LABLINK MEDICAL LABORATORY TESTING ALGORITHM FOR THE DIAGNOSIS OF HIV

Published in January 2016; Reviewed 16th March 2017

The master copy has been signed. Printed copies of this document are not controlled. Document users are responsible for ensuring printed copies are valid prior to use.

Prepared by:

Dr Muhammad Nazri Bin Aziz, Consultant Medical Microbiologist

Mohd. Shazwan Lui Abdullah – Deputy General Manager

Faizah Ahmad Azam, Medical Laboratory Technologist – Integrated Diagnostic Laboratory

Siti Nurfasha Rosli, Medical Laboratory Technologist – Molecular Diagnostic Laboratory

Nabila Ibrahim, Medical Laboratory Technologist – Molecular Diagnostic Laboratory

Shahrizad Bin Mad Shariff – Quality Executive

Abdul Qayyum Bin Abdul Hadi – Public Relations and Marketing Services

Contents

1. Introduction

Malaysia is a country with concentrated HIV epidemic with infection rates remains high above 5% among most-at-risk populations (MARPS) especially among injection drug use/user (PWID), sex worker (SW), transgender (TG) and men sex with men (MSM) population. Based on surveillance data (as of end 2013) Malaysia had a cumulative number of 101, 672 HIV, 20, 235 AIDS cases and 16, 340 death related to HIV/AIDS, thus giving reported people living with HIV (PLHIV) of 85, 332 cases. The annual number of reported new HIV cases has been on a steady decline from a peak of 6, 978 in 2002, to 3, 393 in 2013. The notification rate of HIV also continues to experience a decrease from 28.4 in 2002 to 11.4 cases per 100, 000 populations in 2013.

HIV testing is the gateway to HIV prevention, treatment, care and other support services. People's knowledge of their HIV status through HIV testing services is crucial to the success of the HIV response. The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) have endorsed global goals to achieve 'zero new HIV infections, zero discrimination and zero AIDS-related deaths'. Because of the potential serious medical, social and psychological consequences of misdiagnosis HIV, accurate laboratory diagnosis is essential to identify persons who could benefit from treatment, to reassure persons who are uninfected, and to reduce HIV transmission.

2. Audience

These recommendations describe the types and sequence of laboratory assays used to make the laboratory diagnosis of acute HIV-1 infection, established HIV-1 infection, and HIV-2 infection, as well as monitoring patient on Anti-Retroviral Therapy (ART) using HIV-1 RNA viral load assay. They are intended for use by Lablink Medical Laboratories.

3. Scope

These recommendations are intended for testing of serum or plasma specimens from adults, children aged 2 years or older, as well as for the establishment of diagnosis in HIV exposed infants less than 18 month of age to HIV positive mother. Because maternal antibodies against HIV might be present in uninfected infants born to HIV-infected mothers. These recommendations do not address methods or strategies for screening blood or organ donors for HIV infection.

4. Basic principles for performing HIV testing

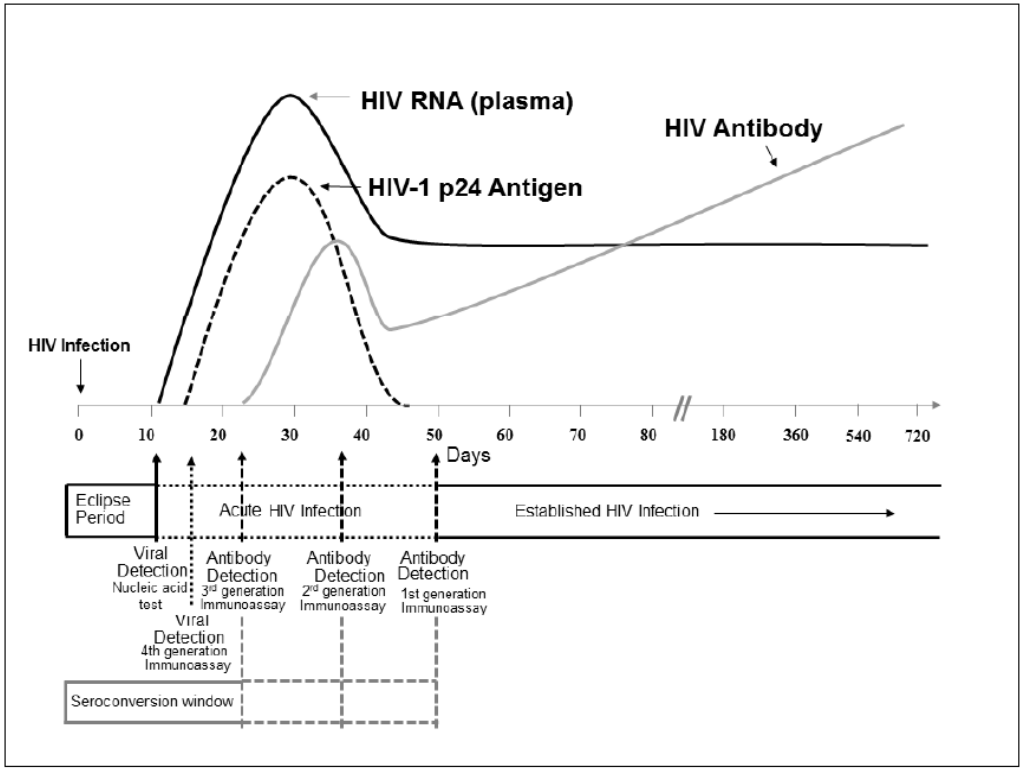
All HIV testing should be performed in accordance with the assay manufacturer's instructions for use (the package insert). Accurate laboratory diagnosis of HIV infection relies on testing algorithms that maximize overall sensitivity and specificity by employing a sequence of tests in combination and applying decision rules for resolving discordant test results. For adults and infants/children over 18 months of age (who were not breastfed or who have stopped breastfeeding at least six weeks earlier), HIV is typically diagnosed through the detection of HIV antibodies (a serological marker) and/or HIV-1 p24 antigen rather than direct detection of the components of the virus itself (virological markers). At Lablink Medical Laboratories, serological assays used for HIV diagnosis detect HIV-1/2 antibodies and HIV-1 p24 antigen. When initial HIV testing is positive, supplemental assays such as HIV-1/2 antibodies particle agglutination test, HIV-1/2 antibodies line immunoassay, and HIV-1 qualitative/quantitative PCR are used to confirm the diagnosis; refer 1. Algorithm for HIV testing in adults and paediatric (Age > 18 months).

