNA in untreated and treated children.</td>
</tr>
<tr>
<td>Study / Authors / Year</td>
<td>Method / Type</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Advantages / Notes</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Panakitsu et al, 1997 [18]</td>
<td>Acid denatured ICD p24 Ag&lt;br&gt;Heat-denatured ICD p24 Ag</td>
<td>85.4%&lt;br&gt;87.8%</td>
<td>100%&lt;br&gt;100%</td>
<td>Advantages of ICD p24Ag include simplicity, rapidity and relatively low cost (paediatric study)</td>
</tr>
<tr>
<td>Patton et al, 2005 [23]</td>
<td>US p24 Ag on DBS (paediatric)</td>
<td>100% sensitivity within 6 weeks of collection (and declining thereafter)</td>
<td>100% specificity within 6 weeks of collection (and declining thereafter)</td>
<td></td>
</tr>
<tr>
<td>Schupbach et al, 2001 [88]</td>
<td>Heat-denatured signal-amplification-boosted p24 Ag EIA (adults)</td>
<td>n/a</td>
<td>n/a</td>
<td>Test suggested as alternative to ART monitoring (20% of cost of RNA monitoring)</td>
</tr>
<tr>
<td>Sherman et al, 2004 [25]</td>
<td>p24 Ag ultrasensitive EIA heat-denatured and boosted by signal amplification test</td>
<td>Overall: 98.1%&lt;br&gt;6 weeks: 95.7%&lt;br&gt;3 months and thereafter: 100%</td>
<td>Overall: 98.7%&lt;br&gt;6 weeks ≤ 7 months: 100%&lt;br&gt;7 months: 93.6%</td>
<td>Test less complex, less expensive equipment, simpler to perform, minimal training, compared with PCR tests&lt;br&gt;HIV subtypes: Detects HIV subtype B and C; further validation of non-B viral subtypes needed</td>
</tr>
<tr>
<td>Schupbach, 2002 [24]</td>
<td>Heat-mediated destruction of Ab and signal amplification p24 Ag test (paediatric)</td>
<td>100% after 10 days of age</td>
<td>99.2% after neutralization</td>
<td>HIV subtypes: 3 studies indicate virus subtypes A-G and O. Validated for subtype B but further studies for non-B needed</td>
</tr>
<tr>
<td>Schupbach, 2003 [89] (J. Int Arch Allergy Immunol)</td>
<td>ICD signal-amplified HIV-1 p24 Ag assay</td>
<td>similarly sensitive in diagnosing paediatric HIV infection as RT PCR</td>
<td>similarly specific in diagnosing paediatric HIV infection as RT PCR</td>
<td>Simple modifications including use of a more efficient virus lysis buffer, heat-mediated destruction of antibodies interfering with antigen detection and tyramide signal amplification have improved the HIV-1 p24 Ag assay. HIV-1 subtypes: test validated for subtype B, but requires further studies for non-B subtypes.</td>
</tr>
<tr>
<td>Study</td>
<td>Methodology</td>
<td>Sensitivity 0-18 months</td>
<td>Sensitivity 0-18 months</td>
<td>Sensitivity 1-2 months and 4-6 months</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Zijenah et al, 2005 [26]</td>
<td>Signal boosted ultrasensitive p24 Ag</td>
<td>96.7%</td>
<td>96.1%</td>
<td></td>
</tr>
<tr>
<td>Sutthent et al., 2003 [20]</td>
<td>Heat-denatured p24 antigen assay modified with a booster step</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Steps to enhance sensitivity of test include development of ultrasensitive p24Ag assay, involving heat-mediated ICD and signal amplification-boosted EIA.

HIV-1 subtype: several tests (see article) validated in countries where subtypes A, B, C, D, C and F predominate. Here: Subtype C.

The diagnostic sensitivity of this test in infants was similar to that of PCR for HIV-1 DNA or RNA using samples from both Europe and Africa; at USD 3 per test, its cost is 10-30 times less expensive than commercially available HIV-1 RNA viral load tests.
### WHO HIV diagnosis in infants and children

