World Health Organization

WHO RECOMMENDATIONS ON THE DIAGNOSIS OF HIV INFECTION IN INFANTS AND CHILDREN:

ANNEXES

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Annex 1 Summary of findings and quality of evidence evaluation for the use of virological testing

Applying the grades of recommendation assessment, development and evaluation (GRADE) methodology, specific questions were identified and formulated according the population targeted, intervention, comparator, outcome and time (PICOT) framework as follows:

1. Should HIV infection in infants be diagnosed by performing an HIV DNA polymerase chain reaction (PCR) test at age 4–6 weeks compared with the gold standard?
2. Should HIV infection in infants be diagnosed by performing an HIV RNA nucleic acid amplification test (NAT) at age 4–6 weeks compared with the gold standard?
3. Should HIV infection in infants be diagnosed by performing an ultrasensitive p24 antigen (Us p24 Ag) test at age 4–6 weeks compared with the gold standard?
4. Is HIV DNA PCR using dried blood spots (DBS) reliable for diagnosing HIV in infants at age 4–6 weeks compared with liquid samples?
5. Is RNA NAT using DBS samples reliable for diagnosing HIV infection in infants at age 4–6 weeks compared with liquid samples?
6. Is the Us p24 Ag test using DBS samples reliable for diagnosing HIV infection in infants at age 4–6 weeks compared with liquid samples?

A review of the literature was performed using the following sites and search engines: PubMed, MEDLINE, American Family Physician, Bandolier, *The Journal of Family Practice*, *BMJ Clinical Evidence*, *The Cochrane Library*, SUM search, National Guideline Clearinghouse, Institute for Clinical Systems Improvement, TRIP Database, abstracts from the Conference on Retroviruses and Opportunistic Infections (CROI) 2005–2008, and abstracts from the conference of the International AIDS Society (IAS) 2005–2008. Different combinations of the following search terms were used: HIV infection, HIV infant diagnosis, HIV virological test, HIV NAT test, HIV DNA assay, HIV RNA assay, p24 Ag assay, dried blood spots. Language or date of publication was not a restriction.

The paper selection process was performed according to the GRADE approach and studies were considered as 'valid' only if the following criteria were met:

- Population of interest (infants <18 months exposed to HIV)
- Uncertain diagnosis (infection status of the population being tested was unknown)
- Consecutive enrolment
- Comparison with an appropriate reference standard.

Therefore, valid studies of diagnostic test accuracy included representative and consecutive patients in whom legitimate diagnostic uncertainty existed; they involved a comparison between the test under consideration and an appropriate reference ('gold') standard. The studies were then listed and briefly described in simple summary tables (Tables A–1 to A–18).

Five studies were selected to assess the DNA PCR assay performance on liquid samples (Tables A–1 to A–3). Data collected on the use of this assay were published between 1993 and 2005. The studies were conducted mainly in high-income countries, and the

performance indicators showed significant variation over time as technologies improved (*1,2,3,4,5*)

For the use of RNA NAT assays on plasma, the evidence used was based on four studies (Tables A–4 to A–6) (*4,6,7,8*) published between 1997 and 2003. Good consistency of results was found across the studies. Only one study (Young et al.) (*7*) was conducted in a low–middle income country.

Data regarding the use of first-generation p24 Ag assay were excluded, given the superiority of the Us p24 Ag assay (Tables A–7 to A–9); nine studies (*9,10,11,12,13,14,15,16,17*) provided evidence on the performance of this assay and all but one (Fiscus et al.) (15) were conducted in low–middle income countries.

The use of DBS samples was reported by seven studies for HIV DNA PCR (Tables A–10 to A–12) (*18,19,20,21,22,23,24*), two studies for RNA-NAT (Tables A–13 to A–15) (*24,25,30*) and four studies for Us p24 Ag assay (Tables A–16 to A–18) (*26,27,28,29*). The studies provide a comparison of the same assay performed on liquid samples or with the current gold standard.

In order to assess the sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) as accuracy indicators, a 2x2 contingency table was obtained from each study restricting data to the performance at age 4–6 weeks, wherever possible. Subsequently, the 2x2 contingency tables were pooled together and accuracy indicators were calculated for each of the assays considered.

Accuracy indicators were defined as surrogate outcomes while preparing the GRADE profile and, for each of the four indicators, valid studies were assessed for limitations using the Quality in Diagnostic and Screening tests (QUADAS) checklist. In addition, the presence of indirectness, inconsistency and impreciseness was assessed, and the final quality of evidence obtained (GRADE profiles).

Table A–1. Summary of evidence for the use of HIV DNA PCR assay

Study	Test used	Specimen	Findings	Sn/Sp	Comments
Kline et al. 1994 [1] USA	In-house DNA PCR versus culture	Venepuncture of infants born to HIV-infected mothers. 144 mother–baby pairs	Birth: 5 positive/5 infected 25 negative/26 uninfected (1 false positive [FP]) 1 month: 2 positive/2 infected 5 negative/5 uninfected	Birth: Sn 100% Sp 96% 1 month: Sn 100% Sp 100% 2 months: Sn 100% Sp 100%	Small numbers
Bremer et al. 1996 [2] Puerto Rico USA	Longitudinal, multicentre study (Roche Amplicor DNA PCR testing kit) Co-culture performed as a gold standard	Peripheral blood specimens routinely collected at follow-up visits. Infants enrolled in the Women and Infants Transmission Study (WITS)	1209 specimens were obtained from 483 infants tested 90 HIV infected (223 samples) 340 not infected (859 samples) 53 indeterminate(127 samples)	At 1 month: Sn 92% Sp 100% PPV 100% NPV 98% Overall 93% agreement between PCR and culture	17 false-positive results probably due to contamination since all these specimens were from the same site. Infants not breastfed.
Nelson et al. 1996 [3] USA	Prospective, blinded study (commercially available DNA PCR test; not specified)	286 seropositive infants and children (1988–1992)	567 tests performed on samples from 286 seropositive subjects 105 infected: 96/105 results positive 181 uninfected: 180/181 results negative	At 8 days–6 months: Sn 95% Sp (not reported) All ages (overall) Sn 91% Sp 99.4% PPV 100%	All 145 initial samples obtained between 5 weeks and 12 months correctly predicted infection status (PPV 100%)
Lambert et al. 2003 [4] Puerto Rico USA	Multicentre randomized controlled trial (commercial DNA PCR assay)	Performed by a single lab (part of virology quality assessment [VQA]) programme proficiency testing)	124 mother–infant pairs 24 infected, 100 uninfected 118 had a test performed at 6 weeks	At 6 weeks: Sn (15/18) 83% Sp (99/100) 99% PPV (16/16) 93.8% NPV (99/102) 97.1%	All women received antiretrovirals (ARVs) during pregnancy (92% azidothymidine (AZT) alone and 8% dual therapy). All infants received postnatal AZT prophylaxis. Most mothers were African, American or Hispanic.

Study	Test used	Specimen	Findings	Sn/Sp	Comments
Sherman et al. 2005 [5] South Africa	Retrospective review (Roche Amplicor HIV-1 DNA PCR version 1.5 assay)	Cohort A: 301 exposed infants Cohort B: 25 abandoned children Cohort C: 443 exposed infants	769 infants tested (58 infected) 1825 tests performed (144 in HIV-infected) 7 FP	<u>At 6 weeks:</u> Sn 98.8% Sp 99.4%	Cohort A: Single-dose nevirapine (SdNVP) in 97%, only 2% were breastfed Cohort B: never received ARVs Cohort C: only some received SdNVP Subtype C most common

Table A–2. Summary of findings for the use of HIV DNA PCR assay

Question: Can HIV DNA PCR be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	92.86; 95%CI [88.49–95.95]	1202 infants (5 studies)	**High**
Specificity (Sp)	99.09; 95% CI [98.28–99.58]		
Positive predictive value (PPV)	95.59; 95% CI [91.79–97.96]		
Negative predictive value (NPV)	98.50; 95%CI [97.53–99.16]		
False negative (FN)	15		
False positive (FP)	9		

Table A–3. GRADE profile for the use of HIV DNA PCR for diagnosis of HIV infection in infants

Question: Should HIV infection in infants be diagnosed by an HIV DNA PCR test at 4–6 weeks?
Population group: Infants exposed to HIV (i.e. infants <18 months of age born to an HIV-positive mother)
Intervention: HIV DNA PCR at 4–6 weeks
Comparator: HIV diagnosed by HIV culture or HIV antibody testing after 18 months of age

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factors	Quality rank
Outcome: **SENSITIVITY** 92.86% (195/210) 95%CI [88.49–95.95]							
5 studies[1,2,3,4,5] (210 infected among 1202 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **SPECIFICITY** 99.09% (983/992) 95% CI [98.28–99.58]							
5 studies[1,2,3,4,5] (210 infected among 1202 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **POSITIVE PREDICTIVE VALUE** 95.59% (195/204) 95% CI [91.79–97.96]							
5 studies[1,2,3,4,5] (210 infected among 1202 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 98.50% (983/998) 95% CI [97.53–99.16]							
5 studies[1,2,3,4,5] (210 infected among 1202 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**

[a] For diagnostic assessment, valid accuracy studies are identified against the Quality in Diagnostic and Screening tests (QUADAS) assessment and provide a high quality of evidence. Studies providing data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), where diagnosis was uncertain and patients were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist.

[c] However, there are concerns about the generalizability of the HIV primers used since HIV subtype B is the one mainly investigated across the selected studies.

Table A–4. Summary of evidence for the use of HIV RNA NAT assay

Study	Test used	Specimen	Findings	Sn/Sp	Comments
Delamare et al. 1997 [6] France	Nucleic acid sequence-based amplification (NASBA) versus antibody (Ab)	96 infants enrolled in French National Prospective study	48 infected 48 uninfected 10 days–3 months: 47/48 positive results 39/39 negative results	0–10 days: Sn 25% Sp 100% 10 days–3 months: Sn 98% Sp 100%	Only 3 mothers received ARVs during pregnancy and none of the infants was treated before the first positive viral detection.
Young et al. 2000 [7] Thailand	Quantitative RNA PCR (Roche Amplicor)	Prospectively tested and retrospective testing of stored specimens All positive samples were retested	395 nonbreastfed exposed infants 22/47 subtype E At 2 months: 47/47 positive 100/100 negative	Birth: Sn 47%, Sp 100% 2 months: Sn 100%, Sp 100% 6 months: Sn 100%, Sp 100%	Short-course antenatal AZT for the mother, no ARVs for the infants 92% subtype E Little difference in the median RNA levels by treatment group
Neisheim et al. 2003 [8] USA	NASBA and RNA PCR (Roche)	156 exposed nonbreastfed infants enrolled in the Perinatal AIDS Collaborative Transmission Study (PACTS)	54 infected 102 uninfected	0–7 days: Sn 29%, Sp 100% 8–28 days: Sn 79%, Sp 100% 29–60 days: Sn 91%, Sp 93% 61–120 days: Sn 96%, Sp 100% 120–180 days: Sn 97%,Sp 100%	Sn and Sp were not affected by maternal or infant AZT treatment even though infant viral load (VL) was lower during the first 6 weeks for those receiving AZT(P=0.005) Lower limit of detection: NASBA 1000 versus Amplicor 400

Study	Test used	Specimen	Findings	Sn/Sp	Comments
Lambert et al. 2003 [4] USA Puerto Rico	NASBA versus Ab/culture	Performed by a single lab (part of VQA programme proficiency testing)	124 mother–infant pairs 24 infected, 100 uninfected	<u>6 weeks:</u> Sn (18/19) 100% Sp (100/100) 99% PPV (18/18) 100% NPV (100/101) 99%	4000 cp/ml lower limit of detection 500 cp/ml lower limit of detection for negative samples that were retested All women received ARVs during pregnancy (92% AZT alone and 8% dual therapy) All infants received postnatal AZT prophylaxis Most mothers were African, American or Hispanic

Table A–5. Summary of findings for the use of HIV RNA NAT

Question: Can HIV RNA NAT be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	97.39; 95%CI [93.44–99.28]	434 infants (4 studies)	**High**
Specificity (Sp)	98.93; 95% CI [96.91–99.78]	434 infants (4 studies)	**High**
Positive predictive value (PPV)	98.03; 95% CI [94.34–99.59]	434 infants (4 studies)	**High**
Negative predictive value (NPV)	98.58; 95% CI [96.41–99.61]	434 infants (4 studies)	**High**
False negative (FN)	3	434 infants (4 studies)	**High**
False positive (FP)	4	434 infants (4 studies)	**High**

Table A–6. GRADE profile for the use of HIV RNA nucleic acid amplification testing (NAT) for diagnosis of HIV infection in infants

Question: Should HIV infection in infants be diagnosed by performing an HIV RNA NAT test at 4–6 weeks?
Population group: Infants exposed to HIV (i.e. infants <18 months born to an HIV-positive mother)
Intervention: HIV RNA NAT at 4–6 weeks
Comparator: HIV diagnosed by HIV culture or DNA PCR or HIV antibody testing after 18 months of age

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factor	Quality rank
Outcome: **SENSITIVITY** 97.39% (149/153) 95% CI [93.44–99.28]							
4 studies[4,6,7,8] (153 infected among 434 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision		**High 4**
Outcome: **SPECIFICITY** 98.93% (278/281) 95% CI [96.91–99.78]							
4 studies[4,6,7,8] (153 infected among 434 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision		**High 4**
Outcome: **POSITIVE PREDICTIVE VALUE** 98.03% (149/152) 95% CI [94.34–99.59]							
4 studies[4,6,7,8] (153 infected among 434 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision		**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 98.58% (278/282) 95% CI [96.41–99.61]							
4 studies[4,6,7,8] (153 infected among 434 infants)	Valid accuracy cohort studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision		**High 4**

[a] For diagnostic assessment, valid accuracy studies are identified against the QUADAS assessment and provide a high quality of evidence. Studies providing data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), where diagnosis was uncertain and patients were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist.