For infants and children under 18 months of age, HIV infection can be diagnosed only by virological testing; maternal HIV antibodies remain in the infant's bloodstream until 18 months of age, making test results from serological assays ambiguous. Virological testing using nucleic acid testing (NAT) technologies can be conducted using dried blood spot (DBS) specimens, which are collected at local sites and sent to centralized laboratory for testing. It is recommended that all HIV-exposed infants have HIV virological testing at 2-3 weeks of age or at the earliest opportunity; refer 2 algorithm for HIV exposed infants, age less than 18 months, to HIV positive mother.

5. Laboratory markers of HIV infection and their detection by diagnostic tests

Analyses of specimens from seroconversion panels have established the dynamics of HIV-1 viremia after infection and the sequential appearance of different laboratory markers. The approximate time at which different markers appear, estimated from different data sources, are outlined schematically in Figure 1.

Figure 1. Sequence of appearance of laboratory markers for HIV-1 infection



Note. Units for vertical axis are not noted because their magnitude differs for RNA, p24 antigen, and antibody. Modified from MP Busch, GA Satten (1997)[50] with updated data from Fiebig (2003),[48] Owen (2008),[49] and Masciotra (2011, 2013).[46,66]

Immediately after HIV infection, low levels of HIV-1 RNA (ribonucleic acid) might be present intermittently, but no viral markers are consistently detectable in plasma. Approximately 10 days after infection, HIV-1 RNA becomes detectable by NAT in plasma and quantities increase to very high levels. Next, HIV-1 p24 antigen is expressed and quantities rise to levels that can be detected by 4th generation immunoassays within 4 to 10 days after the initial detection of HIV-1 RNA. However, p24 antigen detection is transient because, as antibodies begin to develop, they bind to the p24 antigen and form immune complexes that interfere with p24 assay detection unless the assay includes steps to disrupt the antigen-antibody complexes. Next, immunoglobulin (Ig) M antibodies are expressed which can be detected by 3rd and 4th generation immunoassays 3 to 5 days after p24 antigen is first detectable, 10 to 13 days after the appearance of viral RNA. Finally, IgG antibodies emerge and persist throughout the course of HIV infection. First and second generation immunoassays designed to detect only IgG antibodies exhibit considerable variability in their sensitivity during early infection, becoming reactive 18 to 38 days or more after the initial detection of viral RNA.

The pattern of emergence of laboratory markers is highly consistent and allows classification of HIV infection into distinct laboratory stages:

- The eclipse period is the initial interval after infection with HIV when no laboratory markers are consistently detectable.
- The seroconversion window period is the interval between infection with HIV and the first detection of antibodies. Its duration depends on the design of the antibody immunoassay and the sensitivity of the immunoassay during seroconversion.
- Acute HIV infection is the interval between the appearance of detectable HIV RNA and the first detection of antibodies. Its duration also depends on the design of the antibody immunoassay and the sensitivity of the immunoassay during seroconversion.
- Established HIV infection is the stage characterized by a fully developed IgG antibody response sufficient to meet the interpretive criteria for a positive Western blot or IFA.

6. Types of HIV assays available at Lablink Medical Laboratories as at 16th March 2017.

6.1 HIV-1/HIV-2 Ag/Ab Combo Immunoassay (Fourth Generation)

6.2 HIV-1 & HIV-2 Antibodies Particle Agglutination

6.3 HIV-1 & HIV-2 Antibodies Line Immunoassay

6.4 PCR HIV-1 RNA Qualitative Assay (Rapid PCR Xpert HIV-1 RNA Qualitative).

6.5 PCR HIV-1 Viral Load Assay (Rapid PCR Xpert HIV-1 RNA Viral Load)

7. Use of fourth generation serological assays

Fourth generation serological assays that detect both HIV p24 antigen and HIV-1/2 antibodies have the potential to identify infected individuals earlier in the course of disease. In other words, these assays greatly shorten the diagnostic window period.

8. Specimen types used for HIV testing

8.1 Serum : freshly collected whole blood is allowed to coagulate, and the serum fraction is collected away from the clotted red blood cells.

8.2 Plasma : freshly collected wholeblood is added to the recommended anticoagulant, such as EDTA, heparin, or citrate. After centrifugation, the plasma is separated. Use only anticoagulant validated by the assay manufacturer.

