TOOLKIT

HIV MOLECULAR DIAGNOSTICS TOOLKIT TO IMPROVE ACCESS TO VIRAL LOAD TESTING AND INFANT DIAGNOSIS

JULY 2019

HIV TREATMENT AND CARE

World Health Organization

HIV molecular diagnostics toolkit to improve access to viral load testing and infant diagnosis

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1. INTRODUCTION: MOLECULAR DIAGNOSTICS FOR HIV VIRAL LOAD TESTING AND INFANT DIAGNOSIS

Treatment failure monitoring

Monitoring people receiving antiretroviral therapy is important to ensure successful treatment, identify adherence problems and determine whether antiretroviral therapy regimens should be switched in case of treatment failure. In 2013, WHO recommended viral load testing as the preferred monitoring approach to diagnose and confirm antiretroviral therapy failure *(1)*. Compared with clinical or immunological monitoring, viral load provides an early and more accurate indication of treatment failure. Measuring viral load can help to distinguish between drug resistance and non-adherence when coupled with robust enhanced adherence counselling. Further, viral load can serve as a proxy measure for the risk of transmission and effectiveness of prevention interventions at both the individual and population levels.

Updated 2016 WHO guidelines recommend routine viral load monitoring be carried out at 6 months, 12 months after initiation of antiretroviral therapy and then every 12 months thereafter if the person is stable on antiretroviral therapy *(2)*. If viral load is not routinely available, CD4 count and clinical monitoring should be used to assess treatment failure. Further, dried blood spot specimens using venous or capillary whole blood can be used to determine the HIV viral load. A threshold of 1000 copies/mL should be used to determine treatment failure when using dried blood spot specimens, as similarly defined for testing using plasma.

Treatment failure is defined by a persistently detectable viral load exceeding 1000 copies/mL *(2)*: that is, two consecutive viral load measurements within a three-month interval with adherence support between measurements after at least six months of starting a new antiretroviral therapy regimen (Box 1).

In addition, viral load may support differentiated service delivery strategies for people living with HIV, including those who are stable on antiretroviral therapy *(2)*. Stable individuals are defined as those who have received antiretroviral therapy for at least one year and have no adverse drug reactions that require regular monitoring, no current illnesses or pregnancy, are not currently breastfeeding and have good understanding of lifelong adherence and evidence of treatment success (two consecutive viral load measurements below 1000 copies/mL). The package of care for stable individuals can include less frequent clinic visits and medication pickup, community-based care and cessation of CD4 count monitoring if viral load testing is available.

Box 1. Assessing advanced HIV disease

Since CD4 count is the best predictor of disease status and immediate risk of death, it should be used to identify people with advanced HIV disease. Everyone entering or re-entering care should receive a CD4 test at treatment baseline and as clinically indicated for people who are clinically unstable or have symptoms of advanced HIV disease *(2,3)*. Further, it is strongly recommended that people with advanced HIV disease (CD4 count below 200 cells/mm^3 or WHO stage 3 or 4) receive a package of care *(4)*.