<table>
<thead>
<tr>
<th>Study</th>
<th>Test Description</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribas et al, 2003 [55]</td>
<td>Heat-denatured signal-amplified p24 assay (testing kit)</td>
<td>n/a</td>
<td>n/a</td>
<td>Test capable of measuring the plasma level of p24 derived from adult patients infected with B and non-B subtypes as well as recombinant forms of HIV-1 including HIV-1 group O. Test is an affordable alternative to quantitative HIV-1 RNA tests.</td>
</tr>
<tr>
<td>Respess et al, 2005 [90]</td>
<td>Ultrasensitive p24 antigen assay (integrated kit and protocol)</td>
<td>VL 1000-10000: Overall: 43.6% no ART: 56.5% on ART: 34.4%</td>
<td>VL 10000-20000: Overall: 38.9% no ART: 57.1% on ART: 27.3%</td>
<td>Study performed in adults; assay may not have enough sensitivity for routine monitoring of ART but sufficiently sensitive for qualitative paediatric diagnosis.</td>
</tr>
</tbody>
</table>

**Notes:**
- **VL:** Viral Load (copies/mL)
WHO HIV diagnosis in infants and children

ANNEX ### DBS
### Table #. Key characteristics of DBS according to different tests

<table>
<thead>
<tr>
<th>Study</th>
<th>Test used</th>
<th>Potential treatment/ storage of DBS</th>
<th>Sensitivity/specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassol et al, 1992 [58]</td>
<td>In-house HIV DNA PCR</td>
<td>Capillary blood onto S&amp;S 903 filter paper; stored at -20°C.</td>
<td>Samples from infants in Canada, Lower limits of detection: 4-16 HIV DNA copies per 100,000 nucleated cells</td>
<td>Recommends widespread use for early diagnosis of infants but requires further refinements viz: standardization, optimization and automation.</td>
</tr>
<tr>
<td>Comeau et al, 1996 [91]</td>
<td>In-house HIV DNA PCR</td>
<td>Anticoagulated blood spotted onto filter paper, dried &amp; stored in plastic bags at -80°C to +4°C</td>
<td>Samples from infants in USA &amp; Puerto Rico, Sensitivity and specificity at 1-4 months of age ranged between 89-97% and 98-100% respectively, Results unaffected by storage temperature.</td>
<td>DBS HIV DNA PCR is a reliable tool for early diagnosis of HIV infection with important advantages over liquid blood HIV DNA PCR and viral culture</td>
</tr>
<tr>
<td>Biggar et al, 1997 [92]</td>
<td>HIV DNA PCR</td>
<td>Heelpricks into microtainers then onto S&amp;S 903 filter paper, Air-dried and stored at -20°C in impermeable plastic bags with dessicant pellet</td>
<td>Samples from infants in Malawi, Positive predictive value: 98% if both replicates strongly positive, Negative predictive value: 96% if both replicates negative, Lower limit of detection: 5 HIV proviral DNA copies per sample viz. 30 000 nucleated cells</td>
<td>All samples tested in duplicate; positive DBS HIV DNA PCR results after age one month was 98.9% accurate in predicting antibody positivity after age 15 months</td>
</tr>
<tr>
<td>Beck et al, 2001 [93]</td>
<td>In-house nested HIV DNA PCR</td>
<td>Specialised filter paper (FTA card &amp; Gene Guard system); Air-dried, stored at room temperature in plastic</td>
<td>Samples from USA (adults &amp; children): Sensitivity and specificity: 98%</td>
<td>More practical, economical, sensitive and specific method for diagnosis of HIV-1 subtype B</td>
</tr>
<tr>
<td>Study</td>
<td>Technique/DNA PCR</td>
<td>Sample Type</td>
<td>Results</td>
<td>Notes</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------------------</td>
<td>------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Fischer et al, 2004 [11]</td>
<td>In-house nested HIV DNA PCR and HIV-1 DNA PCR (Roche Amplicor v1.5)</td>
<td>Heelstick whole blood on Isocode Card (S&amp;S), dried at room temperature, stored in plastic bag</td>
<td>Sensitivity: 99%, Specificity: 100%</td>
<td>Simple and easy to perform DNA extraction method developed that can easily be implemented under field conditions; HIV-1 subtype A</td>
</tr>
<tr>
<td>Sherman et al, 2005 [57]</td>
<td>HIV-1 DNA PCR Roche Amplicor v1.5</td>
<td>Venous blood on Whatman No. 1 filter paper; air dried, stored in plastic bag with no dessicant sachet at room temperature for 9-19 months</td>
<td>Samples from 6-week old infants in South Africa</td>
<td>Sensitivity: 100%, Specificity: 99.6%</td>
</tr>
<tr>
<td>Luo et al, 2005 [56]</td>
<td>Duplex real time DNA PCR</td>
<td>N/A</td>
<td>Samples from US adults</td>
<td>Sensitivity: 98.1% (95% CI, 95.5%-100%), Specificity: 100% (95% CI, 99%-100%) Positive predictive value (PPV): 100%, Negative predictive value (NPV): 97%</td>
</tr>
<tr>
<td>De Baets et al, 2005 [21]</td>
<td>Ultra-sensitive p24 Ag</td>
<td>Air dried overnight stored in bag with silica gel</td>
<td>Can be performed on filter paper</td>
<td>Sensitivity and specificity 100%</td>
</tr>
<tr>
<td>Patton et al, 2005 [23]</td>
<td>Ultra-sensitive p24 antigen</td>
<td>Whatman no. 1 Air dried at room</td>
<td>Paediatric study</td>
<td>98.8% sensitivity and 100%</td>
</tr>
<tr>
<td></td>
<td>temperature for ≥3 hours; storage in individual plastic bags without desiccant to simulate local conditions</td>
<td>specificity within 6 weeks of collection</td>
<td>within 6 weeks but decline thereafter. HIV-1 subtype C</td>
<td></td>
</tr>
</tbody>
</table>
## ANNEX Comparison of HIV antibody testing technologies: EIA and rapid tests