[c] However, the HIV primers used in the studies selected were mainly for subtype B and different qualitative thresholds have been considered. Moreover, there are concerns about the possible influence of different prevention of mother-to-child transmission (PMTCT) regimens on viral load detectability, even if currently there is no evidence to suggest that this is a problem.

Table A–7. Summary of evidence for the use of HIV ultrasensitive p24 antigen (Us p24 Ag) assay

Study	Test used	Specimen	Findings	Sn/Sp	Comments
Lyamuya et al. 1996 [9] Tanzania	Up24 Ag Heat-mediated destruction of Ab and signal amplification p24 Ag test	DNA PCR as a gold standard 76 infected (125 samples) 101 uninfected (106 samples) Heated plasma or serum samples	123/125 were positive All 18 samples collected at 1–8 weeks were positive All samples of uninfected infants were negative	Sn 98.7% Sp 100%	Not clear if it is ultrasensitive
Sutthent et al. 2003 [10] Thailand	Up24 antigen Heat-mediated destruction of Ab and signal amplification p24 Ag test (paediatric)	121 infants Frozen plasma samples PerkinElmer kit versus RNA-NASBA and DNA PCR	<u>1–2 months:</u> 21/21 correctly identified positive 100/100 correctly identified negative No FP or FN results	1–2 months and 4–6 months: Sn 100% and Sp 100%	The diagnostic sensitivity of this test in infants was similar to that of PCR for HIV-1 DNA or RNA using specimens from both Europe and Africa; at US$ 3 per test, its cost is 10–30 times less expensive than commercially available HIV-1 RNA viral load tests. Subtypes B and E
Sherman et al. 2004 [11] South Africa	Up24 Ag enzyme immunoassay (EIA) heat-denatured and boosted by signal amplification test	Plasma samples collected prospectively PerkinElmer kit	Analysis on 52 plasma samples from 24 infected infants and 151 from 66 uninfected infants Overall: 1 FN, 2 FP At 6 weeks: 1 FN and 0 FP	6 weeks: Sn 95.7% Sp 100 % 3 months: Sn 100% Sp 100%	Mothers and infants received SdNVP <3% reported breastfeeding No infants received antiretroviral therapy (ART) after the first 72 hours of life
Zijenah et al. 2005 [12] Zimbabwe	Us p24 Ag Heat-mediated destruction of Ab and signal amplification p24 Ag test	PerkinElmer kit versus DNA PCR Roche 1.5 Samples from 164 infants Tests conducted in a blind fashion	88/91 correctly identified positive; all uninfected were identified as negative, 3 FN were reported (<400 cp/ml with Amplicor 1.5)	0–18 months: Sn 96.7% Sp 96.1% 0–6 months: Sn 98.1% Sp 96.9%	Patients enrolled from the short-course AZT (short course AZT+ 7 days of AZT for the babies) study and the PACD (no maternal ARVs) All breastfeeding Subtype C

Study	Test used	Specimen	Findings	Sn/Sp	Comments
George et al. 2007 [13] Haiti	Us p24 Ag EIA heat-denatured and with and without signal amplification test	401 frozen plasma samples Kit used: Biomerieux and PerkinElmer versus RT RNA assay 1.1	Us p24 Ag: 43 infected and 157 uninfected, 3 FN and 1 FP VIDAS: 43 infected and 157 uninfected; 2 FN, 1 FP	Us p24 Ag Sn 93%, Sp 99% VIDAS Sn 95%, Sp 99% RT RNA Sn 91% Sp 97%	233 subtype B-exposed ART-naive infants Good accuracy levels reported for the same test done in a district hospital by trained local technician
Elyanu et al. 2007 [14] Uganda	Us p24 Ag EIA Heat-mediated destruction of Ab and signal amplification p24 Ag test	210 HIV-exposed infants were enrolled in a cross-sectional study PerkinElmer Us p24 Ag assay kit	92 infected Of the 121 samples available, 33/37 were correctly identified as positive 82/84 correctly identified negative	1.5–6 months: Sn 89.2% Sp 97.6% PPV 94.3% NPV 95.3%	Compared with Amplicor 1.5 as a gold standard Challenges reported in setting up and quality assurance of the assay Primarily subtypes A, D and C
Fiscus et al. 2007 [15] USA	Us p24 Ag EIA Heat-mediated destruction of Ab and signal amplification p24 Ag test	PerkinElmer kit Stored plasma samples (0–180 days) PCR DNA or culture as a gold standard	802 specimens from 582 infants were tested. 8–30 days: 25/TP, 148/151 TN, 4 FN, 3 FP	Overall: Sn 91.7% Sp 98.5% PPV 94% NPV 97.5%	Excluding infants less than 1 week: Sn 93.7%, Sp 98.3% Only subtype B Large sample size
Nouhim et al. 2007 [16] Cambodia	Us p24 Ag EIA Heat-mediated destruction of Ab and signal amplification p24 Ag test	PerkinElmer kit retrospectively on frozen samples DNA PCR or culture and gold standard	147 exposed Cambodian infants (1–24 months) 42/46 correctly identified positive All 123 correctly identified negative	Sn 91.3% Sp 100%	No breastfeeding
Sohn et al. 2007 [17] Viet Nam	Us p24 Ag EIA Heat-mediated destruction of Ab and signal amplification p24 Ag test	HIV-exposed infants <2 months. Test performed at a local hospital clinical lab versus in-house DNA PCR on 2 separate samples	202 tested 12 infected No FP or FN results	Sn 100% Sp 100%	ART exposure: pre- and postnatal 77.9%, postnatal only 15.3%, or none 6.9% Postnatal given >7 days Formula breastfeeding was the standard practice

Table A–8. Summary of findings with the use of HIV ultrasensitive p24 antigen (Us p24 Ag) assay

Question: Can HIV Us p24 Ag assay be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	95.62; 95%CI [93.25–97.34]	1473 infants (9 studies)	**High**
Specificity (Sp)	99.42; 95% CI [98.75–99.79]	1473 infants (9 studies)	**High**
Positive predictive value (PPV)	98.57; 95% CI [96.92–99.48]	1473 infants (9 studies)	**High**
Negative predictive value (NPV)	98.19; 95%CI [97.19–98.91]	1473 infants (9 studies)	**High**
False negative (FN)	19	1473 infants (9 studies)	**High**
False positive (FP)	6	1473 infants (9 studies)	**High**

Table A–9. GRADE profile for the use of HIV ultrasensitive p24 antigen (Us p24 Ag) on plasma samples for diagnosis of HIV infection in infants

Comparison: Is Us p24 Ag test using DBS samples reliable for diagnosing HIV infection in infants at 4–6 weeks?
Population group: Infants exposed to HIV in low–middle income countries
Intervention: HIV ultrasensitive p24 Ag testing on plasma samples at 4–6 weeks
Comparator: HIV diagnosed by gold standard

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factors	Quality rank
Outcome: **SENSITIVITY** 95.62% (415/434) 95% CI [93.25–97.34]							
9 studies[9,10,11,12,13,14,15,16,17] (434 infected among 1473 infants)	Valid accuracy studies[a] 4	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **SPECIFICITY** 99.42% (1033/1039) 95% CI [98.75–99.79]							
9 studies[9,10,11,12,13,14,15,16,17] (434 infected among 1473 infants)	Valid accuracy studies[a] 4	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **POSITIVE PREDICTIVE VALUE** 98.57% (415/421) 95% CI [96.92–99.48]							
9 studies[9,10,11,12,13,14,15,16,17] (434 infected among 1473 infants)	Valid accuracy studies[a] 4	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 98.19% (1033/1052) 95% CI [97.19–98.91]							
9 studies[9,10,11,12,13,14,15,16,17] (434 infected among 1473 infants)	Valid accuracy studies[a] 4	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**

[a] For diagnostic assessment, valid accuracy studies are identified against the QUADAS assessment and provide a high quality of evidence. Studies providing data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), where diagnosis was uncertain and patients were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist.

[c] However, there are some concerns about the applicability of this test for subtype D. Moreover, there are concerns about the possible influence on detectable viral load of different PMTCT regimens, even if currently there is no evidence to suggest this is a problem.

Table A–10. Summary of evidence for the use of HIV DNA PCR on dried blood spot (DBS) samples

Study	Test used	Specimen/ Potential treatment/storage of DBS	Sn/Sp	Comments
Comeau et al. 1996 [18] Puerto Rico USA	In-house HIV DNA PCR	Whole blood spots versus liquid blood specimen Anticoagulated blood spotted onto filter paper, dried and stored in plastic bags at –80 °C to +4 °C	Samples from infants in the Puerto Rico and the USA Sn and Sp at 1–4 months of age ranged between 89% and 97%, and 98% and 100%, respectively. Results unaffected by storage temperature	DBS HIV DNA PCR offers a reliable tool for early diagnosis of HIV infection with important advantages over liquid blood HIV DNA PCR and viral culture
Biggar et al. 1997 [19] Malawi	Roche Amplicor v1.0 HIV DNA PCR	Whole blood spots versus liquid blood specimen Heel prick into microtainers then onto S&S 903 filter paper. Air-dried and stored at –20 °C in impermeable plastic bag with dessicant pellet	Samples from infants in Malawi PPV: 98% if both replicates strongly positive NPV: 96% if both replicates negative Lower limit of detection: 5 HIV proviral DNA copies per sample, viz. 30 000 nucleated cells	All samples tested in duplicate The use of HIV DNA PCR assay on DBS samples (performed at 4weeks or later) was 98.9% accurate in predicting antibody positivity performed at 15 months
Beck et al. 2001 [20] Peru USA	In-house nested HIV DNA PCR	Whole blood spots versus liquid blood specimen Specialized filter paper (FTA card and Gene Guard system); air-dried, stored at room temperature in plastic bag with silica gel dessicant	Samples from the USA (adults and children): Sn and Sp: 98% Samples from Peru (adults and infants): Sn: 99% Sp: 100%	Practical, economical, sensitive and specific method for diagnosis of HIV-1 subtype B
Fischer et al. 2004 [21] Rwanda	In-house nested HIV DNA PCR and HIV-1 DNA PCR (Roche Amplicor v1.5)	Whole blood spots versus liquid blood specimen Heel stick whole blood on Isocode Card (S&S), dried at room temperature, stored in plastic bag	Samples from infants in Rwanda In-house nested PCR assay Sn 90%, Sp 94% HIV-1 DNA PCR v1.5 <4 months: Sn 100%, Sp 98% >4 to <12 months: Sn 100%, Sp 97% >12 months to <26 months: Sn 100%, Sp 100%	Simple and easy to perform DNA extraction method developed that can easily be implemented under field conditions HIV-1 subtype A was the subtype detected in these samples

Study	Test used	Specimen/ Potential treatment/storage of DBS	Sn/Sp	Comments
Sherman et al. 2005 [22] South Africa	HIV-1 DNA PCR Roche Amplicor v1.5	Blood spots versus liquid blood specimen (RNA and DNA) 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	Samples from 6-week-old infants in South Africa Sn: 100%, Sp: 99.6%	DBS collection for HIV DNA PCR testing increases access to early diagnosis in low-resource settings Subtypes C and A of HIV-1 were detected
Ngo-Giang-Huong et al. 2008 [23] Thailand	In-house real-time DNA PCR versus Roche Amplicor HIV-1 DNA test v1.5	Venous blood on Whatman No. 1 filter paper; air-dried, stored in plastic bag with no dessicant sachet at room temperature.	1319 DBS in Thailand (nonbreastfed). Real-time DNA PCR and Roche DNA PCR results were 100% concordant	Compared with HIV serology results, the Roche test sensitivity was 98.6% (95% CI: 92.6–100.0%) and its specificity at 4 months of age was 99.7% (95% CI: 99.2– 99.9%).
Leelawiwat et al. 2009 [24] Thailand	HIV-1 DNA PCR Roche Amplicor v1.5 for DBS HIV-1 DNA PCR Roche Amplicor v1.0 for whole blood samples	2-month-old infants (Thailand) 809 nonbreastfeeding infants were enrolled but samples collected only from 741 (both whole blood and DBS samples) at 2 months Venous blood on Whatman No. 1 filter paper; air-dried, stored in plastic paper envelop and bag with dessicant sachet at room temperature for 9–19 months	56/56 correctly identified positive 106/106 correctly identified negative	PMTCT for mother and babies: short-course AZT or short-course AZT plus SdNVP 2000 cp/ml lower limit of detection