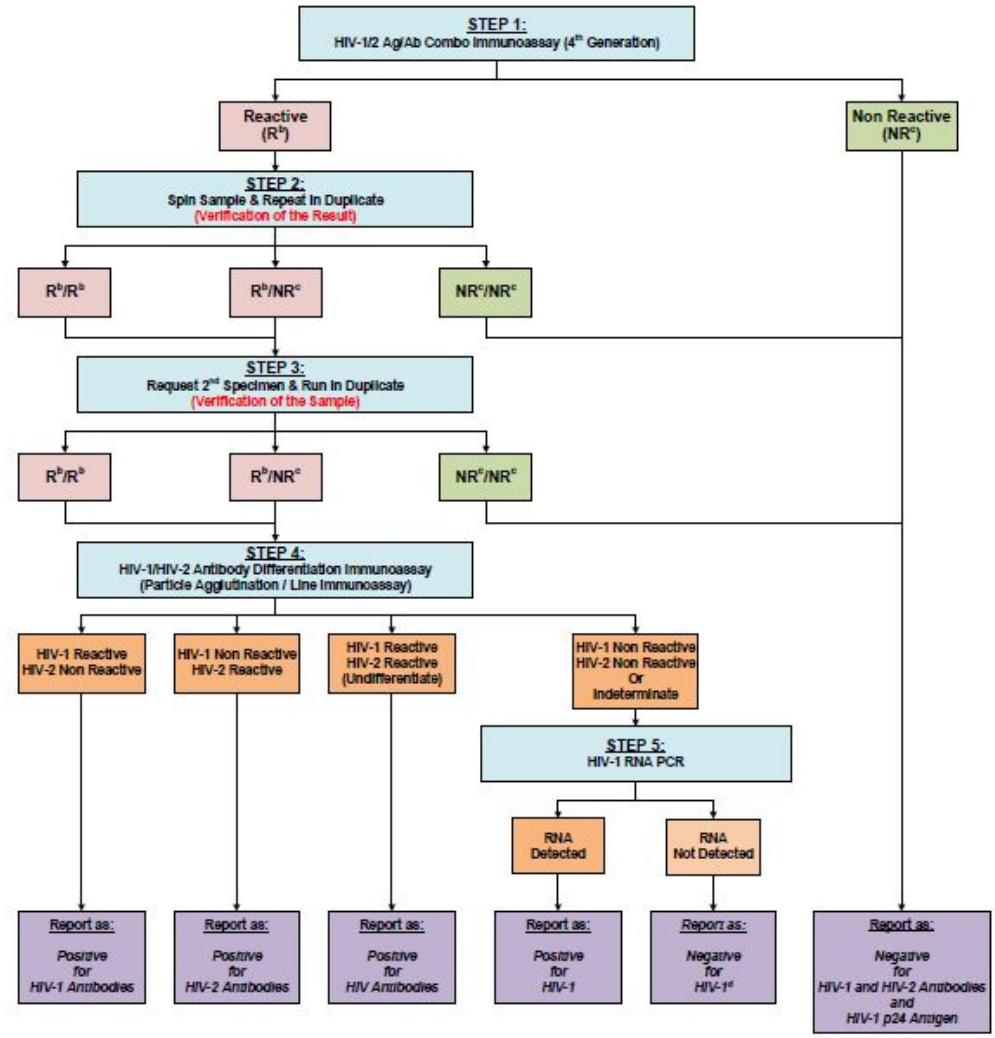
9. Lablink Medical Laboratory HIV Testing Algorithms

9.1 HIV Testing Algorithm in adults and childrens after 18 months of age – refer Algorithm 1.

9.2 HIV Testing Algorithm in infants less than 18 months of age – refer Algorithm 2.

Algorithm 1 : HIV Testing Algorithm in Adults and Infants Older than 18 Months.

1. Algorithm for HIV Testing in Adult and Paediatric (Age > 18 Months[a])



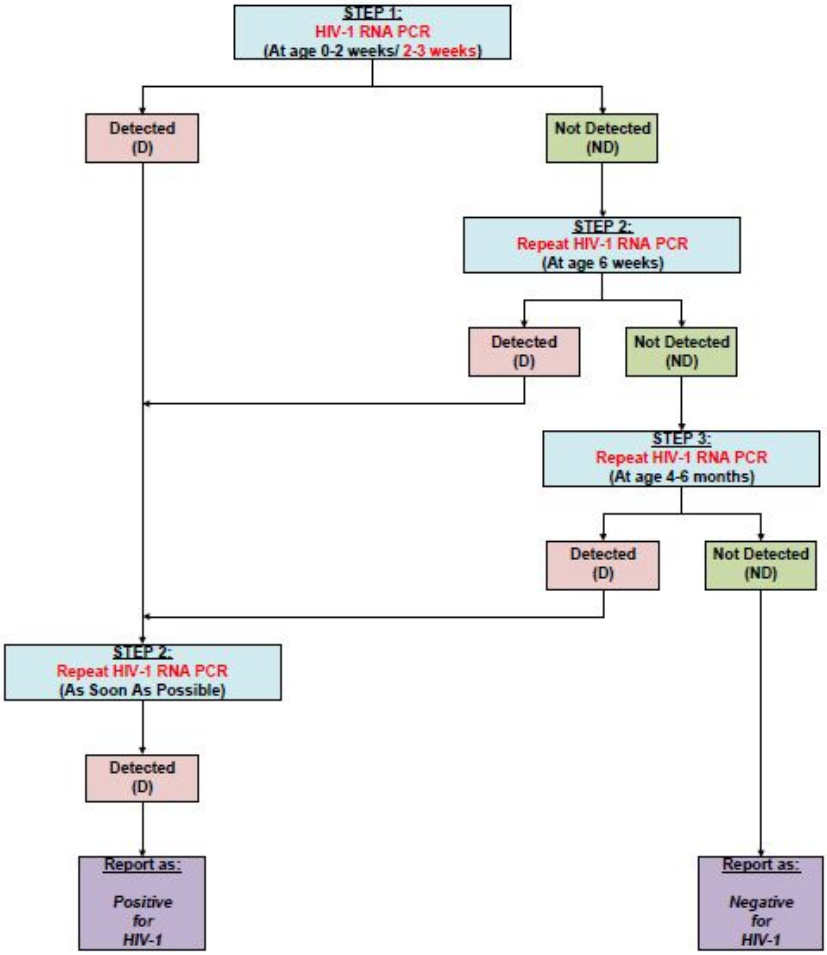
[a] Mother not traceable, HIV status unknown in Non-Breastfed Infants.