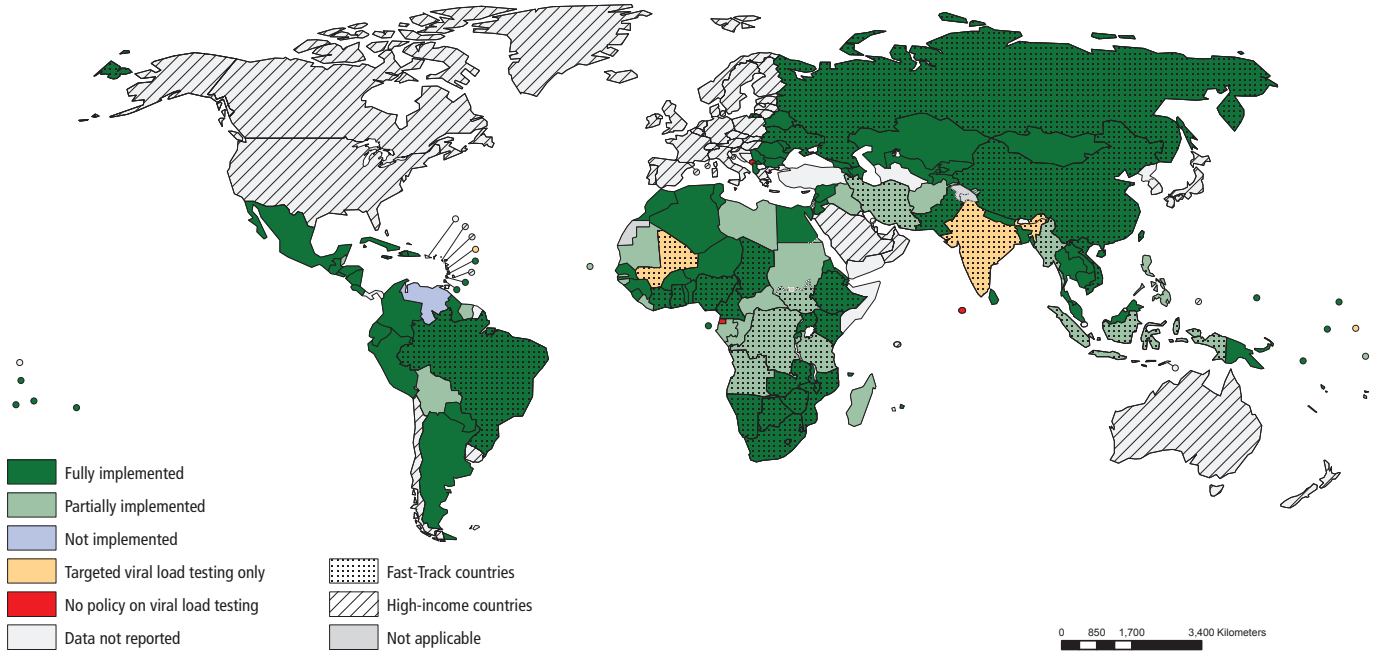
Many national guidelines now recommend and are scaling up access to viral load testing for treatment monitoring (Fig. 1).

The proportion of yearly viral load tests performed has increased significantly since 2013 (Fig. 2) *(5)*. About 15 million viral load tests were conducted in 2017, and projections suggest that nearly 29 million tests may be performed in 2022. Despite increasing volumes, the total coverage of the demand of viral load testing remained below 60% in 2017.

As national viral load test volumes are large and continue to grow, this will add significantly more costs to national testing budgets. Fortunately, several recent pricing commitments have been negotiated to support viral load testing expansion and access *(6–8)*.

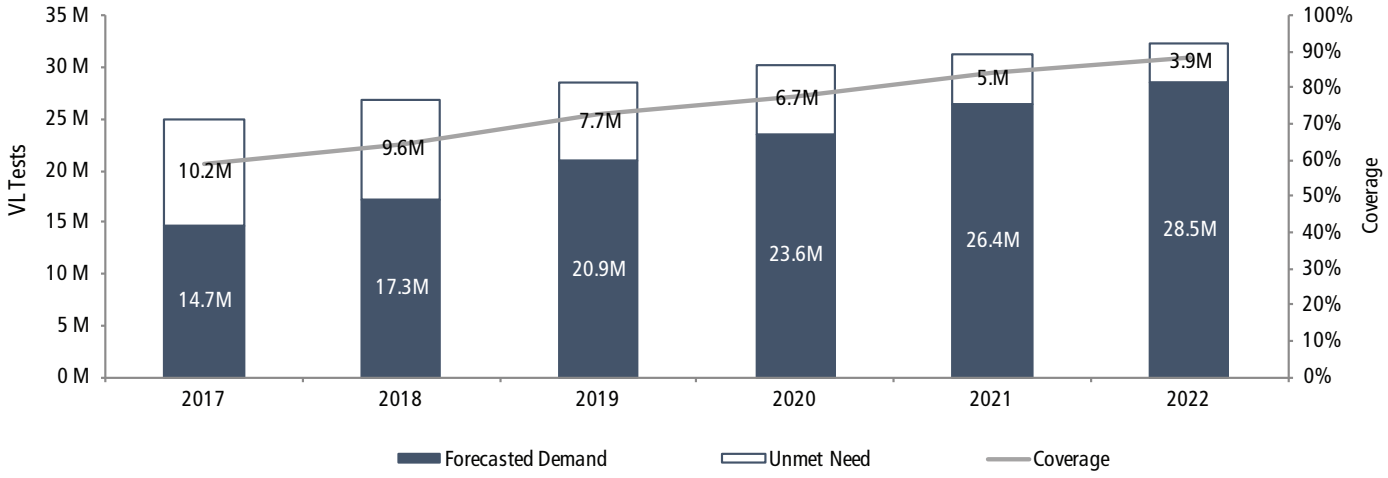
Numerous technologies, both laboratory-based and near-point-of-care assays, currently exist to support the scaling up of viral load testing and infant diagnosis. Several additional technologies are being developed *(9,10)*.