Table A–11. Summary of findings for the use of HIV DNA PCR on dried blood spot (DBS) samples

Question: Can HIV DNA testing on DBS samples be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	94.73; 95%CI [92.97–96.16]	4085 infants (7 studies)	**High**
Specificity (Sp)	98.53; 95% CI [98.06–98.92]	4085 infants (7 studies)	**High**
Positive predictive value (PPV)	94.15; 95% CI [92.32–95.66]	4085 infants (7 studies)	**High**
Negative predictive value (NPV)	98.68; 95%CI [98.23–99.05]	4085 infants (7 studies)	**High**
False negative (FN)	43	4085 infants (7 studies)	**High**
False positive (FP)	48	4085 infants (7 studies)	**High**

Table A–12. GRADE profile for the use of HIV DNA PCR using DBS samples for diagnosis of HIV infection in infants

Question: Is HIV DNA PCR using DBS reliable for diagnosing in infants at 4–6 weeks?
Population group: Infants exposed to HIV (i.e. infants <18 months born to an HIV-positive mother) in low–middle income countries
Intervention: HIV DNA PCR testing on DBS specimen at 4–6 weeks
Comparator: HIV diagnosed by DNA PCR in whole blood samples

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factors	Quality rank
Outcome: **SENSITIVITY** 94.73% (773/816) 95% CI [92.97–96.16]							
7 studies[18,19,20,21,22,23,24] (816 infected among 4085 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **SPECIFICITY** 98.53% (3221/3269) 95% CI [98.06–98.92]							
7 studies[18,19,20,21,22,23,24] (816 infected among 4085 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **POSITIVE PREDICTIVE VALUE** 94.15% (773/821) 95% CI [92.32–95.66]							
7 studies[18,19,20,21,22,23,24] (816 infected among 4085 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 98.68% (3221/3264) 95% CI [98.23–99.05]							
7 studies[18,19,20,21,22,23,24] (816 infected among 4085 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**

[a] For diagnostic assessment, valid accuracy studies are identified against the QUADAS assessment and provide a high quality of evidence. Studies providing data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), where diagnosis was uncertain and patients were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist

[c] However, there are some concerns about the applicability of this test for subtype D. Moreover, there are concerns about the possible influence on detectable viral load of different PMTCT regimens, even if currently there is no evidence to suggest this is a problem.

Table A–13. Summary of evidence for the use of RNA nucleic acid amplification testing (NAT) on dried blood spot (DBS) samples

Study	Technique	Specimen	Finding	Sn/Sp	Comments
Leelawiwat et al. 2009 [24] Thailand	Nuclisens RNA NASBA assay 1000 cp/ml lower limit of detection assumed. VL was measured with Amplicor 1.5	2-month-old infants (Thailand) 809 nonbreastfeeding infants were enrolled but for 741 a pair of whole blood and DBS samples was collected at 2 months	56/56 correctly identified positive 106/106 correctly identified negative No FP/FN results High correlation for VL measurement between DBS and plasma samples; however, in DBS VL was 0.4 log lower (less when corrected by haematocrit)	Sn 100% Sp 100%	PMTCT for mother and babies: short-course AZT or short-course AZT plus SdNVP
Kerr et al. 2009 [25] Multicentre	Gen-Probe Aptima HIV-1 RNA qualitative assay	DBS versus whole blood 50 µl of whole blood per spot on Whatman 903 cards (heel stick) Samples collected in Dominican Republic (B), Haiti (B), Malawi (C), South Africa (C), Tanzania (HIVNET024- A,C,D), Trinidad (B), North Carolina, USA (subtype B), Viet Nam (CRF01-AE)	291 DBS from infants and children born to HIV-infected mothers 128/129 TP 162/162 TN 1 FN stored for 4 years and had the lowest VL in HIVNET024 samples (10.954 in May 2003)	Sn 99.2% Sp 100%	In whole blood spots intracellular RNA or proviral DNA might be detected as well increased sensitivity and a lower limit of detection. Timing of test not specified
Stevens et al. 2009 [30]	Ultra-high-throughput, automated using the Gen-Probe Aptima HIV-1 screening assay. Roche Amplicor as comparator	Western blot EDTA samples (500) 500 DBS samples Randomly used	The sensitivity of the assay with western blot (WB) and DBS samples was 100%, and the specificities were 99.4% and 99.5% for DBS and WB, respectively	WB Sp 99.4% Sn 100% DBS Sp 99.5% Sn 100%	Improved Sn using TMA technology Ability to process 1900 samples in a 24-hour period makes the Aptima assay an attractive option

Table A–14. Summary of findings for the use of RNA nucleic acid amplification testing (NAT) on dried blood spot (DBS) samples

Question: Can HIV RNA NAT on DBS samples be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	99.45; 95%CI [96.96–99.9]	485 infants (2 studies)	**High**
Specificity (Sp)	100; 95% CI [99.02–100]	485 infants (2 studies)	**High**
Positive predictive value (PPV)	100; 95% CI [98.35–100]	485 infants (2 studies)	**High**
Negative predictive value (NPV)	99.67; 95%CI [98.19–99.99]	485 infants (2 studies)	**High**
False negative (FN)	4	485 infants (2 studies)	**High**
False positive (FP)	1	485 infants (2 studies)	**High**

Table A–15. GRADE profile for the use of RNA nucleic acid amplification testing (NAT) on dried blood spots (DBS)

Comparison: Is RNA NAT using DBS samples reliable for diagnosing HIV infection in infants at 4–6 weeks?
Population group: Infants exposed to HIV in low–middle income countries
Intervention: HIV RNA NAT on DBS samples at 4–6 weeks
Comparator: HIV diagnosed by gold standard test on whole blood samples

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factors	Quality rank
Outcome: **SENSITIVITY** 99.45% (180/181) 95% CI [99.96–99.99]							
2 studies[24,25] (181 infected among 485 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **SPECIFICITY** 100% (304/304) 95% CI [99.02–100]							
2 studies[24,25] (181 infected among 485 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **POSITIVE PREDICTIVE VALU**E 100% (180/180) 95% CI [98.35–100]							
2 studies[24,25] (181 infected among 485 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 99.67% (304/305) 95% CI [98.19–99.99]							
2 studies[24,25] (181 infected among 485 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**

[a] For diagnostic assessment, valid accuracy studies provide a quality of evidence as high as randomized controlled trials (RCTs) considering patients' important outcomes. Therefore, those studies that provide data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), whose diagnosis was uncertain and they were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist.

[c] However, different qualitative thresholds have been considered. Moreover, there are concerns about the possible influence of different PMTCT regimens on the detectability of viral load, even if currently there is no evidence to suggest that this is a problem.

Table A–16. Summary of evidence for the use of ultrasensitive p24 antigen (Us p24 Ag) assay on dried blood spot (DBS) samples

Study	Technique	Specimen	Finding	Sn/Sp	Comments
De Baets et al. 2005 [26] Democratic Republic of Congo	Us p24 Ag on venous plasma and capillary plasma stored on filter paper and liquid sample versus DNA PCR (in-house on DBS)	941 children (1 month–12 years) 153 under 18 months (13 infected)	<18 months: 5/5 positive results on capillary plasma on filter paper (total 87 performed) 12/13 positive results on venous plasma (150 performed)	Sn 100% Sp 100%	This study assessed the accuracy of different diagnostic algorithms
Patton et al. 2006 [27] South Africa	Modified Us p24 Ag test kit on DBS versus NAT (DNA Amplicor v1.5 or NASBA and Nuclisens RNA)	Specimens from 141 children (0–12 years) 83 infected and 58 uninfected (with DNA PCR)	No FP 1 indeterminate and 1 FN	Sn 98.8% Sp 100%	Relationship between p24 Ag values in DBS and plasma was assessed in a small group with known VL (r=0.76)
Patton et al. 2008 [28] South Africa	Modified Us p24 Ag test kit on DBS versus NAT (DNA Amplicor v1.5 or NASBA and Nuclisens RNA)	W. paper 1 without (121) versus paper 903 with (125) desiccant 147 6-week-old babies plus 99 known HIV-infected children	DBS available for 75 (<6 weeks) 14/147 found positive were HIV exposed	Paper 903 with dessicant: Sn 98.3% at 6 weeks Sp 100% at 6 weeks Paper 1 without dessicant: Sn 96.5% at 6 weeks Sp 100% at 6 weeks	Paper 1 could be considered acceptable when 903 not available

Study	Technique	Specimen	Finding	Sn/Sp	Comments
Cachafeiro et al. 2009 [29] Dominican Republic Malawi South Africa USA Viet Nam	Modified Us p24 Ag test kit Compared with Roche Amplicor v1.5 DNA PCR or in-house (South Africa, USA and Viet Nam) or NuclisensQT/BioMerieux RNA PCR (Dominican Republic, Malawi)	Whatman paper SS 903 Stored with desiccant in zip-locked bags at room temperature or –20 ℃ and then shipped to the USA Specimens from 100 infants, 1–6 weeks of age Controls from non HIV-infected infants	4/5 correctly identified as positive 95/95 correctly identified as negative	1–6 weeks: Sn (4/5) 83% Sp (95/95) 100% PPV 100% NPV 98.9%	Sn and Sp were also analysed according to: time since DBS collection (sensitivity decreased with storage); and country of origin with presumed different subtypes. ARV exposure in infancy: USA 6 weeks of AZT; Malawi SdNVP plus 1 week of AZT

Table A–17. Summary of findings for the use of ultrasensitive p24 antigen (Us p24 Ag) assay on dried blood spot (DBS) samples

Question: Can HIV Us p24 Ag on DBS samples be used at 6 weeks to diagnose HIV in HIV-exposed infants?

Outcomes	Values and uncertainty around these	Number of participants (studies)	Quality of evidence
Sensitivity (Sn)	97.37; 95%CI [94.86–99.91]	537 infants (4 studies)	**High**
Specificity (Sp)	100; 95% CI [99.29–100]	537 infants (4 studies)	**High**
True positive (PPV)	100; 95% CI [97.34–100]	537 infants (4 studies)	**High**
Negative predictive value(NPV)	99.30; 95%CI [97.96–99.85]	537 infants (4 studies)	**High**
False negative (FN)	3	537 infants (4 studies)	**High**
False positive (FP)	0	537 infants (4 studies)	**High**

Table A–18. GRADE profile for the use of HIV ultrasensitive p24 antigen (Us p24 Ag) on dried blood spot (DBS) samples for the diagnosis of HIV infection in infants

Question: Is the Us p24 Ag test using DBS samples reliable in diagnosing HIV infection in infants at 4–6 weeks?
Population group: Infants exposed to HIV in low–middle income countries
Intervention: Us p24 Ag on DBS samples at 4–6 weeks
Comparator: HIV diagnosed by gold standard test on whole blood samples

Number of studies	Design	Limitations	Consistency	Directness or generalizability	Imprecise or sparse data	Other factors	Quality rank
Outcome: **SENSITIVITY** 97.37% (111/114) 95% CI [92.50–99.46]							
4 studies[26,27,28,29] (121 infected among 577 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **SPECIFICITY** 100% (423/423) 95% CI [99.29–100]							
4 studies[26,27,28,29] (121 infected among 577 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **POSITIVE PREDICTIVE VALUE** 100% (111/111) 95% CI [97.34–100]							
4 studies[26,27,28,29] (121 infected among 577 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**
Outcome: **NEGATIVE PREDICTIVE VALUE** 99.30% (423/426) 95% CI [97.96–99.85]							
4 studies[26,27,28,29] (121 infected among 577 infants)	Valid accuracy studies[a] **4**	No serious limitations[b]	No serious inconsistency	No serious indirectness[c]	No serious imprecision	No publication bias	**High 4**

[a] For diagnostic assessment, valid accuracy studies provide a quality of evidence as high as that from randomized controlled trials (RCTs) considering patients' important outcomes. Therefore, those studies that provide data about the use of a recognized gold standard test in the population of interest (HIV-exposed infants), whose diagnosis was uncertain and they were consecutively enrolled, were selected and considered to start from a high quality.

[b] Assessed with the QUADAS checklist.

[c] However, there are some concerns about the applicability of this test for subtype D, as well as about the possible influence on detectable viral load of different PMTCT regimens, even if currently there is no evidence to suggest that this is a problem.