### Table 1. Comparison of HIV antibody testing technologies: EIA and rapid tests

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EIA</th>
<th>Rapid tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection (sample/type specimen)</td>
<td>HIV antibodies in plasma/serum (venepuncture)</td>
<td>Several can also detect HIV antibodies in whole blood (finger-prick samples)</td>
</tr>
<tr>
<td>Accuracy (sensitivity, specificity)</td>
<td>Varies with test; EIA and rapid tests give similar diagnostic performances</td>
<td></td>
</tr>
<tr>
<td>Laboratory equipment</td>
<td>Micropipette, washer, incubator, spectrophotometer</td>
<td>None to minimal (micropipette)</td>
</tr>
<tr>
<td>Laboratory personnel</td>
<td>Skilled laboratory technician</td>
<td>Can be performed by any health care worker who has been adequately trained, including counsellors</td>
</tr>
<tr>
<td>Ease of performance*</td>
<td>Level 4</td>
<td>Level 1-3, depending on test type</td>
</tr>
<tr>
<td>Time to perform</td>
<td>&gt;2 hours (plus waiting time for patients)</td>
<td>Mostly 10-30 minutes</td>
</tr>
<tr>
<td>Shelf - life</td>
<td>Usually 12 months</td>
<td>Usually 12 months</td>
</tr>
<tr>
<td>Storage conditions</td>
<td>2-8 °C</td>
<td>Some 2-8 °C; most 2-30 °C</td>
</tr>
<tr>
<td>Cost per test**</td>
<td>US$ 0.40-1.20</td>
<td>US$ 0.47-2.0</td>
</tr>
<tr>
<td>Volume of tests</td>
<td>Mostly suitable for medium- to large-volume testing, i.e. &gt;40-90 samples per testing tray</td>
<td>Most kits are suitable for small- and large-volume testing, i.e. 1-100 samples per day</td>
</tr>
<tr>
<td>Quality Assurance</td>
<td>It is recommended to include QC specimens in every run.</td>
<td>QC specimens should be performed by preference daily, but certainly once weekly</td>
</tr>
</tbody>
</table>
Annex XX Timing of antibody seroreversion in infants and children of HIV-infected mothers

<table>
<thead>
<tr>
<th>Study</th>
<th>Timing of seroreversion in HIV-uninfected infants</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andiman et al., 1990 [69]</td>
<td>Mean and median time 7 months</td>
<td>Half-life of passive antibody was 38 days</td>
</tr>
<tr>
<td>Chantry et al., 1995 [9]</td>
<td>As determined by EIA: Mean time: 11.6 months</td>
<td>Time to seroreversion confirms 1994 CDC definition; recommendation to use Western Blot as confirmatory test only after 18 months of age</td>
</tr>
<tr>
<td></td>
<td>(range: 17.9 - 82 weeks) As determined by Western Blot: Mean time: 15.8 months (range: 44.9 - 94.1 weeks)</td>
<td></td>
</tr>
<tr>
<td>Moodley et al., 1995 [74]</td>
<td>As determined by EIA: 100% by 15 months 94.5% by</td>
<td>By 9 months of age - significant difference between antibody decay rates in infected and uninfected children; 95.8% of uninfected children had ≥ 50% decay in antibody titers between 6 and 9 months (sensitivity: 97.8%; specificity: 93.8%).</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td>Sherman GG, Jones SA., 2005 [61]</td>
<td>As determined by EIA: 59% at median of 12.2 months (range 11.2 – 18 months)</td>
<td>(Specificity of test at median of 12.2 months: 59%)</td>
</tr>
<tr>
<td>Siranivin, S</td>
<td>As determined by PA;</td>
<td>Semiquantitative tests were useful in</td>
</tr>
<tr>
<td>Atamiasirikul, K., 2000 [94]</td>
<td>53% at 7-8 months; 67% at 9 months; 100% at 12 months. As determined by qualitative tests: 16% at 7-8 months; 11% at 9 months; 74% at 12 month.</td>
<td>Diagnosis between the age of 6-12 months; interpretation must take into consideration AIDS related manifestations and history of breastfeeding.</td>
</tr>
</tbody>
</table>