	Range (COI)	Result	Risk Group	Confirmatory Panel
[b] EIA :	0.9 – 10.0	Equivocal	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	10.1 – 100.0	Weakly Reactive	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	>100.0	Reactive	High	Particle Agglutination Only
			Low (Medico-legal Cases, Specialist Request or Risk Unknown)	Particle Agglutination and Immunoblot
[c] EIA :	<0.9	Non Reactive	Not Applicable	Not Applicable

[d] Consider repeat testing if clinically indicated. If there is a reason to suspect recent HIV-2 infection, additional testing for HIV-2 RNA should be considered.

Algorithm 2 : Algorithm for HIV exposed Infants less than 18 months to HIV Positive Mother

2. Algorithm for HIV Exposed Infants (Age < 18 Months) to HIV Positive Mother – Molecular Testing



NOTE :

For babies more than 18 months of age, please follow Algorithm 1.

10. Detail list of tests for the diagnosis of HIV available at Lablink Medical Laboratories as at 15th of January 2016.

No.	Test / Objective	Specimen	Container	Transportation Requirement	Method	Note
10.1	**Test:** HIV-1 and HIV-2 Ag/Ab Combo [4TH Generation Immunoassay] **Objective:** Serological detection of HIV-1 p24 antigen and HIV-1 and HIV-2 IgM and IgG antibodies. **Note:** Use as screening test for HIV infection	Venous Blood (Serum and Plasma)	Plain Tube or Plain Tube with Serum Separator. OR Li-heparin tube OR EDTA tube OR Li- heparin with plasma separator	4 weeks at 2–8 °C 7 days at 25 °C 3 month at -20°C (frozen 5 times only)	EIA – Enzyme Immunoassay	**Note 1:** HIV-1 p24 antigen can be detected by 4th generation immunoassay within 4 to 10 days after the initial detection of HIV-1 RNA by PCR or after 14 days of HIV infections. **Note 2:** IgM antibodies can be detected by 3 to 5 days after p24 antigen is first detectable, 10 to 13 days after the appearance of HIV-1 RNA or after 17 to 19 days of HIV infections. Finally, IgG antibodies emerge and persist throughout the course of HIV infection. **Note 3:** Please refer Figure 1 and HIV Algorithm 1a, 1b and 1c.

No.	Test	Specimen	Container	Transportation Requirement	Method	Note
10.2	**Test:** HIV-1 and HIV-2 Antibodies Particle Agglutination **Objective:** Serological detection of HIV-1 and HIV-2 IgM and IgG antibodies. **Note:** Use as supplementary assay for confirmation of HIV infection.	Venous Blood (Serum and Plasma)	Plain Tube or Plain Tube with Serum Separator. OR EDTA Tube or EDTA Tube with Plasma separator OR Citrate tube OR Heparin tube	7 days at 2-8 °C >7 days at -20°C (frozen 3 times only)	Particle Agglutination	**Note 1:** IgM antibodies can be detected by 3 to 5 days after p24 antigen is first detectable, 10 to 13 days after the appearance of HIV-1 RNA or after 17 to 19 days of HIV infections. Finally, IgG antibodies emerge and persist throughout the course of HIV infection. **Note 2:** Please refer Figure 1 and HIV Algorithm 1a and 1d.

No.	Test	Specimen	Container	Transportation Requirement	Method	Note
10.3	**Test:** HIV-1 and HIV-2 Line-Immunoassay (LIA) **Objective:** Serological detection of 2 types HIV-1 antibodies, 2 types of HIV-2 antibodies and 3 types of HIV-1/HIV-2 antibodies. **Note:** Use as supplementary assay for confirmation of HIV infection.	Venous blood (Serum)	Plain Tube or Plain Tube with Serum Separator. OR EDTA Tube or EDTA Tube with Plasma Separator. OR Citrate tube OR Heparin tube	7 days at 2-8 °C >7 days at -20°C (frozen 3 times only)	Line Immunoassay (LIA)	**Note 1:** Five HIV-1 antigens are applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O peptides are present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 are applied to detect antibodies to HIV-2 **Note 2:** IgM antibodies can be detected by 3 to 5 days after p24 antigen is first detectable, 10 to 13 days after the appearance of HIV-1 RNA or after 17 to 19 days of HIV infections. Finally, IgG antibodies emerge and persist throughout the course of HIV infection. **Note 3:** Please refer Figure 1 and HIV Algorithm 1a and 1d.

No.	Test	Specimen	Container	Transportation Requirement	Method	Note
10.4	**Test:** Rapid Xpert® HIV-1 RNA Qualitative **Objective:** Qualitative Detection of HIV-1 RNA. **Note:** Use as screening and confirmatory tests for HIV-1 infection in children < 18 months of age. Use as supplementary assay for confirmation of HIV-1 infection in adults.	Specimens: **Adult/ Paediatric** Whole blood (200 ul / 0.2 ml)	**Adult/ Paediatric** EDTA Tube or EDTA microtainer tube	**Whole Blood** **Note 1** Whole blood may be held at 15–30°C for up to 8 hours or at 2–8 °C for up to 72 hours, prior to preparing and testing the specimen.	Real Time PCR	**Note 1** Immediately after HIV infection, Day 1 – day 9, low levels of HIV-1 RNA (ribonucleic acid) might be present intermittently, but no viral markers are consistently detectable in plasma. **Note 2** Approximately 10 days after infection, HIV-1 RNA becomes detectable by NAT in plasma and quantities increase to very high levels. HIV-1 RNA plasma peaks about 30-days after infection, and then decreases until about 40 days when the level plateaus. **Note 3** Please refer Figure 1, HIV Algorithm 1a, 1d and HIV Algorithm 2.