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Annex 2 GRADE profiles for clinical algorithms

A2.1 Methodology

Based on the new WHO paediatric ART guidelines, all HIV-infected children should be initiated on ART during the first year of life and should, therefore, be identified as early as possible. Since virological tests are still unaffordable and/or unavailable in many settings, it was necessary to investigate:

- How well HIV-exposed infants (needing special follow up) can be identified by using antibody tests
- How well HIV-infected infants (needing ART in the first year of life) can be identified by using clinical algorithms (possibly combined with a rapid test)
- How well HIV-uninfected infants or HIV-exposed infants who have seroreverted (not needing special follow up) can be identified by using rapid tests
- What would be the best age to conduct such screening?

These questions were reframed according to the PICOT framework, a format and structure for developing research questions (*1*).

1. Is the accuracy of applying a clinical algorithm (with or without performing a rapid test) to identify HIV infection in a child younger than 18 months comparable with that of the gold standard infant test (PCR or retrospectively determined serostatus)?
2. Is the accuracy of one rapid test performed to exclude HIV infection in an infant less than 18 months comparable with that of the gold standard infant test (PCR or retrospectively determined serostatus)?
3. Is the accuracy of one rapid test performed to identify HIV-exposure in certain* young infants comparable with that of the gold standard?**
4. What would be the best age to conduct such screening (also taking into account information about seroreversion in untreated infants)?

* The definition of 'certain' young infants includes infants of mothers with unknown serostatus and may include infants from 'certain' HIV-negative mothers. In high HIV-prevalence areas or high-risk groups, we may want to also include mothers who tested HIV negative less than 3 months prior to delivery, depending on the need for repeat testing.

** The gold standard for the identification of HIV exposure should be the serostatus of the mother. Additional information from the test performance as compared with the infant gold standard diagnostic test will also be provided.

A review of the literature was performed, using the following sites and search engines to identify suitable literature: PubMed, Bandolier, the *Journal of Family Practice*, *BMJ Clinical Evidence*, *The Cochrane Library*, SUM search, National Guideline Clearinghouse, Institute for Clinical Systems Improvement and Medadvocates. Various combinations of the following terms were used: serological, rapid*, antibody, test, algorithm, diagnosis*, seroreversion, AIDS or SIDA, HIV or VIH and children, enfants, pediatr*, infants. A total of 2096 citations (published before 10 October 2008) were screened. Subsequently, hand searches of related and referenced articles were done. There were no restrictions on language, age of the children or date of publication. Abstracts and reports were included only if they were referenced.

All identified studies reporting on the accuracy of clinical algorithms or serological tests in infants and children or allowing the calculation of some measure of accuracy, as well as studies providing information on seroreversion in infants and children, were listed and described.

For the pooled analysis, the figures from the 2x2 contingency tables of each individual study were entered into Excel spreadsheets. The information about seroreversion was also entered in spreadsheets to facilitate further calculations. Studies with insufficient or unclear information were excluded from this analysis, as well as studies with incorrect comparisons due to very poor methodology (e.g. a comparison of the performance of a clinical algorithm against children identified by the CDC definition of AIDS) and studies that had remained unpublished for more than three years. In studies where the performance of different algorithms was evaluated in the same study population, only the data from the groups with the best accuracy were used for further analyses. In studies where the performance of different tests was evaluated in the same study population, only the data for the groups including the highest number of HIV-infected infants were used for further analyses.

Certain studies were pooled to compare and evaluate the accuracy of a diagnostic tool in a larger group of infants. GRADE PRO analysis was prepared for a selected number of case scenarios, based on the most interesting statistics from the pooled analysis (*23*). Information from the Newcastle–Ottawa quality assessment scale for cohort studies and the Standards for the Reporting of Diagnostic accuracy studies (STARD) initiative were kept in mind during evaluation of the quality of evidence (*3*).

A2.2 GRADE PRO analyses of studies on clinical algorithms

Key outcomes to be considered for recommendations include:

- Factors related to the performance of the tool:

  Sensitivity, specificity, PPV, NPV, LR+, LR– and interobserver agreement

- Factors related to possible task-shifting from paediatricians to non-paediatricians, mainly PHCWs:

  Competence/quality assurance/agreement in conclusion between paediatricians and non-paediatricians, acceptability of clinical algorithms (among PHCWs and the population), PHCWs' self-confidence in applying an algorithm and time needed to apply the algorithm and the cost of applying it.

A2.3 Review of the literature on clinical algorithms for infants and children

Table A–19 summarizes the 22 studies that assessed the accuracy of a clinical algorithm. While a variety of clinical algorithms was assessed, most studies reported on the accuracy of a WHO clinical case definition or a modified version of it.

All these observational studies, except one, were performed in African settings (with HIV-1 subtypes A and C predominantly). Other subtypes (B or HIV-2), as well as differences in environmental factors, have been associated with different patterns of disease progression, possibly resulting in better outcomes of clinical algorithms.

Most of the studies (14/22) selected only inpatients and some included only very ill infants (e.g. those admitted to the intensive care unit). For 17/22 studies, the algorithm was applied by paediatricians or study physicians of tertiary-level hospitals, rather than by primary and secondary health-care workers among outpatients, such as children under five years of age attending clinics.

While the study population included children from birth to the age of 18 years, all studies included infants younger than 18 months but the age distribution, which is closely related to the HIV prevalence rate, however, was most often not described. Three studies included only infants younger than 18 months.

The HIV-prevalence rates among the study populations ranged between 4% and 84%. The studies included different percentages of young infants, used different inclusion criteria, and were performed in different countries and in different decades. Only 8/22 studies used a gold standard that is currently an acceptable gold standard for infants younger than 18 months.

The reported sensitivities ranged between 9% and 89%, specificities between 42% and 99%, and PPVs between 3% and 95%.

Table A–19. List of studies on clinical algorithms in infants and children

No.	Reference	Country and timing of research	Type of study	Study population	Health-care level	Age	HIV pre-valence (%)	Type of investigator	Gold standard
1	Bahwere et al. (4)	Malawi 2002–2005	Information from a prospective and a retrospective cohort	Malnourished children enrolled in a community-based therapeutic programme	Community	<5 years	4.3	Trained nurses and health surveillance assistants	DNA PCR (for those <12 months and confirmations before 18 months) + rapid tests on children and parents
2	Jones et al. (5)	South Africa 2005	Retrospective chart review	Patients attending a PMTCT clinic	Secondary	0–12 months	8.6	18 MDs experienced in local paediatrics (2/3 paediatricians)	EIA +DNA PCR
3	Thurstans S. (*46*)	Malawi 2002–2003	Retrospective chart review	Children who attended a nutritional rehabilitation unit	Tertiary	6–59 months	68.5	Nurses and MDs	DNA PCR in those <12 months, rapid tests in those >18 months
4	Joubert et al. (*7*)	South Africa 2002	Cohort study	Inpatients	Tertiary	0–13 years	43.1	Paediatricians?	EIA + p24 Ag
5	Horwood et al. (*8*)	South Africa 2003	Cohort study	Outpatients, excluding known HIV-infected children	Secondary	2–59 months	28.7	IMCI trainer (MD and nurse) versus a (blinded) paediatrician	EIA + VL test
6	Van Gend et al. (9)	South Africa 2000	Cohort study	Inpatients in medical ward and intensive care unit	Tertiary	0–13 years	30.8	Paediatricians?	EIA + p24 Ag
7	Yeung et al. (10)	South Africa 1996–1997	Prospective cohort study	Inpatients	Secondary	0–5 years	25.6	Study physician	Antibody test + (p24 Ag and/or PCR for those <6 months) or IgG3 for those >6 months

No.	Reference	Country and timing of research	Type of study	Study population	Health-care level	Age	HIV pre-valence (%)	Type of investigator	Gold standard
8	Bakaki et al. (*11*)	Uganda 1994–1996	Cohort study	Inpatients with sepsis and suspected HIV (42% <1 month)	Tertiary	3 days–18 months	21.3	Investigator (blinded to the serostatus)	EIA + PCR
9	Atakouma et al. (12)	Togo 1994–1995	Cohort study	Inpatients	Tertiary	2 months–12 years	84.2	Paediatricians?	EIA + rapid test (HIV Chek)
10	Agbèré et al. (13)	Togo 1994–1995	Cross-sectional study	Inpatients	Tertiary	2 months–12 years	84.2	Paediatricians?	EIA + rapid test (HIV Chek)
11	Jeena et al. (14)	South Africa 1993–1994	Retrospective cohort study	Inpatients at the ICU with post-mortem biopsies available	Tertiary	<12 years	14.0	Paediatricians?	EIA
12	Chintu et al. (15)	Zambia 1993	Cohort study	Inpatients	Tertiary	>6 months	21.6	Paediatricians?	EIA + WB (+ HIV test repeated at 18 months)
13	Kline et al. (16)	USA 1993	Retrospective chart review	Infants with documented intra-uterine HIV exposure	Tertiary	<24 months	20	Paediatricians?	Viral culture + p24Ag test
14	Otieno et al. (17)	Kenya 1992	Cross-sectional study	Inpatients	Tertiary	15 days–12 years	14.0	Investigator	EIA + CD4/CD8 ratio <0.6
15	Vetter et al. (18)	Côte d'Ivoire 1991–1992	Cohort study	Inpatients	Tertiary	28 days–15 years	8.2	Paediatricians?	EIA + WB
16	Msellati et al. (19)	Rwanda 1990	Cohort study	Inpatients	Tertiary	0–18 years	15.0	Paediatricians?	EIA + WB

No.	Reference	Country and timing of research	Type of study	Study population	Health-care level	Age	HIV pre-valence (%)	Type of investigator	Gold standard
17	Eur. Coll. Study (20)	Europe 1990	Retrospective cohort study	Infants with documented intra-uterine HIV exposure	Tertiary	<18 months	15.7	Paediatricians?	EIA + WB, serological test result at 18 months, culture + p24Ag, symptoms
18	Lepage et al. (21)	Rwanda 1986	Cross-sectional study	Inpatients	Tertiary	1 months –14 years	15.4	Paediatricians (blinded to the serostatus)	EIA + WB + p24 Ag (if <16 months)
19	Colebunders et al. (22)	DR Congo 1986	Cross-sectional study	Inpatients (children with measles excluded)	Tertiary	1 months –12 years	10.7	3 physicians (blinded to the serostatus)	EIA + WB
20	Harms et al. (23)	Rwanda 1985	Cohort study	Inpatients	Tertiary	<13 years	44.4	Paediatricians?	EIA + WB
21	Müller et al. (2324)	Uganda 1985–1989	Retrospective chart review	Suspect immunocompromised in- and outpatients	Tertiary	0–8 years	48.8 (in) 50.6 (out)	Paediatricians?	EIA
22	Jonckheer et al. (25)	Belgium 1981–1987	Retrospective chart review	Symptomatic patients of African origin (in- and outpatients)	Tertiary	0–9 years	46.0	Paediatricians?	CDC definition of AIDS + serological test

Summary of search findings

- 22 observational studies
- All but one in African settings, 1981–2005
- Investigator: paediatrician at tertiary level (17/22)
- Study population: mainly inpatients (14/22)
- Age group: 0–18 years (percentage of infants unclear), 3/22 in infants only
- HIV prevalence: 4–84%
- Not always acceptable gold standard (8/22)

- Variety of clinical algorithms
- Not equal stratifications
- Wide variation in reported test performance:
- Sensitivity: 9–89%, specificity: 42–99%, PPV: 3–95%

Table A–20 provides the GRADE profile for clinical algorithms without antibody testing. This was based on the pooled information of 15 observational studies performed in children up to 18 years of age.

Table A–20. GRADE profiles for clinical algorithms without antibody testing

Question: Should a clinical algorithm (without rapid testing) versus a gold standard infant test be used for the identification of HIV infection in infants <12 months of age?
Settings: In resource-limited settings without access to virological testing
Bibliography: References 4–*18*).