**Notes:** PA - particle agglutination; MEIA - microparticle enzyme immunoassay.
ANNEX #. DIAGNOSIS OF HIV-2 INFECTION IN INFANTS

ANNEX: ## WHO algorithm infant screening.
Maternal Status UNKNOWN and Child undergoes HIV Antibody Testing (e.g., HIV exposure unknown and child screened at immunization or well-child clinic)

What is Age of Child at Time of HIV Antibody Testing?

- **Birth - 9 months**
  - HIV Antibody Test
    - Negative: Child not HIV-exposed
    - Positive
      - HIV Viral Test
        - Positive: Infected*
        - Negative
          - Yes
            - Current or Recent (within 6 Weeks) Breastfeeding?
              - Yes
                - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding
                  - Positive: Infected*
                  - Negative: Uninfected
              - No: Uninfected
                - HIV Antibody Test
                  - Positive: Infected*
                  - Negative: Uninfected
          - No: Uninfected
            - HIV Antibody Test
              - Positive: Infected*
              - Negative: Uninfected
        - Current or Recent (within 6 Weeks) Breastfeeding?
          - Yes
            - HIV Antibody Test
              - Positive: Infected*
              - Negative: Uninfected
          - No: Uninfected
            - HIV Antibody Test
              - Positive: Infected*
              - Negative: Uninfected
    - HIV Viral Test
      - Positive: Infected*
      - Negative
        - Yes
          - Current or Recent (within 6 Weeks) Breastfeeding?
            - Yes
              - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding
                - Positive: Infected*
                - Negative: Uninfected
            - No: Uninfected
              - HIV Antibody Test
                - Positive: Infected*
                - Negative: Uninfected
      - No: Uninfected
        - HIV Antibody Test
          - Positive: Infected*
          - Negative: Uninfected
  - HIV Viral Test
    - Positive: Infected*
    - Negative
      - Yes
        - Current or Recent (within 6 Weeks) Breastfeeding?
          - Yes
            - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding
              - Positive: Infected*
              - Negative: Uninfected
          - No: Uninfected
            - HIV Antibody Test
              - Positive: Infected*
              - Negative: Uninfected
      - No: Uninfected
        - HIV Antibody Test
          - Positive: Infected*
          - Negative: Uninfected
- **9-18 months**
  - HIV Antibody Test
    - Positive
      - HIV Viral Test
        - Positive: Infected*
        - Negative: Uninfected
    - Negative: Child not HIV-exposed
  - Current or Recent (within 6 Weeks) Breastfeeding?
    - Yes
      - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding
        - Positive: Infected*
        - Negative: Uninfected
    - No: Uninfected
      - HIV Antibody Test
        - Positive: Infected*
        - Negative: Uninfected
- **>18 months**
  - HIV Antibody Test
    - Positive: Infected*
    - Negative: Child not HIV-exposed
  - Current or Recent (within 6 Weeks) Breastfeeding?
    - Yes
      - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding
        - Positive: Infected*
        - Negative: Uninfected
    - No: Uninfected
      - HIV Antibody Test
        - Positive: Infected*
        - Negative: Uninfected

Negative: Child not HIV-exposed
Positive: Infected*
Uninfected

Note: *=Indicates need for cultures/other diagnostic tests

Current or Recent (within 6 Weeks) Breastfeeding?

- Yes
- No: Uninfected

HIV Viral Test

- Positive: Infected*
- Negative

HIV Antibody Test

- Positive: Infected*
- Negative: Child not HIV-exposed

What is Age of Child at Time of Antibody Test?