No.	Test	Specimen	Container	Transportation Requirement	Method	Note
10.5	**Test:** Rapid Xpert® HIV-1 RNA Viral Load (VL) **Objective:** Quantitative Detection of HIV RNA. **Note:** Use as an aid in assessing viral response to anti-retroviral treatment as measured by changes in plasma HIV-1 RNA levels.	Specimens: Plasma 3 - 5 ml	EDTA Tube or EDTA with Plasma Separator.	**Note 1** Whole blood may be held at 15–30°C for up to 8 hours or at 2–8 °C for up to 72 hours, prior to preparing and testing the specimen. **Note 2** After centrifugation, plasma may be held at 15–30 °C for up to 24 hours or at 2–8°C for up to 6 days, prior to testing.	Real Time PCR	**Note 1** The HIV-1 VL Assay, performed on the GeneXpert® Instrument Systems, is an in vitro diagnostic test designed for the rapid quantitation of HIV-1 in human plasma from HIV-1 infected individuals over the range of 40 to 10, 000, 000 copies/mL, and is validated for specimens across Group M Subtypes A, B, C, D, AE, F, G, H, AB, AG, J, K, and Group N and Group O. **Note 2** The HIV-1 VL assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to anti-retroviral treatment as measured by changes in plasma HIV-1 RNA levels. **Note 3** The HIV-1 VL Assay is not intended to be used as a donor screening test for HIV-1. **Note 4** The HIV-1 VL Assay can ONLY be used as a supplementary diagnostic test to confirm the presence of HIV-1 infection if the COI value of HIV-1/HIV-2 Ag/Ab ECLIA > 100 COI (Roche & Abbott) or >12 COI (Centaur).

Algorithm 1a: Step 1 – HIV-1/HIV-2 Combo Immunoassay (4th Generation)

1. Algorithm for HIV Testing in Adult and Paediatric (Age > 18 Months[a])

STEP 1:
HIV-1/2 Ag/Ab Combo Immunoassay (4th Generation)

- **Reactive (R[b])** → **STEP 2:** Spin Sample & Repeat in Duplicate (Verification of the Result)
- **Non Reactive (NR[c])**

[a] Mother not traceable, HIV status unknown in Non-Breastfed Infants.

	Range (COI)	Result	Risk Group	Confirmatory Panel
[b] EIA :	0.9 – 10	Equivocal	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	10.1 – 100	Weakly Reactive	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	> 100	Reactive	High	Particle Agglutination Only
			Low (Medico-legal Cases, Specialist Request or Risk Unknown)	Particle Agglutination and Immunoblot
[c] EIA :	< 0.9	Non Reactive	Not Applicable	Not Applicable

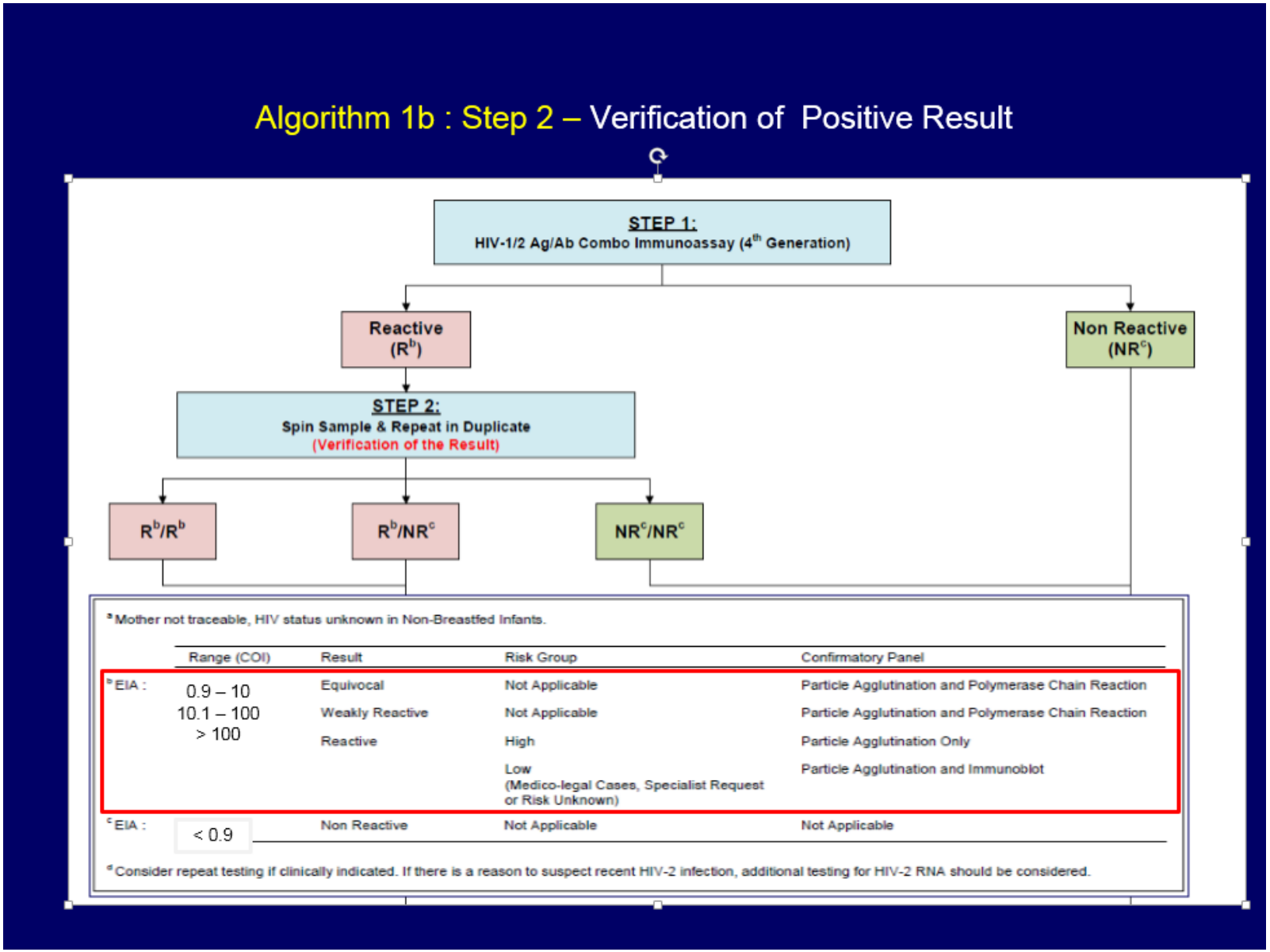
[d] Consider repeat testing if clinically indicated. If there is a reason to suspect recent HIV-2 infection, additional testing for HIV-2 RNA should be considered.

HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected.

No laboratory evidence of HIV infection.

Sample can be reported as nonreactive for HIV.

If recent HIV exposure is suspected, redraw and repeat algorithm.



[a] Mother not traceable, HIV status unknown in Non-Breastfed Infants.

	Range (COI)	Result	Risk Group	Confirmatory Panel
[b] EIA :	0.9 – 10	Equivocal	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	10.1 – 100	Weakly Reactive	Not Applicable	Particle Agglutination and Polymerase Chain Reaction
	> 100	Reactive	High	Particle Agglutination Only
			Low (Medico-legal Cases, Specialist Request or Risk Unknown)	Particle Agglutination and Immunoblot
[c] EIA :	< 0.9	Non Reactive	Not Applicable	Not Applicable

[d] Consider repeat testing if clinically indicated. If there is a reason to suspect recent HIV-2 infection, additional testing for HIV-2 RNA should be considered.

1. HOW TO REPORT NON-REACTIVE HIV RESULT?

Run 1	Run 2	Run 3	Result	Comments
NR (<0.9)	NA	NA	Non-Reactive	HIV-1 p24 Antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection. If recent HIV exposure is suspected, kindly repeat test after 2 – 4 weeks.
NR (<0.9)	NR (<0.9)	NR (<0.9)	Non-Reactive	HIV-1 p24 Antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection. If recent HIV exposure is suspected, kindly repeat test after 2 – 4 weeks.

NOTE: Refer Algorithm 1a and 1b. NR – Non-Reactive; NA – Not Applicable.

2. HOW TO REPORT PRELIMINARY HIV RESULT?

2.1 EQUIVOCAL RESULT;

Run 1	Run 2	Run 3	Result	Comments
EQ (0.9-10.0)	EQ (0.9-10.0)	EQ (0.9-10.0)	Equivocal (EQ)	**PRELIMINARY HIV RESULT** Most likely biological false positive result in the absence of risk factors. If recent high-risk exposure is suspected, the result may indicate of early infection. Kindly sent a repeat specimen for sample verification and confirmation of HIV status, if clinically indicated. Assays for HIV confirmation include; 1. Enzyme Immunoassay (EIA) Second Sample 2. Particle Agglutination (PA) test; 2. PCR HIV-1 RNA Qualitative Assay

NOTE: Refer Algorithm 1a and 1b. EQ – Equivocal; PA – Particle Agglutination, TAT – Turn-Around-Time.

2.2 WEAKLY REACTIVE RESULT

Run 1	Run 2	Run 3	Result	Comments
WR (10.1-100)	WR (10.1-100)	WR (10.1-100)	Weakly Reactive (WR)	**PRELIMINARY HIV RESULT** Laboratory evidence indicates high probability of HIV infection. Kindly sent a repeat specimen for sample verification and confirmation of HIV status, if clinically indicated. Assays for HIV confirmation include; 1. EIA (Enzyme Immunoassay) Second Sample 2. Particle Agglutination (PA) Test; 3. PCR HIV-1 RNA Qualitative Assay