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	A clinical algorithm (without rapid testing)	A gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
Factors associated with the performance of a clinical algorithm as a diagnostic tool												
Sensitivity compared with the gold standard (at different HIV-prevalence rates)[a]												
15	Observational studies	Serious[b]	Serious[c]	Very serious[d]	No serious imprecision	None	659/1453 (45.4%)	1453/1453 (4%)	Relative risk (RR) 0.45 (0.43–0.48)	2 fewer per 100	Very low	Critical
								16%		8 fewer per 100		
								40%		22 fewer per 100		
Specificity compared with the gold standard (at different HIV-prevalence rates)												
15	Observational studies	Serious	Serious	Very serious	No serious imprecision	None	6836/7616 (89.8%)	7616/7616 (4%)	RR 0.90 (0.89– 0.9)	0 fewer per 100	Very low	Important
								16%		1 fewer per 100		
								40%		4 fewer per 100		
PPV compared with the gold standard (at different HIV-prevalence rates)												
15	Observational studies	Serious	Serious	Very serious	No serious imprecision	None	659/1439 (45.8%)	1439/1439 (4%)	RR 0.46 (0.43–0.48)	2 fewer per 100	Very low	Critical
								16%		8 fewer per 100		
								40%		21 fewer per 100		

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	A clinical algorithm (without rapid testing)	A gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
NPV compared with the gold standard (at different HIV-prevalence rates)												
15	Observational studies	Serious	Serious	Very serious	No serious imprecision	None	6836/7630 (89.6%)	7630/7630 (4%) 16% 40%	RR 0.90 (0.89–0.9)	0 fewer per 100 1 fewer per 100 4 fewer per 100	Very low	Important
Likelihood ratio (LR)+ (true positives versus false positives)[e]												
15	Observational studies	Serious	No serious inconsistency	Very serious	No serious imprecision	None	659/1453 (45.4%)	780/7616 (10.2%)	Odds ratio (OR) 4.43 (4.06–4.83)[e]		Low	Critical
LR– (false negatives versus true negatives)[e]												
15	Observational studies	Serious	No serious inconsistency	Very serious	No serious imprecision	None	794/1453 (54.6%)	6836/7616 (89.8%)	OR 0.61 (0.58–0.64)[e]		Low	Important
Interobserver agreement (among non-paediatricians and paediatricians)[f]												
1	Observational study	No serious limitations	No serious inconsistency	Serious indirectness	No serious imprecision	None	35/42 (83.3%)	39/42 (92.9%)	RR 0.90 (0.76–1.05)[f]	9 fewer per 100	Moderate	Important
Factors associated with task-shifting from paediatricians to non-paediatricians (preferably PHCWs)												
Competence/quality assurance (QA) (real and perfect agreement with paediatrician's conclusion)[f]												
1	Observational study	No serious limitations	No serious inconsistency	Serious	No serious imprecision	None	561/690 (81.3%)	690/690 (100%)	RR 0.81 (0.78–0.84)[f]	18 fewer per 100	MODERATE	IMPORTANT

[a] The choice of control risk was considered low if <5% (arbitrarily opted for 4%), median at 16% (=the median value for the 15 studies) and high at 40% (Horwood et al. [2003] found 37.6% in Kwa-Zulu Natal, South Africa). The absolute effect was assessed for 100 and not 1000 infants (the default setting) to facilitate understanding.

[b] Studies used a mixture of gold standards (and QA), inclusion criteria, populations and clinical algorithms. This was shown to possibly result in different figures, but not necessarily in different conclusions.

[c] The sensitivities within different (sub) populations range between 9% and 89%, specificities between 42% and 99%, and PPV between 3% and 95%.

[d] The algorithm is not applied by primary health-care workers (PHCWs) in a population that is limited to infants <12 months.
Horwood et al. (2003) demonstrated that performance by non-paediatricians may result in different figures, but not necessarily in different conclusions: sensitivity was 56% (non-P) versus 72% (P), specificity was 85% versus 90%, PPV was 60% versus 75%, NPV was 83% versus 89%, and LR+ was 3.7 versus 7.5. Performance in infants <12 months old, however, may result in different conclusions. This study did not assess the performance of PHCWs but in highly qualified Integrated Management of Childhood Illness (IMCI) trainers (1 professional nurse and 1 general practitioner).

[e] LR+ between 2.0 and 5.0 means: 'small change' (increase in odds of HIV if the clinical algorithm is positive). There are many false positives.
LR– between 0.5 and 1.0 means: 'rarely important change' (decrease in odds of HIV if the algorithm is negative). Symptoms occur late or are being missed.

[f] Cohen Kappa coefficients: for agreement between IMCI trainers it was 0.51, for agreement between paediatricians it was 0.73, and for agreement between paediatricians and IMCI trainers it was 0.52 (P<0.001 in all cases). (K>0.75= excellent agreement, 0.4<K<0.75= intermediate, K<0.4= poor agreement).

Table A–21 provides the GRADE profile for the evaluation of clinical symptoms in seropositive infants 6 months of age. This was based on the pooled information of two observational studies (*26, 27*). This profile is used as a proxy for 'the use of an algorithm after performing antibody testing'.

The evidence for possible task-shifting was derived from one observational study that provided information on the interobserver agreement, agreement between the conclusions made by paediatricians and those by non-paediatricians (even though this included highly qualified IMCI trainers, namely, one professional nurse and one general practitioner, rather than PHCWs); as well as the difference in some indicators of test performance.

Table A–21. GRADE profile for clinical algorithms with antibody testing

Question: Should a clinical algorithm with rapid testing versus a gold standard infant test be used to identify HIV infection in infants <12 months of age?
Settings: In resource-limited settings without access to virological testing
Bibliography: References (*26, 27*).

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	A clinical algorithm with rapid testing	A gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
Factors associated with the performance of a clinical algorithm as a diagnostic tool in seropositive infants												
Sensitivity compared with the gold standard (at different HIV-prevalence rates)												
2	Observational studies	Serious[a]	No serious inconsistency	Serious[b]	Serious	None	37/77 (48.1%)	77/77 (4%)	RR 0.48 (0.37–0.59)	2 fewer per 100	Low	Critical
								16%		8 fewer per 100		
								40%		20 fewer per 100		
Specificity compared with the gold standard (at different HIV-prevalence rates)												
2	Observational studies	Serious[a]	No serious inconsistency	Serious[b]	Serious	None	370/387 (95.6%)	387/387 (4%)	RR 0.96 (0.93–0.97)	0 fewer per 100	Low	Important
								16%		0 fewer per 100		
								40%		1 fewer per 100		
PPV compared with the gold standard (at different HIV-prevalence rates)												
2	Observational studies	Serious[a]	No serious inconsistency	Serious[b]	Serious	None	37/54 (68.5%)	54/54 (4%)	RR 0.68 (0.55–0.73)	1 fewer per 100	Low	Critical

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	A clinical algorithm with rapid testing	A gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
								16%		5 fewer per 100		
								40%		12 fewer per 100		
NPV compared with the gold standard (at different HIV-prevalence rates)												
2	Observational studies	Serious[a]	No serious inconsistency	Serious[b]	Serious	None	370/410 (90.2%)	410/410 (4%)	RR 0.90 (0.67–0.93)	0 fewer per 100	Low	Important
								16%		1 fewer per 100		
								40%		4 fewer per 100		
LR+ (true positives versus false positives)[c]												
2	Observational studies	Serious	No serious inconsistency[c]	Serious[c]	Serious[c]	None	37/77 (48.1%)	17/387 (4.4%)	OR 10.94 (6.51–18.39)		Low	Critical
LR– (false negatives versus true negatives)[c]												
2	Observational studies	Serious	No serious inconsistency[c]	Serious[c]	Serious[c]	None	40/77 (51.9%)	370/387 (95.6%)	OR 0.54 (0.44–0.67)		Low	Important
Interobserver agreement (among non-paediatricians and paediatricians)[d]												
1	Observational study	No serious limitations	No serious inconsistency	Serious indirectness	No serious imprecision	None	35/42 (83.3%)	39/42 (92.9%)	RR 0.90 (0.76–1.05)	9 fewer per 100	Moderate	Important
Factors associated with task shifting from paediatricians to non-paediatricians (preferably PHCWs)												
Competence/QA (real and perfect agreement with paediatrician's conclusions)[d]												
1	Observational study	No serious limitations	No serious inconsistency	Serious indirectness	No serious imprecision	None	561/690 (81.3%)	690/690 (100%)	RR 0.81 (0.78–0.84)	18 fewer per 100	Moderate	Important

[a] The study population included infants 6 months of age only but recruitment was done at several sites. The investigators were assumed to be paediatricians. Enzyme immunoassay (EIA) and western blot (WB) in the mother, rather than rapid tests in the infant, were used to assess HIV exposure.

[b] The sample size is very small, the confidence intervals are large.

[c] LR+ >10 indicates a large change/increase in odds of the disease among those who have a positive algorithm. LR+ between 5 and 10 indicates a moderate change; LR– between 0.2 and 0.5 indicates a small but sometimes important change/decrease in the probability of HIV among those who have a negative algorithm.

[d] Cohen Kappa coefficient for agreement between non-paediatricians was 0.51, for agreement between paediatricians it was 0.73, and for agreement between paediatricians and non-paediatricians it was 0.52. (P<0.001), indicating 'intermediate agreement' for all cases). Non-paediatricians were not PHCWs but highly qualified IMCI trainers (one professional nurse and one general practitioner). If K >0.75, there is 'excellent agreement'.

A2.4 GRADE PRO analyses of studies on antibody testing

Key outcomes to be considered for recommendations include:

- *Factors related to the performance of the tool:*

  Sensitivity, specificity, PPV, NPV, LR+, LR– and interobserver agreement

- *Factors related to possible task-shifting from laboratory to non-laboratory personnel (mainly PHCWs):*

  Competence/quality assurance/agreement between rapid test results reported by non-laboratory and those by laboratory personnel, competence in phlebotomy, PHCWs' self-confidence in phlebotomy and performing rapid tests, acceptability of phlebotomy and rapid testing in infants among PHCWs and the population, costs and time needed for phlebotomy and performing rapid tests, and occupational risks.

Summary of search findings

- 12 observational studies (1989–2007) on the use of serological tests in infants and children, studies performed for different purposes
- Investigators: laboratory personnel
- Study population: Children 0–13 years, variable HIV prevalence rates
- Not always compared with acceptable gold standard. Mixed range of serological assays used, not all currently accepted and available in the market
- Samples: fresh/frozen samples/DBS – oral fluid/plasma
- Reported assay performance differed by type of rapid test and age group.

Table A–22 provides the GRADE profile for the identification of HIV exposure in newborns using one rapid test (Determine).

Table A–22. GRADE profile for the identification of HIV exposure in newborns

Question: Should one rapid test in 'certain'* newborns versus gold standard test in the mothers be used for the identification of HIV exposure?

Settings: Resource-limited settings without access to virological testing (or rather in all settings?)

Bibliography: References (*8*, *27*–40).

* The definition of 'certain' young infants includes infants from mothers with unknown serostatus and may include infants from 'certain' HIV-negative mothers. In high HIV-prevalence areas or high-risk groups, we may want to also include mothers who tested HIV negative less than a few months prior to delivery, depending on the need for repeat testing.

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test in certain newborns	Gold standard test in the mothers	Relative (95% CI)	Absolute	Quality	Importance
Factors associated with test performance in newborns												
Sensitivity versus perfect identification of maternal status (at different HIV-prevalence rates)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	220/221 (99.5%)	221/221 (4%)	RR 0.99 (0.97–0.99)	0 fewer per 1000	Moderate	Critical
								16%		1 fewer per 1000		
								40%		3 fewer per 1000		
Specificity versus perfect identification of maternal status (at different HIV-prevalence rates)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	1554/1561 (99.6%)	1561/1561 (4%)	RR 0.99 (0.99–1.00)	0 fewer per 1000	Moderate	Important
								16%		1 fewer per 1000		
								40%		3 fewer per 1000		
PPV versus perfect identification of maternal status (at different HIV-prevalence rates)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	220/227 (96.9%)	227/227 (4%)	RR 0.97 (0.94–0.98)	1 fewer per 1000	Moderate	Critical
								16%		4 fewer per 1000		
								40%		11 fewer per 1000		

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test in certain newborns	Gold standard test in the mothers	Relative (95% CI)	Absolute	Quality	Importance
NPV versus perfect identification of maternal status (at different HIV-prevalence rates)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	1554/1555 (99.9%)	1555/1555 (4%) 16% 40%	RR 0.99 (0.99–1.00)	0 fewer per 1000 1 fewer per 1000 3 fewer per 1000	Moderate	Important
LR+ (true positives versus false positives)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	220/221 (99.5%)	7/1561 (0.4%)	OR 221.99 (106.0–464.9)		Moderate	Critical
LR– (false negatives versus true negatives)												
2	Observational studies	No serious limitations	No serious inconsistency	Serious[a]	No serious imprecision	None	1/221 (0.5%)	1554/1561 (99.6%)	OR 0.005 (0.001–0.032)		Moderate	Important
Interobserver agreement – not reported												
0	-	-	-	-	-	None	0/0 (0%)	0/0 (0%)	-	-		Important
Factors associated with task shifting (laboratory versus non-laboratory personnel, ideally PHCWs)												
Competence/QA (real and perfect agreement if rapid tests are used)[b]												
4	Observational studies	No serious limitations	No serious inconsistency	No serious indirectness	No serious imprecision		415/422 (98%)	422/422 (100%)	Range from 97.9 to 99.3		High	Important
Competence in phlebotomy – not reported												
0	-	-	-	-	-	None			-	-		Important
Self-confidence in phlebotomy and testing among PHCWs – not reported												
0	-	-	-	-	-	None			-	-		Important
Acceptability of phlebotomy and rapid testing in newborns (versus mothers) among PHCWs and population[c]												
2	Any other evidence	Serious[c]	No serious inconsistency	No serious indirectness	No serious imprecision	None	481/789 (61%)	478/789 (60.6%)	RR 1.006 (0.93–1.09)	3 more per 1000		Important
Time needed for phlebotomy + rapid test – not reported												
0	-	-	-	-	-	None			-	-		Important
Cost of phlebotomy and rapid test – not reported												
0	-	-	-	-	-	None			-	-		Critical
Occupational risk[d]												
3	Any other evidence[d]					None			-	mean 5 times per year being pricked		Important

[a] The studies assessed the performance of enzyme immunoassay (EIA) + WB in newborns, rather than the performance of one rapid test only. Rapid test – performance is expected to be similar to EIA – performance in newborns has not yet been assessed with currently available rapid tests.