Fig. 1. National policy on routine viral load testing for monitoring ART and level of implementation for adults and adolescents in low- and middle-income countries (situation as of July 2019)



Source: Global AIDS Monitoring (UNAIDS/WHO/UNICEF) and WHO HIV Country Intelligence Tool, 2019

Fig. 2. Estimated viral load forecast in low- and middle-income countries globally



Source: 2018 CHAI HIV Market Report.

Infant diagnosis

Infant diagnosis consists of testing throughout the exposure period of HIV-exposed infants. Depending on the age, this can comprise either nucleic acid–based testing or serological testing. More specifically, early infant diagnosis refers specifically to nucleic acid-based testing of infants within two months of birth. See Annex 1 for the infant diagnosis algorithm.

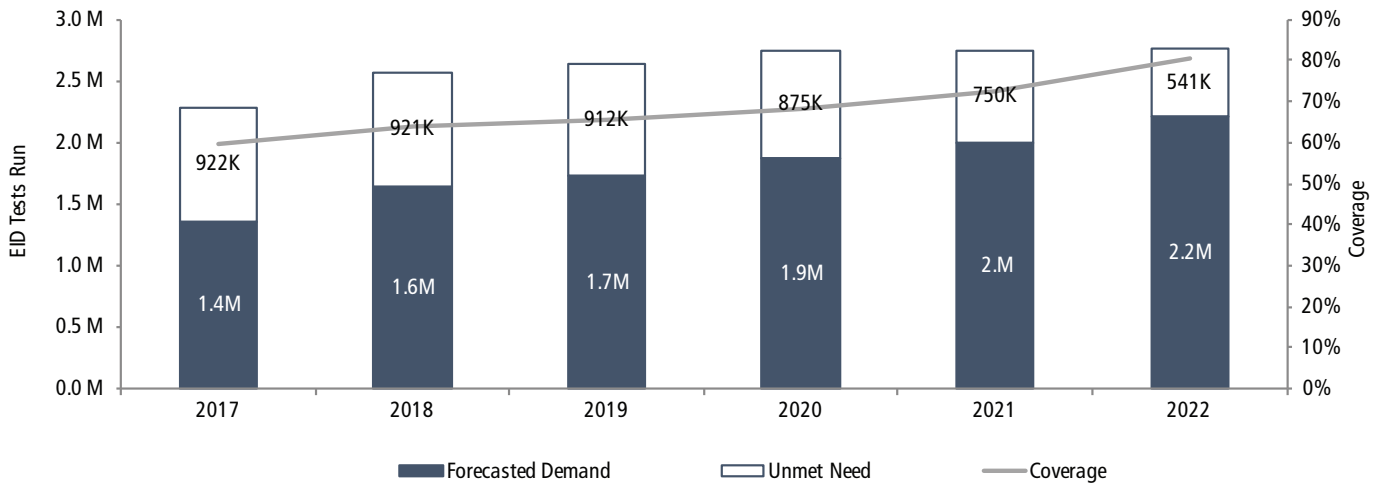
Coverage of early infant diagnosis (testing within two months of birth) has remained stagnant in recent years, with about 51% of HIV-exposed infants receiving a nucleic acid test within the first two months of life in 2018 *(11)*. The proportions of HIV-exposed infants tested at nine months or at the end of the exposure period have been difficult to gather.