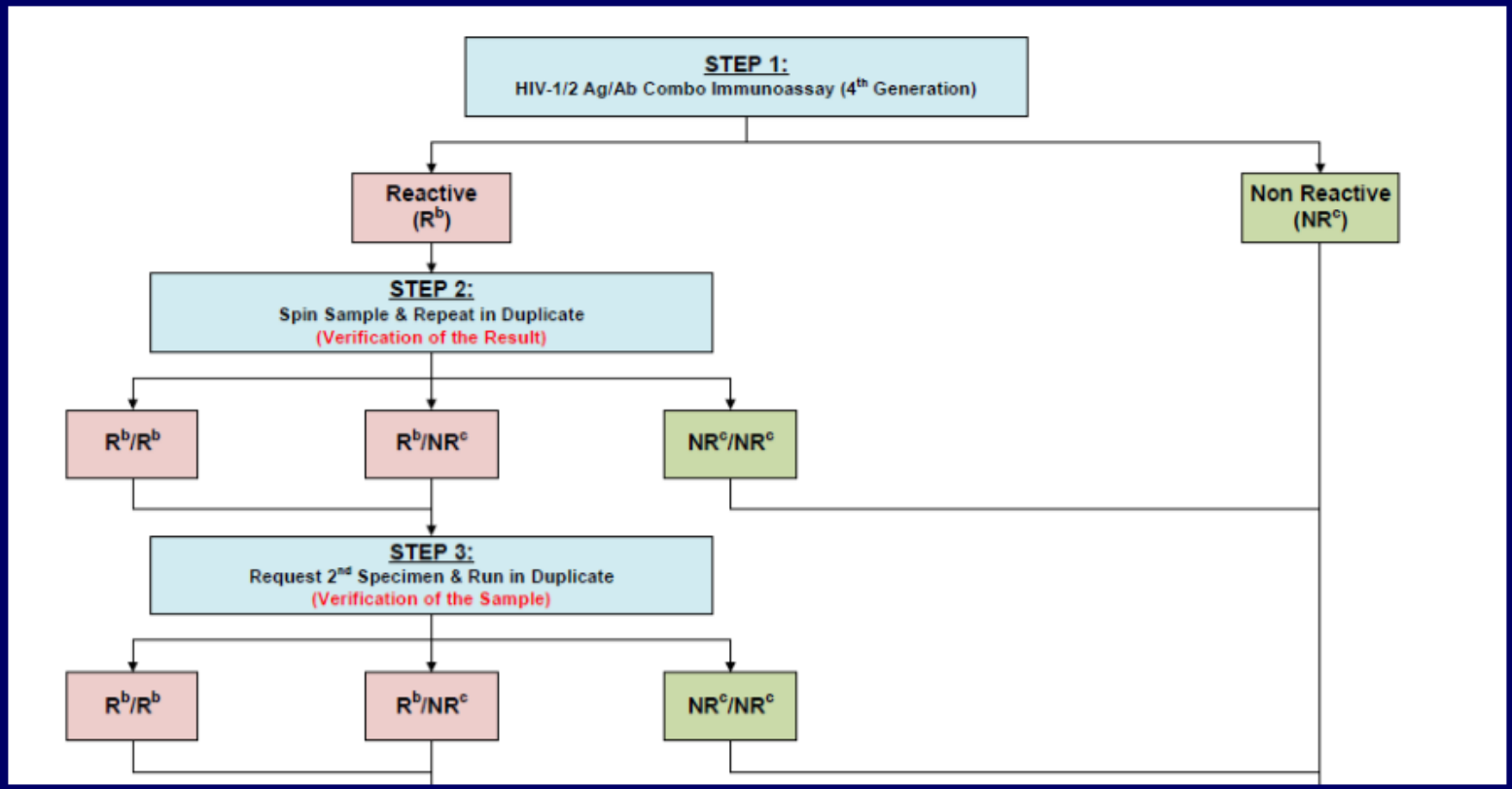
NOTE: Refer Algorithm 1a and 1b. WR – Weakly Reactive; PA – Particle Agglutination, TAT – Turn-Around-Time.

2.3 REACTIVE HIV RESULT

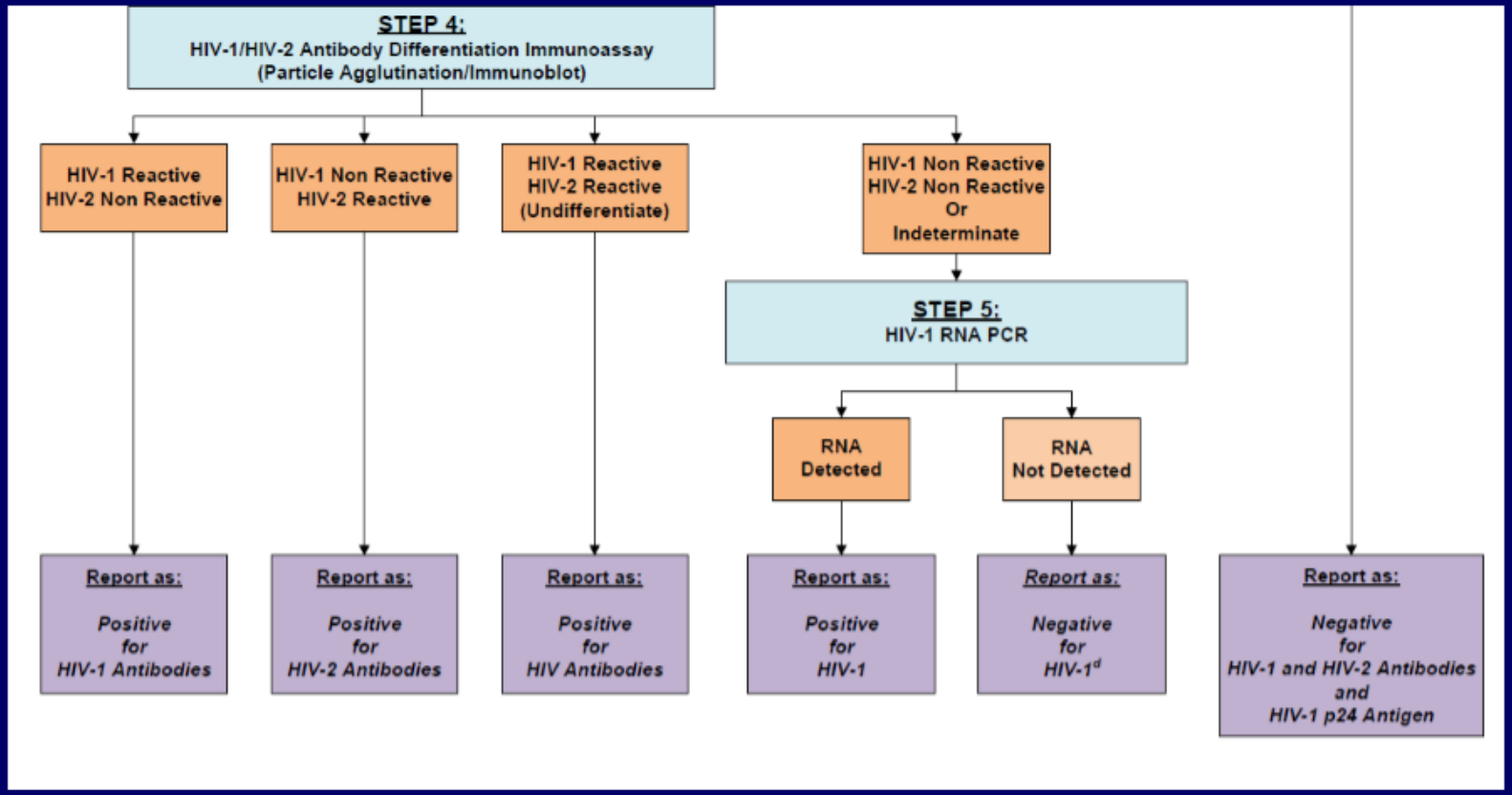
Run 1	Run 2	Run 3	Result	Comments
R (>100)	R (>100)	R (>100)	Reactive (R)	**PRELIMINARY HIV RESULT** Positive for HIV antibodies. Laboratory evidence of HIV infection Kindly sent a repeat specimen for sample verification and confirmation of HIV status.
				High Risk Group Assays for HIV confirmation is EIA (Enzyme Immunoassay) SECOND SAMPLE and Particle Agglutination (PA) Test.
				Low Risk Group Assays for HIV confirmation include; **PANEL 1** 1. Enzyme Immunoassay (EIA) SECOND SAMPLE and Particle Agglutination (PA) test, AND 2. HIV Line Immunoassay (LIA) / Immunoblot Assay. **PANEL 2** 1. Enzyme Immunoassay (EIA) SECOND SAMPLE and Particle Agglutination (PA) test, AND 2. PCR HIV-1 RNA Qualitative Assay or PCR HIV-1 Viral Load Assay.