[b] The studies provide information on the field performance of Determine (most results), Unigold, Capillus, Oraquick and Hemastrip. There was less than 3% discrepancy if applied by trained non-laboratory personnel. The duration of training, however, fluctuated between half a day and 1 week. In a study in Ethiopia, the agreement was 97.7% (95%CI: 94.6–99.2%) for positive samples and 99.0% (95%CI: 96.6–99.8%) for negative samples.

[c] Two studies evaluated test uptake in newborns. These studies were conducted in the USA at a time when there was no access to ART. The data demonstrated that newborn test uptake was closely related and slightly higher than antenatal care (ANC) test uptake, but the latter is expected to change over time with increasing access to ART.

[d] One study estimated the occupational risk at secondary health-care level as an average of 5 times per year being pricked and up to 9 times per ear being splashed. Two cross-sectional analyses (in primary and tertiary settings) reported a prevalence rate for occupational injuries of 13.7% and 13%, respectively.

Table A–23 provides the GRADE profile for the exclusion of HIV infection using EIA in infants aged 12–18 months.

Table A–23. GRADE profile for the exclusion of HIV infection in infants aged 12–18 months

Question: Should one rapid test at 12–18 months versus gold standard infant tests be used for the exclusion of HIV infection?
Settings: Resource-limited settings without access to virological testing
Bibliography: References (*8*, *39*–40)

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test at 12–18 months	Gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
Factors associated with test performance of the Determine test in infants aged 12–18 months												
Sensitivity compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	19/19 (100%)	19/19 (4%)	RR 1.0 (0.83–1.0)	0 fewer per 1000	Very low	Important
								16%		0 fewer per 1000		
								40%		0 fewer per 1000		
Specificity compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	94/129 (72.9%)	129/129 (4%)	RR 0.73 (0.65–0.80)	10 fewer per 1000	Very low	Critical
								16%		43 fewer per 1000		
								40%		107 fewer per 1000		
PPV compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	19/54 (35.2%)	54/54 (4%)	RR 0.35 (0.24–0.49)	26 fewer per 1000	Very low	Important
								16%		104 fewer per 1000		
								40%		260 fewer per 1000		

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test at 12–18 months	Gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
NPV compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	94/94 (100%)	94/94 (4%) 16% 40%	RR 1.0 (0.96–1.00)	0 fewer per 1000 0 fewer per 1000 0 fewer per 1000	Very low	Critical
LR+ (true positives versus false positives)												
3	Observational study	No serious limitations	No serious inconsistency[a]	No serious indirectness	Very serious[b]	None	19/19 (100%)	35/129 (27.1%)	OR 3.69 (2.78–4.90)		Low	Important
LR- (false negatives versus true negatives)												
3	Observational study	No serious limitations	No serious inconsistency[a]	No serious indirectness	Very serious[b]	None	0/19 (0%)	94/129 (72.9%)	OR 0.03 (0.002–0.53)		Low	Critical
Interobserver agreement – not reported												
0	-	-	-	-	-	None			-	-		Important
Factors associated with task shifting (laboratory versus non-laboratory personnel, ideally PHCWs)												
Competence/QA (real and perfect agreement)[c]												
4	Observational study	No serious limitations	No serious inconsistency	No serious indirectness	No serious imprecision		415/422 (98.3%)	422/422 (100%)	Range from 97.9 to 99.3		High	Important
Occupational risks[d]												
3	Any other evidence					None						Important

[a] There is unexplained heterogeneity in the results of studies using the same rapid test.

[b] Information available only from very small groups with small subpopulations.

[c] The studies inform about the field performance of Determine (most results), Unigold, Capillus, Oraquick and Hemastrip. There was less than 3% discrepancy if applied by trained non-laboratory personnel. The duration of training, however, varied between half a day and 1 week. In a study in Ethiopia, the agreement was 97.7% (95%CI: 94.6–99.2%) for positive samples and 99.0% (95%CI: 96.6–99.8%) for negative samples.

[d] One study estimated the occupational risk at secondary health-care level as an average of 5 times per year for being pricked and up to 9 times per year for being splashed. Two cross-sectional analyses (in primary and tertiary settings) reported a prevalence rate for occupational injuries of 13.7% and 13%, respectively.

Table A–24 provides the GRADE profile for the performance of HIV antibody testing in infants aged 6–9 months compared with a gold standard infant diagnostic test. This was based on three observational studies.

Table A–24. GRADE profile for the exclusion of HIV infection in infants aged 6–9 months

Question: Should one rapid test at 6–9 months versus gold standard infant test be used for the exclusion of HIV?
Settings: Resource-limited settings without access to virological testing
Bibliography: References (*8*, *39*–40).

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test at 6–9 months	Gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
Factors associated with test performance of the Determine test in infants aged 6–9 months												
Sensitivity compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	22/22 (100%)	22/22 (4%)	RR 1.0 (0.85–1.0)	0 fewer per 1000	Very low	Important
								16%		0 fewer per 1000		
								40%		0 fewer per 1000		
Specificity compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	50/121 (41.3%)	121/121 (4%)	RR 0.41 (0.32–0.50)	23 fewer per 1000	Very low	Critical
								16%		94 fewer per 1000		
								40%		236 fewer per 1000		
PPV compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	22/93 (23.7%)	93/93 (4%)	RR 0.24 (0.16–0.33)	30 fewer per 1000	Very low	IMPORTANT
								16%		121 fewer per 1000		
								40%		304 fewer per 1000		

Quality assessment							Summary of findings					
							Number of patients		Effect			
No. of studies	Design	Limitations	Inconsistency	Indirectness	Imprecision	Other considerations	One rapid test at 6–9 months	Gold standard infant test	Relative (95% CI)	Absolute	Quality	Importance
NPV compared with gold standard infant test (at different HIV-prevalence rates)												
3	Observational study	No serious limitations	Serious[a]	No serious indirectness	Very serious[b]	None	50/50 (100%)	50/50 (4%) 16% 40%	RR 1.00 (0.93–1.0)	0 fewer per 1000 0 fewer per 1000 0 fewer per 1000	Very low	Critical
LR+ (true positives versus false positives)												
3	Observational study	No serious limitations	No serious inconsistency[a]	No serious indirectness	Very serious[b]	None	22/22 (100%)	71/121 (58.7%)	OR 1.70 (1.47–1.98)		Low	Important
LR- (false negatives versus true negatives)												
3	Observational study	No serious limitations	No serious inconsistency[a]	No serious indirectness	Very serious[b]	None	0/22 (0%)	50/121 (41.3%)	OR 0.053 (0.003–0.82)		Low	Critical
Factors associated with task shifting (laboratory versus non-laboratory personnel, ideally PHCWs)												
Competence/QA (real and perfect agreement)[c]												
4	Observational study	No serious limitations	No serious inconsistency	No serious indirectness	No serious imprecision		415/422 (98.3%)	422/422 (100%)	Range from 97.9 to 99.3		High	Important
Acceptability of phlebotomy and RT in infants (versus their carers)[d]												
2	Any other evidence					None						Important
Occupational risks[e]												
3	Any other evidence					None						Important

[a] There is unexplained heterogeneity between the results of studies using the same rapid test.

[b] Information available only from very small groups with small subpopulations.

[c] The studies inform about the field performance of Determine (most results), Unigold, Capillus, Oraquick and Hemastrip. There was less than 3% discrepancy if applied by trained non-laboratory personnel. The duration of training, however, fluctuated between half a day and 1 week. In a study in Ethiopia, the agreement was 97.7% (95%CI: 94.6–99.2%) for positive samples and 99.0% (95%CI: 96.6–99.8%) for negative samples.

[d] Surveys revealed that 85–92% of the parents of hospitalized children would consent to HIV screening for medical reasons of the hospitalized child or their siblings, while only 62–70% would consent to screening for themselves.

[e] One study estimated the occupational risk at secondary health-care level as an average of 5 times per year for being pricked and up to 9 times per year for being splashed. Two cross-sectional analyses (in primary and tertiary settings) reported a prevalence rate for occupational injuries of 13.7% and 13%, respectively.

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Annex 3 Summary of guideline group decisions regarding evidence and risk–benefit analyses

Risk–benefit analyses for recommendations 1 and 2 are in the body of the report and tables.

Recommendation 3 is sourced already.

<table>
<tr><td colspan="3">Recommendation 4
In infants and children undergoing viral testing, the following assays are strongly recommended for use:
• HIV DNA in whole blood
• HIV DNA in DBS
• HIV RNA in plasma (except for infants on ART)
• HIV RNA in DBS (except for infants on ART)
• Us p24 HIV Ag in plasma (except for infants on ART or where subtype D is common)
• Us p24 HIV Ag in DBS (except for infants on ART or where subtype D is common)</td></tr>
<tr><td colspan="3">Population:
Infants (<18 months) exposed to HIV</td></tr>
<tr><td colspan="3">Intervention:
Virological testing with assays of at least 98% Sn and 99% Sp</td></tr>
<tr><td colspan="3">Action:
If reactive and <12 months, start treatment and perform confirmatory test at the initiation of ART.
If not reactive, HIV infection is unlikely unless still breastfeeding.</td></tr>
<tr><td>Factor</td><td>Decision</td><td>Explanation</td></tr>
<tr><td>Quality of evidence</td><td>Strong</td><td>Published evidence:
HIV DNA in whole blood: GRADE evidence HIGH
HIV DNA in DBS: GRADE evidence HIGH
HIV RNA in plasma: GRADE evidence HIGH
HIV RNA in DBS: GRADE evidence HIGH
HIV p24 Ag in plasma: GRADE evidence: HIGH
HIV p24 Ag in DBS: GRADE evidence: HIGH
Concerns exist about the performance of RNA and Us p24 Ag:
• in the context of PMTCT-related ARV exposure, however, currently there is no evidence to suggest it is a problem
• regarding thresholds for the use of the quantitative assay, particularly for DBS samples
• where subtype D is prevalent for p24 Ag
• b-DNA test is not recommended as Sp is less than 98%
• as most DBS RNA assays also detect DNA
• as quantitative RNA is also useful for clinical management.
HIV DNA testing will be needed to resolve discordant HIV DNA and HIV RNA test results once on ART.</td></tr>
<tr><td>Benefits or desired effects</td><td rowspan="2">Strong (benefits outweigh risks)</td><td>Early diagnosis
Use of DBS facilitates early diagnosis in remote settings.</td></tr>
<tr><td>Risks or undesired effects</td><td>Lack of standardization and quality assurance could affect assay performance.
The performance of some assays might be subtype dependent.
Laboratories will not know whether patient has been exposed to ARVs.</td></tr>
<tr><td>Values and preferences</td><td>Strong</td><td>Us p24 Ag: currently kits are not commercially packed together affecting laboratory supplies and delivery.
The use of DBS sample allows easy collection and transport.</td></tr>
<tr><td>Costs and feasibility</td><td>Weak</td><td>Concerns about sustainability of existing RNA commercial platforms
May be more easily available and more useful in the long term</td></tr>
<tr><td>Overall ranking of recommendation</td><td colspan="2">Strength of recommendation
Strong</td></tr>
</table>