- 9-18 months
- >18 months

7/31/2006
ANNEX ## WHO Algorithm maternal screening
**Testing Identifies Infected Mother** (e.g., mother identified by PMTCT; or mother screened at immunization or well-child clinic instead of testing infant)

**Mother is Known to be HIV Antibody Positive**

**What is Age of Child at Time of Initial Evaluation?**

- **6 weeks - 9 months**
  - HIV Viral Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

- **9 - 18 months**
  - HIV Antibody Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

- **>18 months**
  - HIV Antibody Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

**What is Age of Child at 6 Weeks After Complete Cessation Breastfeeding?**

- **<9 months**
  - HIV Viral Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

- **>9 months**
  - HIV Antibody Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

**Current or Recent (within 6 Weeks) Breastfeeding?**

- **Yes**
  - HIV Viral Test:
    - **Positive:** Infected*

- **No**
  - HIV Viral Test:
    - **Positive:** Infected*
  - HIV Antibody Test:
    - **Positive:** Infected*
    - **Negative:** Uninfected

**What is Age at Time of Antibody Test?**

- **9-18 months**
  - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding:
    - **Positive:** Infected*
    - **Negative:** Uninfected

- **>18 months**
  - HIV Antibody Test 6 Weeks After Complete Cessation Breastfeeding:
    - **Positive:** Infected*
    - **Negative:** Uninfected
### ANNEX# TABLE #. SIGNS, SYMPTOMS OR CONDITIONS SUGGESTIVE OF HIV INFECTION