NOTE: Refer Algorithm 1a and 1b. R – Reactive; PA – Particle Agglutination, TAT – Turn-Around-Time.

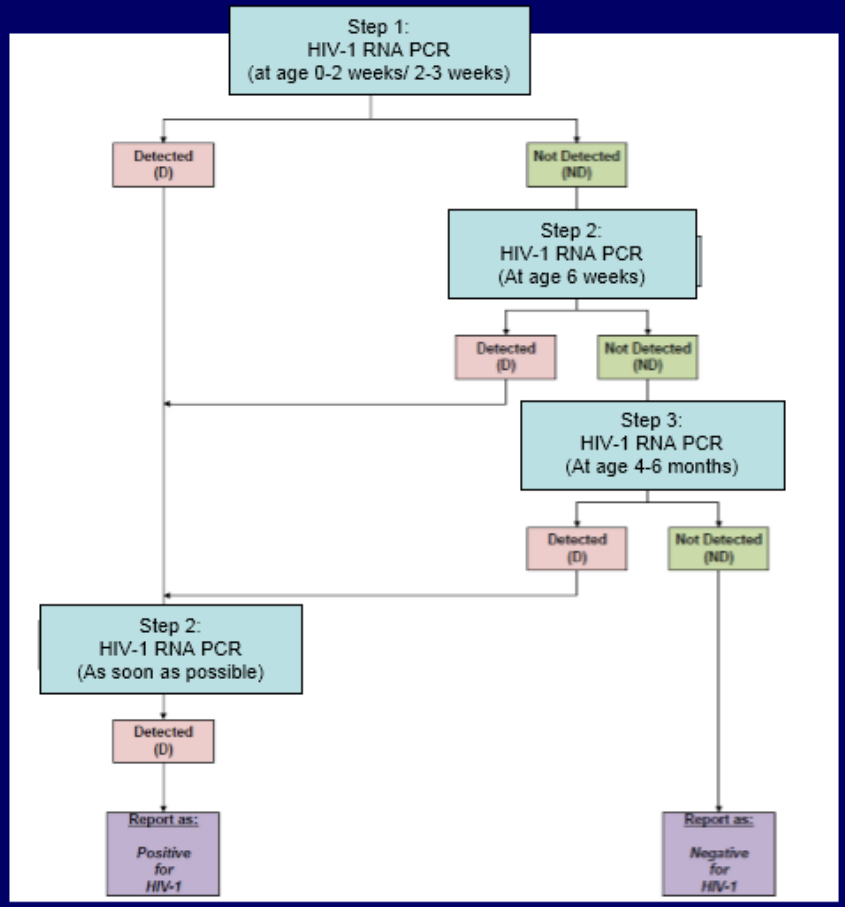
Algorithm 1c : Step 3 – Verification of Sample



Algorithm 1d: Step 4 – Assays for Confirmation of HIV Infection



Algorithm 2 : Molecular testing for HIV Exposed Infants (Age < 18 Months) to HIV Positive Mother



Detection of HIV-1 RNA is indicative of active HIV infection

Early diagnosis of HIV in infants Is made possible using molecular based tests and allows for rapid implementation of ART in infected children

11. **REFERENCES:**

1. Centers for Disease control and Prevention and association of Public Health Laboratories: **Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations**. Available at http://stacks.cdc.gov/view/cdc/23447. Published June 2014.
2. World health Organization. Guidelines. **Consolidated Guidelines on HIV Testing Services 2015.** 5Cs: Consent, Confidentiality, Counselling, Correct Results and Connection. July 2015.
3. Deniz Gökengin et al. 2014 European Guideline on HIV Testing. International Journal of STD & AIDS. Available at http://std.sagepub.com/content/early/2014/04/22/0956462414531244. Published online 22 April 2014.
4. Roche Cobas HIV combi PT (HIV-1 antigen and total antibodies to HIV-1 and HIV-2) package insert (v7).
5. FUJIREBIO SERODIA HIV-1/2 (Passive Particle-Agglutination Test for detection of antibodies to HIV-1 and-or HIV-2) package insert.
6. FUJIREBIO INNO-LIA HIV I/II Score package insert (28672 v2)
7. Xpert® HIV-1 Qualitative; 301-3068, Rev. C March 2015 Package Insert (REF: GXHIV-QA-CE-10)
8. Xpert® HIV-1 Viral Load Assay; 301-3068, Rev. C January 2015 Package Insert.(REF: GXHIV-VL-CE-10)