Current forecasts for nucleic acid testing suggest moderate growth and sustained volumes through 2022 (Fig. 3) *(5)*. About 1.4 million infant nucleic acid tests were performed in 2017, with more than 2 million projected to be needed for 2022.

Since 2010, several key recommendations have been made to support access to and expanded scale-up of infant diagnosis *(2,12)*.

- An indeterminate range should be used to improve the accuracy of all nucleic acid–based infant diagnosis assays (strong recommendation, moderate-quality evidence).
- Among infants with an initial positive nucleic acid test result, it is strongly recommended that antiretroviral therapy be started without delay and, at the same time, a second specimen be collected to confirm the initial positive test (strong recommendation, low-quality evidence).
- 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 algorithm used for adults to determine final diagnosis (strong recommendation, high-quality evidence).
- In generalized epidemic settings, infants and children with unknown HIV status who are admitted for inpatient care or attending malnutrition or TB clinics should be routinely tested for HIV (strong recommendation, low-quality evidence).
- In generalized epidemic settings, infants and children with unknown HIV status should be offered HIV testing in outpatient or immunization clinics (conditional recommendation, low-quality evidence).
- Nucleic acid–testing technologies that are developed and validated for use at or near to the point of care can be used for infant HIV testing (conditional recommendation, low-quality evidence).
- Addition of nucleic acid testing at birth to existing infant diagnosis approaches can be considered to identify HIV infection among HIV-exposed infants (conditional recommendation, low-quality evidence).
- Consideration should be given to replace serological testing at nine months of age with nucleic acid–based testing.

Fig. 3. Estimated infant diagnosis forecast in low- and middle-income countries globally



Source: 2018 CHAI HIV Market Report.