Recommendation 5		
It is strongly recommended that all HIV-exposed infants have HIV virological testing at 4–6 weeks of age or at the earliest opportunity thereafter.		
Population: All HIV-exposed infants (<18 months of age)		
Intervention: Virological testing at 4–6 weeks		
Action: If reactive and <12 months, start treatment and send confirmatory test. If non-reactive, HIV infection unlikely, continue follow up and perform HIV serological test at 9–18 months of age.		
Factor	**Decision**	**Explanation**
Quality of evidence	Strong	Natural history and mortality data support early ART initiation. The CHER study[1] reported 75% reduction in mortality with early diagnosis and immediate treatment regardless of clinical, immunological and virological stage.
Benefits or desired effects	Strong	Early diagnosis improves infant follow up and HIV care. Early treatment reduces infant mortality and morbidity. Modelling suggests that 4–6 weeks is optimal to increase detection rates and avoid increasing HIV-attributable mortality.
Risks or undesired effects		Concerns about mislabelling the specimen and other human errors (>5%) Infants may be tested but unable to access ART. Other aspects of the child health programme may suffer.
Values and preferences	Strong	Ethical obligation to identify and treat early if prevention failed Denial, neglect of child and stigma enhanced by identification of HIV in infant
Costs and feasibility	Weak	<u>Increased by:</u> Health system constraints in collecting specimen and returning results Lack of sufficient capacity/infrastructure to do a different or second test in many countries Emotional costs for families and staff <u>Reduced by:</u> Avoiding morbidity-related costs Processing a large number of specimens as unit cost per test decreases with increasing numbers of specimens processed
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 6		
In infants with an initial positive virological test result, ART should be started without delay and a second specimen collected at the same time to confirm the initial positive virological test result. Do not delay ART. Initiation of ART saves lives and ART should not be delayed while waiting for the results of the confirmatory test.		
Population: Infants and children with a first positive virological test result		
Intervention: Confirmatory test on a separate specimen preferably taken at or just before starting ART		
Action: If reactive, continue ART. If not reactive, a third test will be required to resolve the discordance between the two earlier viral tests.		
Factor	**Decision**	**Explanation**
Quality of evidence	Strong	Serial testing with tests that have at least 95% Sn minimize the chances of FP results Depends on prevalence in population being tested Natural history and mortality data support early ART initiation. In the CHER study,[1] 16% versus 4% deaths were deferred with immediate ART; 75% reported reduction in mortality with early diagnosis and immediate treatment of infants who were well at diagnosis.
Benefits or desired effects	Strong (benefits outweigh risks)	Using a separate sample reduces human error (e.g. mislabelling of first specimen, etc.). Early diagnosis improves infant follow up and HIV care. Confirmatory testing reduces FP results and unnecessary ART. Early treatment reduces infant mortality and morbidity.
Risks or undesired effects		Concerns about mislabelling the specimen and other human errors (estimate may be as high as 5%) Infants may be tested but are often unable to access ART in a timely fashion (e.g. <25% access reported by Clinton Foundation). Other aspects of the child health programme may suffer due to repeat infant testing (human and financial resources).
Values and preferences	Strong	Ethical obligation to identify and treat early if prevention failed, as well as not treat unnecessarily Denial, neglect of child and stigma enhanced by identification of HIV in infant, therefore exclusion of FP results has great value The importance of a confirmatory test is greater in low-prevalence settings. For example, if using tests with 99% Sn and 98% Sp the following would be found: 30% prevalence: treat 311 for 297 truly infected: 14/311 treated unnecessarily 5% prevalence: treat 137 for 55 truly infected : 38/137 treated unnecessarily 1% prevalence: treat 3 for 1 truly infected: 2/3 treated unnecessarily
Costs and feasibility	Strong (despite overall increase in cost)	<u>Increased by:</u> Health system constraints in collecting specimen and disseminating results Lack of sufficient capacity/infrastructure in many countries to do a different or second test Emotional costs for families and staff <u>Reduced by:</u> Interruption of unnecessary treatment and care for those who have FP results Unit cost for per test decreases with increasing numbers of specimens processed
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 7		
Access to virological testing in infants should be accompanied by the test results being returned to the clinic and child/mother/carer as soon as possible, but at the latest within four weeks of specimen collection. Positive results should be fast tracked to the mother/baby pair as soon as possible to enable prompt initiation of ART.		
Population: All infants undergoing virological testing		
Intervention: Test result returned at the latest within four weeks of specimen collection		
Action If results are delayed, priority should be given to tracking and getting results to caregiver/infant to enable prompt initiation of ART for infected children.		
Factor	**Decision**	**Explanation**
Quality of evidence	Strong	Delays in specimen handling and return of test results causes critical delays in confirming HIV infection and commencing life-saving ART and HIV care. Natural history and mortality data support early ART initiation. The CHER study[1] reported 75% reduction in mortality with early diagnosis and immediate treatment regardless of clinical, immunological and virological stage.
Benefits or desired effects	Strong	To avoid death and make testing useful, results need to be delivered and care started in as short a time as possible. Most deaths in the CHER study[1] were early, sudden and without symptoms. Testing uptake may improve if results are given quickly.
Risks or undesired effects		Maintaining confidentiality May decrease rates of offering testing if health-care workers are aware of the need to give results quickly and see this as increasing their work
Values and preferences	Strong	May improve health systems' ability to ensure children get into care Mothers and families likely to prefer quicker results
Costs and feasibility	Strong	Costs might be reduced as morbidity-related costs are avoided.
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 8

It is strongly recommended that all infants with unknown or uncertain HIV exposure being seen in health-care facilities at or around birth or at the first postnatal visit (usually 4–6 weeks) or other child health visit have their HIV exposure ascertained.

Population:

Infants with unknown or uncertain HIV exposure being seen in health-care facilities at the first postnatal or other child health visit

Intervention:

1. Determine HIV status of the mother in this pregnancy through review of records, maternal or caregiver questioning (STRONG recommendation).
2. If maternal HIV testing has not been done or the HIV status of the mother remains unclear for the duration of the pregnancy, an HIV serological test in the mother should be performed after obtaining informed consent (STRONG recommendation).
3. If the mother is unavailable or does not consent to testing, a single HIV serological test in the infant should be performed to detect HIV exposure (STRONG recommendation).

Action:

- If the infant is seen <72 hours after delivery and exposure documented, post-exposure prophylaxis (PEP) should be given, and mothers should be counselled on safe infant-feeding practices according to the national/local recommendations.
- For infants first seen at 4–6 weeks or the earliest thereafter and in whom HIV exposure is documented, HIV virological testing should be performed and the mother should receive safe infant-feeding counselling.

Factor	Decision	Explanation
Quality of evidence	Strong	Effective interventions to prevent new infection in the mother and infant are available. Interventions save lives and reduce mortality and hospitalization. A negative test excludes HIV exposure. The mother needs care and treatment.
Benefits or desired effects	Strong (benefits outweigh risks)	Prefer to confirm HIV exposure by assessment of the mother or testing In HIV exposed, cotrimoxazole saves lives. An appropriate infant-feeding choice can be offered. Benefits, especially in high-prevalence settings Facilitates a higher detection of exposed infants Opportunity for diagnosis of HIV infection and care for the mothers
Risks or undesired effects		Caution if older infant and breastfeeding or other exposure not known or recent May miss incident HIV infection (window period) May need to retest mother in high-incidence areas (due to infection acquired late in pregnancy) A positive HIV serological test may be misinterpreted by the mother as the child being HIV infected In low-prevalence settings there is a higher likelihood that a positive result is an FP result.
Values and preferences	Weak	Potential harms – FP results and associated anxiety Prevalence in children will determine how acceptable testing is to children or their parents. Health-care workers might be reluctant to test because of increased workload. Mothers may be more willing to accept infant testing than maternal testing.
Costs and feasibility	Weak	In low-prevalence areas the value of detecting rare conditions should be balanced against the costs. Opportunity costs are higher in low-prevalence settings. Retesting cost needs to be considered (tested early in pregnancy).
Overall ranking of recommendation	Strength Of recommendation **Strong**	

Recommendation 9		
It is strongly recommended that HIV-exposed infants who are well have HIV serological testing at around 9 months of age (or at the time of the last immunization visit). Infants who have reactive serological assays at 9 months should have a viral test to identify infected infants who need ART.		
Population: HIV-exposed children who are well and aged 9–18 months		
Intervention: HIV serological testing at 9 months		
Action: If non-reactive and no exposure via breastfeeding within the past six weeks, infant uninfected; discontinue co-trimoxazole and discharge from programme If reactive and infant is well, repeat at 18 months of age. If infant is sick, perform virological test if available.		
Factor	**Decision**	**Explanation**
Quality of evidence	Quality is low but felt could support a strong recommendation	At 9 months of age, maternal HIV antibodies are no longer detectable in between 40% and 50% of HIV-exposed children.
Benefits or desired effects	Benefits outweigh risks	Leads to early exclusion of HIV where possible Follow up for HIV and cotrimoxazole can be stopped. Virological testing for seropositive infants will help identify infected infants early.
Risks or undesired effects		If very recent exposure to HIV cannot be ruled out, a negative serological test does not exclude HIV infection (window period).
Values and preferences	Strong	Reduces the mother's anxiety regarding the child being infected
Costs and feasibility	Strong	Avoids HIV care-related costs for HIV-exposed but -uninfected child. Inexpensive intervention
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 10		
It is strongly recommended that infants with signs or symptoms suggestive of HIV infection should have HIV serological testing and, if positive (reactive), virological testing.		
Population: Sick infants and children (4 weeks–9 months of age)		
Intervention: Serological testing followed by virological testing when positive		
Factor	**Decision**	**Explanation**
Quality of evidence	Weak	There are concerns as not all rapid tests can be used in this case. Serological assays used must be highly sensitive, e.g. current Determine test. Further research is necessary. Impaired immune system of the baby could reduce HIV antibody detection in the baby. Persisting maternal antibodies could affect the serological test result. The performance of the clinical algorithm depends on the health-care worker.
Benefits or desired effects	Weak	In sick children who are HIV infected, the earliest possible start of HIV treatment and care reduces mortality.
Risks or undesired effects		Possible FN results (*see above*) FP results, especially in settings where the result cannot be further confirmed.
Values and preferences	Strong	If clinical suspicion is still high, may need to treat while seeking further testing HIV infection is an unlikely cause of signs and symptoms such as cough fever, diarrhoea, pneumonia, tuberculosis in low-prevalence settings. However, in settings where the prevalence is >5%, HIV infection is a more likely cause of such signs and symptoms.
Costs and feasibility	Strong	Depends on the HIV prevalence
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 11		
In breastfeeding infants or children, it is strongly recommended that breastfeeding not be discontinued before performing any kind of diagnostic HIV test.		
Population: HIV-exposed infants who are breastfeeding.		
Intervention: Do not discontinue breastfeeding at the time of virological or HIV serological testing.		
Action: Breastfeeding should be continued for HIV-infected infants and ART started. In infants who have negative results, safer infant-feeding options will need to be considered.		
Factor	**Decision**	**Explanation**
Quality of evidence	Strong	Transmission rates through breastfeeding are established: Mixed feeding 0–6 months: 1.5%/month Exclusive breastfeeding 0–6 months: 0.75%/month Breastfeeding 7–36 months: 0.75%/month Breastfeeding mother on ART: 0.3%/month Mixed feeding and abrupt cessation of breastfeeding are recognized to increase the risks of transmission.
Benefits or desired effects versus risks or undesired effects	Benefits greater than risks	The risk of transmission due to breastfeeding is known. The risk of death or morbidity for the nonbreastfed, HIV-infected or HIV-exposed infant is increased. Very abrupt cessation of breastfeeding leads to risk for the HIV-infected or -uninfected infant and is therefore undesirable. Infant-feeding decisions are more easily made if based on the results of testing, and not prior to receiving the results. May increase the rates of returning for results
Values and preferences	Strong	May be more acceptable to mothers Some health-care workers feel breastfeeding at any time is unacceptable for HIV-positive women.
Costs and feasibility	Strong	May reduce health systems costs due to infant morbidity, and reduce mortality in infected infants
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 12		
It is strongly recommended that children (18 months or older) with suspected HIV infection or HIV exposure have HIV serological testing performed according to the standard diagnostic HIV serological algorithm used in adults.		
Population: All children 18 months or older suspected to be HIV infected or recommended to have HIV testing based on other clinical or exposure criteria		
Intervention: HIV serological testing according to the diagnostic algorithm		
Action: If reactive, confirms HIV infection; provide HIV care and treatment. If non-reactive, excludes HIV; discharge from programme.		
Factor	**Decision**	**Explanation**
Quality of evidence	Strong	There is no evidence to suggest that HIV serological testing performs differently in children. Concerns about time to seroreversion in breastfeeding children Concerns about fourth-generation assays
Benefits or desired effects	Benefits outweigh risks	An HIV-positive result is most likely to indicate infection; young children need access to the full package of care as soon as possible.
Risks or undesired effects		FP results more likely if fourth-generation assays used as very sensitive
Values and preferences	Strong	Acceptable to patients Health-care providers at all levels have been shown to be able to use simple rapid tests. Biggest fear is taking blood and giving results to parents
Costs and feasibility	Strong	Simple inexpensive intervention
Overall ranking of recommendation	Strength of recommendation **Strong**	

Recommendation 13		
In sick infants in whom HIV infection is being considered as an underlying cause of symptoms and signs and virological testing is not available, perform HIV serological testing and use the clinical algorithm for presumptive clinical diagnosis of HIV infection.		
Population: Sick infants and children (18 months of age) in settings where virological testing not available		
Intervention: Perform HIV serological testing and follow the clinical algorithm for presumptive diagnosis of HIV infection.		
Factor	**Decision**	
Quality of evidence	Weak	Concerns as not all rapid tests can be used in this case Serological assays used must be highly sensitive, e.g. current Determine. Further research necessary Impaired immune system of the baby could reduce HIV antibody detection in the baby. Persisting maternal antibodies could affect serological test result. The performance of the clinical algorithm depends on the health-care worker.
Benefits or desired effects	Weak	In sick children who are HIV infected, start of HIV treatment and care at the earliest possible reduces mortality.
Risks or undesired effects		Possible FN results (*see above*) FP results especially in settings where the result cannot be further confirmed
Values and preferences	Strong/weak	Saves lives Do not stop child from getting ART because not able to perform virological testing If clinical suspicion is still high may need to treat while seeking further testing HIV infection is an unlikely cause of signs and symptoms in low-prevalence settings. However, in settings where the prevalence is >5%, HIV infection is a more likely cause of signs and symptoms.
Costs and feasibility	Strong/weak	Depends on the prevalence
Overall ranking of recommendation	Strength of recommendation **Strong** for high-prevalence settings **Weak** for low-prevalence settings (<1% maternal ANC seroprevalence)	

References

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Annex 4 Characteristics of a screening test

Characteristics of a screening test	HIV serological testing
1. The condition should be an important health problem.	✔
2. There should be a treatment for the condition.	✔
3. Facilities for diagnosis and treatment should be available.	✔
4. There should be a latent or asymptomatic stage of the disease.	✔
5. There should be a test for the condition.	✔
6. The test should be acceptable to the population.	✔
7. The natural history of the disease should be adequately understood.	✔
8. There should be an agreed policy on who to treat.	✔
9. The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.	To be determined
10. Case-finding should be a continuous process, not just a 'once and for all' project.	✔
11. Test used should be sensitive.	✔

Likelihood ratios

Likelihood ratio positive (LR+):

The odds that a positive test result would be found in a patient with, versus without, a disease.