<table>
<thead>
<tr>
<th>Sign/condition</th>
<th>Description</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. NON-SPECIFIC SIGNS AND SYMPTOMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main non-specific signs and symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic otitis</td>
<td>While otitis is frequent in both HIV-infected and uninfected children, persistent or recurrent otitis, particularly with ear discharge lasting more than two weeks, is over five times more frequent in HIV-infected than uninfected children.</td>
<td>Bakaki et al., 2001 [95].</td>
</tr>
<tr>
<td>Persistent or recurrent diarrhoea</td>
<td>Diarrhoea is frequent in infants regardless of HIV infection status. Prolonged diarrhoea lasting more than 2 - 4 weeks is about 5 times more frequent in HIV-infected than uninfected infants.</td>
<td>Bakaki et al., 2001 [95].</td>
</tr>
<tr>
<td>Growth faltering</td>
<td>Persistent abnormalities in growth, including weight loss or gradual but steady deterioration in weight gain from expected growth as indicated on the child’s growth chart, can be an early sign of HIV infection but is frequently found in infants and children in resource-limited settings. Signs have been observed in 90-100% of infected untreated children but also in 60-100% of uninfected children. Early, severe and sustained impairment in weight, height and head circumference growth have been observed among HIV-infected compared with HIV-uninfected children. Low weight for age and low height for age (stunting) were more common than low weight for height (wasting); development of wasting was associated with a history of severe and/or persistent infections.</td>
<td>Blanche et al., 1997; Spira et al., 1999 [96, 97]; Bailey et al., 1999; Bakaki et al., 2001 [95, 98].</td>
</tr>
<tr>
<td><strong>Non-specific signs and symptoms as reported from other studies</strong></td>
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<td>Chronic cough, malnutrition and generalized lymphadenopathy</td>
<td>These non-specific clinical signs can be seen in both HIV-infected and uninfected infants; they have been observed in 90-100% of infected untreated children but also in 60-100%.</td>
<td>Blanche et al., 1997; Spira et al., 1999 [96, 97].</td>
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<tr>
<td>Clinical syndrome</td>
<td>Description</td>
<td>Reference(s)</td>
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<tr>
<td>Acute respiratory infection, malaria, malnutrition, meningitis, anaemia, or diarrhoeal disease</td>
<td>Clinical syndromes found, irrespective of age or HIV infection status, in more than 80% of children; however, HIV-infected children were somewhat more likely than uninfected children to be hospitalized for malnutrition and respiratory infections.</td>
<td>Vetter et al., 1996 [99]</td>
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<tr>
<td>Fever, cough, diarrhoea, ear discharge, oral ulcers and skin rash</td>
<td>When present for 14 days or longer these signs were all significantly more common in HIV-1-infected than in HIV-uninfected children.</td>
<td>Kawo et al., 2000 [100]</td>
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<tr>
<td>Severe bacterial infections</td>
<td>Development of serious bacterial infection (particularly recurrent serious bacterial infections) requiring hospitalization, including severe pneumonia (see IMCI classification), meningitis, sepsis, abscess, or cellulites. Infection due to <em>Streptococcus pneumoniae</em> is the major aetiology of severe bacterial infections in HIV-infected and uninfected children in developing countries. Other bacterial pathogens, including <em>Salmonella</em> species and <em>Haemophilus influenzae</em> are also seen.</td>
<td>Dray-Spira et al., 2000 [101].</td>
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<tr>
<td>Persistent (&gt;30 days despite treatment) or recurrent oral thrush</td>
<td>After the neonatal period the presence and persistence of oral thrush in children not on antibiotic treatment is unusual; however, oral thrush is seen in 20-60% of HIV-infected infants. Thrush appears as creamy white curdlike patches, with inflamed underlying mucosa with punctate or diffuse erythema exposed after removal of the exudate, and can be found on the oropharyngeal mucosa, palate and tonsils.</td>
<td>Taha et al., 1995; Dray-Spira et al., 2000; Bakaki et al., 2001 [95, 101, 102]</td>
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<tr>
<td>Chronic parotitis</td>
<td>Chronic parotid enlargement is defined as one-sided or bilateral parotid swelling (just in front of the ear) with or without pain and fever and persisting for more than 2 weeks. Parotitis was significantly more common in HIV-infected than uninfected children, reported in 40-50% of infected children compared to 3-8%</td>
<td>Dray-Spira et al., 2000; Bakaki et al., 2001 [95, 101]</td>
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<tr>
<td>Condition</td>
<td>Description</td>
<td>References</td>
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<td>of uninfected children. Parotid enlargement is more commonly seen in infected children over 18 months than younger children.</td>
<td></td>
<td>Taha et al., 1995; Dray-Spira et al., 2000; Bakaki et al., 2001 [95, 101, 102]</td>
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<tr>
<td>Generalized persistent lymphadenopathy</td>
<td>Development of persistent, non-inguinal lymphadenopathy in infancy, consisting of enlarged lymph nodes (&gt;1.5 cm) in two or more regions other than the inguinal area without any apparent underlying cause. Common feature of HIV infection, seen in 85-90% of infected children.</td>
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<td>Hepatosplenomegaly</td>
<td>Persistent enlargement of liver (&gt;2 cm) or spleen (&gt;1 cm) outside of the neonatal period in the absence of other aetiologies such as hepatitis C or cytomegalovirus infection, is also common in HIV-infected children.</td>
<td>Taha et al., 1995; Bakaki et al., 2001 [95]</td>
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<tr>
<td>Persistent and/or recurrent fever</td>
<td>Fever &gt;38°C lasting more than 1 week or recurring more than once over a period of a week. Common in HIV-infected infants; prolonged fever has been reported in 22-56% of HIV-infected infants in studies in Africa.</td>
<td>Bakaki et al., 2001 [95]</td>
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<td>Neurologic dysfunction</td>
<td>Progressive neurologic impairment, microcephaly, delay in achievement of developmental milestones, hypertonia, or encephalopathy are seen more frequently in HIV-infected than uninfected children. In a study in Uganda, 30% of HIV-infected infants developed motor abnormalities and 26% cognitive abnormalities in the first 12 months of life, compared to 11% and 6% of HIV-exposed but uninfected infants.</td>
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<td>Herpes zoster (shingles)</td>
<td>Single dermatomal zoster (painful rash with blisters confined to one dermatome on one side). While uncommon, seen in about 2% of infected children compared to 0.2% of HIV-exposed but uninfected children in a study in Uganda.</td>
<td>Bakaki et al., 2001 [95]</td>
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<tr>
<td>HIV dermatitis</td>
<td>This is manifest primarily as an</td>
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<td>WHO HIV diagnosis in infants and children</td>
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<td>Erythematous papular rash that may consist of discrete, macular, hyperpigmented and pruritic lesions, and is persistent and unresponsive to treatment. Subcutaneous abscesses and impetigo may have increased frequency as well. Generalized dermatitis is frequent in HIV-infected infants in developing countries, reported in 70-87% of infected compared to 20-35% of uninfected infants.</td>
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<td>Dray-Spira et al., 2000; Bakaki et al., 2001 [95, 101]</td>
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</tbody>
</table>
References


WHO HIV diagnosis in infants and children


[27] Schupbach J, Boni J, Bisset LR, Tomasik Z, Fischer M, Gunthard HF, et al. HIV-1 p24 antigen is a significant inverse correlate of CD4 T-cell change in patients with