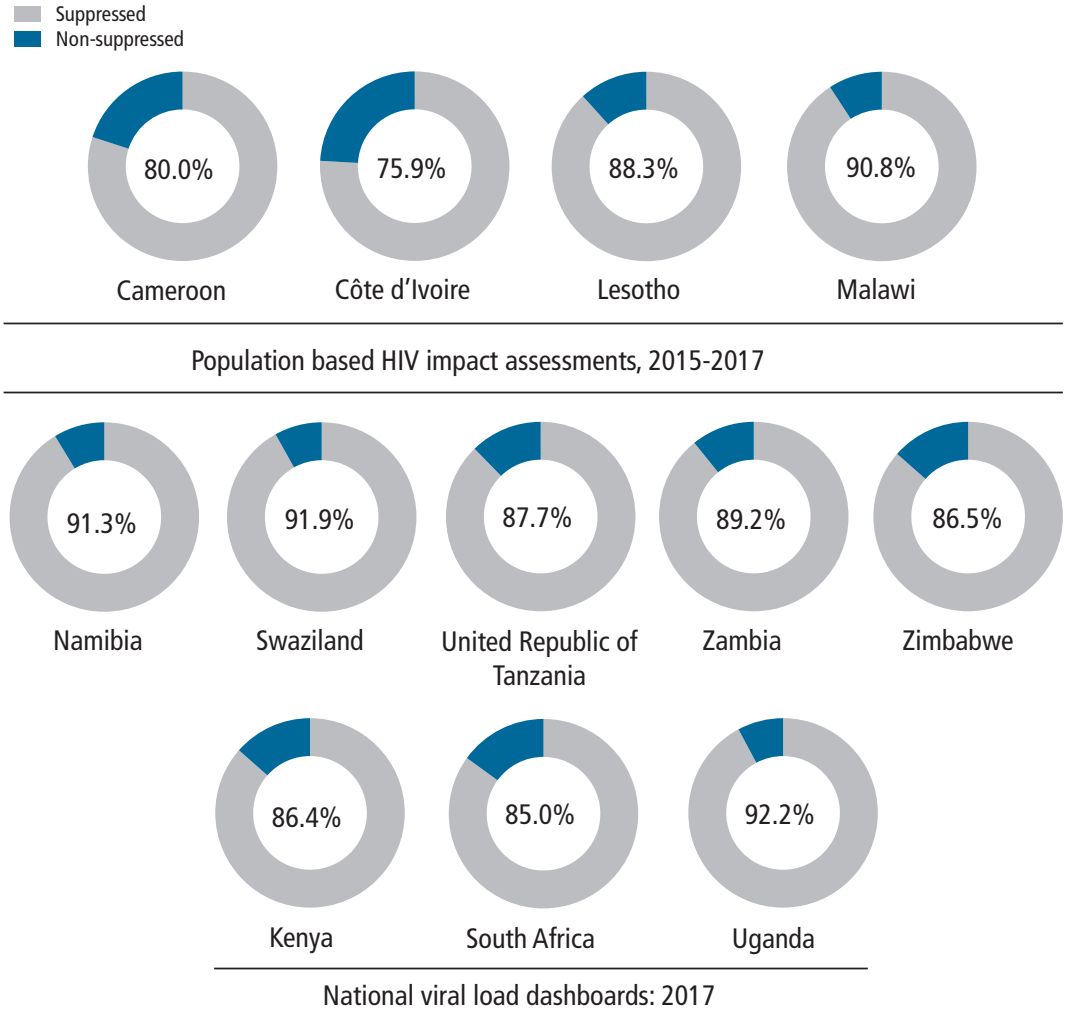
2. USING VIRAL LOAD TEST RESULTS TO SUPPORT THE CLINICAL MANAGEMENT OF PEOPLE LIVING WITH HIV RECEIVING ANTIRETROVIRAL THERAPY

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* provided a strong recommendation for using viral load testing routinely as the preferred antiretroviral drug monitoring tool. WHO recommends viral load testing at six months after initiating antiretroviral therapy, at 12 months and then annually thereafter to enable early detection of treatment failure, prevent drug resistance, identify people with high viral loads with poor adherence and avoid inappropriate switching of treatment regimens *(2)*. In 2014, UNAIDS launched the 90–90–90 treatment targets to be accomplished by 2020, aimed at helping to end the AIDS epidemic as a public health threat. The third 90 aims to ensure that 90% of the people receiving antiretroviral therapy have suppressed viral loads *(13)*.

Antiretroviral therapy and treatment adherence provides remarkable and sustained clinical benefits, even among people with advanced HIV disease. Evidence from national viral load dashboards and the population-based HIV impact assessment *(14)* results suggests that the suppression rates among people living with HIV on antiretroviral therapy are generally 85–92% (Fig. 4).

It is important to understand viral suppression rates at the population level to identify potential hot-spots of

Fig. 4. Viral load suppression rates in selected countries



transmission, inform national targets and provide targeted programmatic quality improvement efforts, but perhaps more importantly, at the patient level to provide enhanced care and support to people living with HIV. Clinically stable people with undetectable viral loads can be provided with differentiated service delivery options that reduce clinic visits and allow for three- or six-monthly drug prescriptions. Further, viral load testing is critical to ensure that those with detectable viral loads greater than 1000 copies/mL are provided enhanced adherence counselling and more closely monitored to determine whether they need to switch to second-line treatment (Fig. 5). The treatment monitoring algorithm is meant to support clinicians and patients in determining whether elevated viral loads or suspicion of treatment failure is caused by drug resistance or poor adherence. It is undesirable for both people living with HIV and programmes to unnecessarily switch people to more expensive and less-well-tolerated second-line regimens when they are simply non-adherent, primarily because adherence issues will not necessarily improve upon switching. However, continuing on a failing drug regimen when the root cause is drug resistance can lead to further drug resistance, additional immune deterioration and possibly clinical effects.