The probability of a test result being positive in a person with the disease divided by the probability of a test result being positive in a person without the disease.

Likelihood ratio positive (LR+) = Sensitivity / (1 – Specificity)

LR(+) = [TP / (TP + FN)] / [FP / (FP + TN)]

Likelihood ratio negative (LR–):

The odds that a negative test result would be found in a patient without, versus with, a disease.

The probability of a test result being negative in a person who has the disease, divided by the probability of a negative test result in a person who does not have the disease.

Likelihood ratio negative (LR–) = (1– Sensitivity) / Specificity

LR(–) = [FN / (TP + FN)] / [TN / (FP + TN)]

Table A–25. Impact of likelihood ratios

LR(+)	LR(–)	Impact on likelihood
10	0.1	Excellent
6	0.2	Very good
2	0.5	Fair
1	1	Useless

Table A–26. Likely performance of HIV assays (HIV serological or HIV virological tests) with a sensitivity of 98% and specificity of 98% at various prevalence levels

HIV prevalence in population being tested (%)	1	2	5	10	20	30	50
No. of positive results per 10 000 tests (TP+FP)	296	392	680	1160	2120	3080	5000
No. truly infected in 10 000	100	200	500	1000	2000	3000	5000
No. uninfected in 10 000	9900	9800	9500	9000	8000	7000	5000
No. uninfected testing positive/10 000 tested (FP)	198	196	190	180	160	140	100
No. infected testing negative/10 000 tested (FN)	2	4	10	20	40	60	100
No. uninfected testing negative (TN)	9702	9604	9310	8820	7840	6860	4900
No. infected testing positive (TP)	98	196	490	980	1960	2850	4900
PPV (%)	33.1	50	72.1	84.5	92.5	95.5	98
NPV (%)	100	100	99.9	99.8	99.5	99.1	98

Table A–27. Serial testing using tests with a sensitivity of 98% and specificity of 98%

HIV prevalence in the population initially being tested (%)	1-test algorithm PPV (%)	1-test algorithm NPV (%)	2-test algorithm PPV (%)	2-test algorithm NPV (%)
1	33.1	100	96	99
2	50	100	98	98
5	72.1	99.9	99.2	95
10	84.5	99.8	99.6	90
20	92.5	99.5	99.8	80
30	95.5	99.1	99.9	70
50	98	98	100	50

Table A–28. Likely performance of HIV assays (HIV serological or HIV virological tests) with a sensitivity of 98% and specificity of 99% at various prevalence levels

HIV prevalence in population being tested (%)	1	2	5	10	20	30	50
No. of positive results per 10 000 tests (TP+FP)	197	294	585	1070	2040	3010	4950
No. truly infected in 10 000	100	200	500	1000	2000	3000	5000
No. uninfected in 10 000	9900	9800	9500	9000	8000	7000	5000
No. uninfected testing positive/10 000 tested (FP)	99	98	95	90	80	70	50
No. infected testing negative/10 000 tested (FN)	2	4	10	20	40	60	100
No. uninfected testing negative (TN)	9801	9702	9405	8910	7920	6930	4950
No. infected testing positive (TP)	98	196	490	980	1960	2850	4900
PPV (%)	49.7	66.7	83.8	91.6	96.1	97.7	99
NPV (%)	100	100	99.9	99.8	99.5	99.1	98

Table A–29. Serial testing using tests with a sensitivity of 98% and specificity of 99%

Prevalence in the population initially being tested (%)	1-test algorithm PPV (%)	1-test algorithm NPV (%)	2-test algorithm PPV (%)	2-test algorithm NPV (%)
1	49.7	100	99	98
2	66.7	100	99.5	96.1
5	83.8	99.9	99.8	90.6
10	91.6	99.8	99.9	82
20	96.1	99.5	100	66.9
30	97.7	99.1	100	54.1
50	99	98	100	33.6

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Annex 5 Studies reporting the prevalence of HIV infection in populations of infants and children undergoing HIV testing

Study	Population	Prevalence
Rabie H et al. (2007) [1]	Children admitted to paediatric ICU in Tygerberg, S. Africa	47/465, or 10%, were HIV infected
Ojukwu JU et al. (2007) [2]	Children admitted to the hospital in Ebonyi State, Nigeria	31/282 or 11% were HIV infected. <1 year 5.2% 1–5 years 2.5% 6–10 years 2.1% 11–15 years 1.0%
Rogerson SR et al. (2004) [3]	Children admitted to the inpatient paediatric ward in Blantyre, Malawi, March 2000	187/991 or 18.9% were HIV infected. Prevalence was: malnutrition 40%, Lower respiratory tract infection (LRTI) 29% Sepsis 28% Malaria 11% Surgical admissions 11%
Pillay K et al. (2001) [4]	160 paediatric hospital admissions, Durban, South Africa	100/160 or 62.5% were HIV infected; 63/91 children <12 months, 69.2% were HIV infected
Meyers TM et al. (2000) [5]	6 months serial paediatric admissions under 5 years, Soweto, South Africa	507/549 tested for HIV, 29.2% were found to be HIV infected
Yeung S et al. (2000) [6]	Consecutive children admitted to South African district hospital 1996–1997	281, 26% HIV infected
Zwi KJ et al. (1999) [7]	Admissions to paediatric wards in urban regional hospital, South Africa 1992–1997	Prevalence of HIV infection 1992 2.9% 1997 20%
Malnutrition		
Fergusson P et al. (2008) [8]	Meta-analysis studies of children with severe acute malnutrition in sub-Saharan Africa; 17 countries	4891 children, 29.2% were HIV infected
Thurstans S et al. (2008) [9]	Severely malnourished children admitted to nutrition rehabilitation units in Malawi	523 children, 21.6% were HIV infected
Chinkhumba J et al. (2008) [10]	Children 6–59 months with severe acute malnutrition admitted to nutritional rehabilitation unit in Malawi	79/454 children aged 6–59 months, 17.4% were HIV-infected
Ndagije F et al. (2007) [12]	Severely malnourished Rwandan children	52/112 children aged 2 months to 5 years, 46.4% were HIV infected
Babirekere-Iriso E et al. (2006) [12]	Bacteraemia in children with severe malnutrition in Kampala, Uganda	Of 134 children aged 6–59 months, 44% were HIV infected
Angami et al. (2004) [13]	Hospital-based study among malnourished children, India	Of 175 children, 21.7% were HIV infected
Ticklay IM et al. (1997) [14]	Malnourished children >15 months admitted to ward in Harare, Zimbabwe, 1993–1994	Of 140, 48.6% were HIV infected
Prazuck T et al. (1993) [15]	Severely malnourished children in Burkina Faso	Of 433, 13.8% were HIV infected
Mgone CS et al. (1991) [16]	Severely malnourished children >18 months in Dar es Salaam	Of 102 children, 25.5% were HIV infected
Tuberculosis		
Hesseling AC et al. (2009) [17]	Infants with culture-positive TB in Western Cape, South Africa; 2004–2006	53 out of 245 or 21.6% were HIV infected
Schaaf HS et al. (2007) [18]	596 cases of culture-confirmed TB in children from Cape Town, South Africa, 2003–2005	133/414 children, 32.1% had HIV infection
Hussain T et al. (2007) [19]	Paediatric patients with active TB disease attending outpatient clinic in Agra, India	23/270, or 8.5% had HIV infection

Study	Population	Prevalence
Soeters M et al. (2005) [20]	Children admitted to a TB hospital in the Western Cape	138 children with TB; 43 or 31% were HIV infected
Shahab T et al. (2004) [21]	Children <12 years with active TB diagnosed between 1999 and 2000 in Aligarh, India	250 consecutive children <12 years; 2% were HIV infected
Jeena PM et al. (2002) [22]	Children with culture-proven pulmonary TB in Durban, South Africa	57/118 cases of TB; 48% were HIV infected
Madhi SA et al. (2000) [23]	Children hospitalized with TB in Johannesburg, South Africa	Of 161 children, 42% were HIV infected
Espinal MA et al. (1996) [24]	Consecutively enrolled children aged 18–59 months with new-onset, clinically diagnosed TB; Santo Domingo, Dominican Republic	Of 189, 11 or 5.8% were HIV infected
Chintu C et al. (1993) [25]	Hospitalized children with TB aged 1 month to 14 years in Lusaka, Zambia	Of 237 with clinical diagnosis of TB, 88 or 37% had HIV infection (compared with 10.7% of control children)
Diarrhoea		
Nte AR et al. (2008) [26]	Children with persistent diarrhoea requiring hospitalization in Port Harcourt, Nigeria	Of 99 with known HIV status, 44.4% were HIV infected
Chhagan MK et al. (2006) [27]	Children hospitalized with diarrhoeal disease in Durban, South Africa, 2001	Of 1145 children, 68% were in HIV infected
Tumwine JK et al. (2005) [28]	Children aged <60 months admitted between 2002 and 2003 in Uganda with persistent diarrhoea (>14 days)	Of 243, 91 or 37.4% were HIV infected
Bacteraemia or meningitis		
Bachou H et al. (2006) [29]	Severely malnourished children with bacteraemia in Kampala, Uganda	Of 411 severely malnourished children, 151 or 36.7% were HIV infected
Brent AJ et al. (2006) [30]	*Salmonella* bacteraemia in children admitted to hospital in Kenya	Of 166 cases of non-typhoidal *Salmonella* bacteraemia, HIV infection was present in 18%
Madhi SA et al. (2000) [31]	Children <12 years old hospitalized with pneumococcal bacteraemia in Johannesburg, South Africa	Of 225, 146 or 64.9% were HIV infected
Madhi SA et al. (2001) [32]	Children less than 12 years old in Johannesburg, South Africa admitted to hospital with proven or suspected bacterial meningitis	Of 147, 62 or 42.2% were HIV infected
Lower respiratory tract infection (LRTI)		
Madhi SA et al. (2002) [33]	80 children hospitalized from1997 to 1999 with parainfluenza virus isolated in a cohort study of etiology of LRTI in South Africa	Of 80, 24 or 30% HIV infected
Madhi SA et al. (2002) [34]	Children hospitalized for LRTI with influenza virus isolated in a cohort study of etiology of LRTI in South Africa, 1997–1999	25/116, or 21.6% were HIV infected
Madhi SA et al. (2000) [35]	1215 children aged 2–60 months admitted with severe LRTI hospitalized in 1997–1998 in Johannesburg, South Africa	45.1% had HIV infection
Madhi SA et al (2000) [36]	990 children age 2–60 months hospitalized with severe LRTI and prospectively enrolled 1997–1998 in Johannesburg, South Africa	44.6% were HIV infected
Malaria		
Malamba S et al. (2007) [37]	847 children <5 years old with severe malarial anaemia in Kampala, Uganda	78/847 or 9.2% were HIV infected
Bronzan RN et al. (2007) [38]	1119 children admitted for severe malaria in Blantyre, Malawi between 1996 and 2005 and who had HIV-infection status determined	16% had HIV infection, highest in those with severe malarial anaemia, at 20.4%

Study	Population	Prevalence
Measles		
Moss WJ et al. (2008) [39]	1227 children with confirmed measles and known HIV-infection status hospitalized in Lusaka, Zambia between 1998 and 2003	189/1227 or 15% had HIV infection (median age 12 months)

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