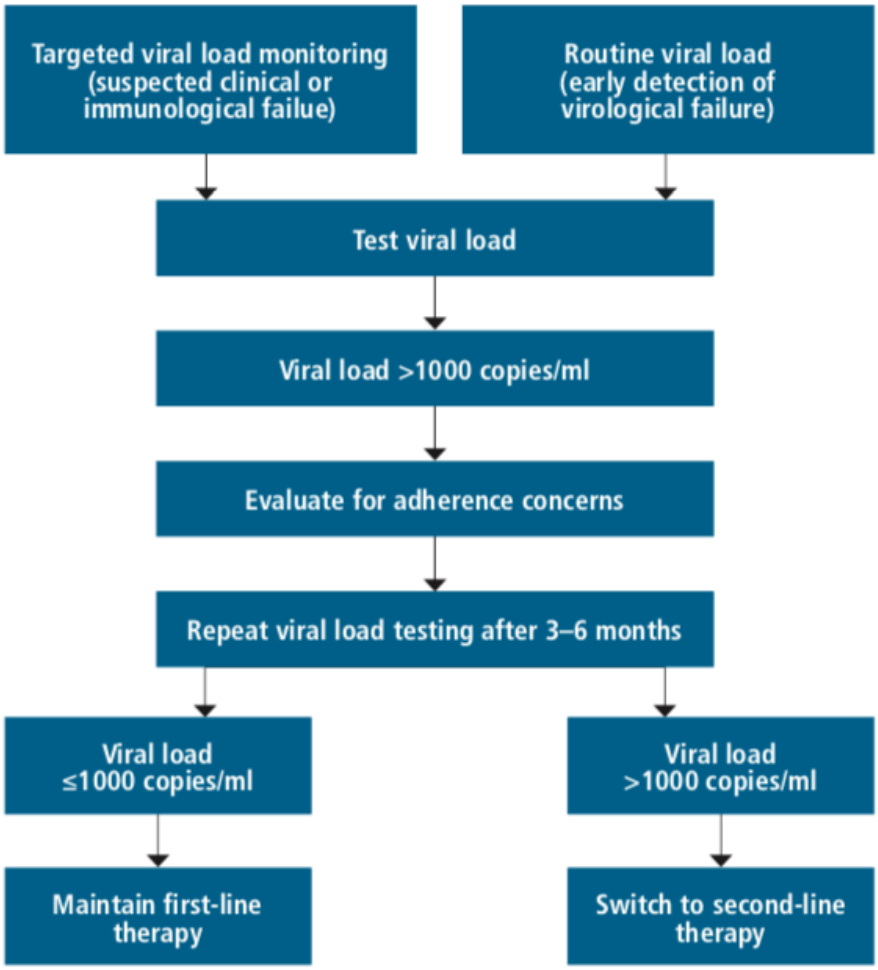
Are viral load test results being used to make clinical decisions?

Viral load testing has been significantly scaled up in recent years, from 7 million tests in 2013 to 15 million tests in 2017. However, performing viral load tests should not be the main consideration of viral load programmes. Programmes may want to also focus on how viral load test results are used to inform clinical decision-making.

Médecins Sans Frontières carried out an in-depth analysis of the execution of key steps in the viral load treatment monitoring algorithm across six countries and 149 clinical sites supported by their programmes *(15)*. Among people with an elevated initial viral load (18% mean), an average of 68% received at least one enhanced adherence counselling session, 52% received a second follow-up viral load test, 34% re-suppressed (<1000 copies/mL) and 33% of those eligible switched to second-line treatment.

These results are further supported by a preliminary analysis of publicly available data published on national dashboards in three countries in eastern Africa *(16)*. Despite

Fig. 5. WHO treatment failure monitoring algorithm

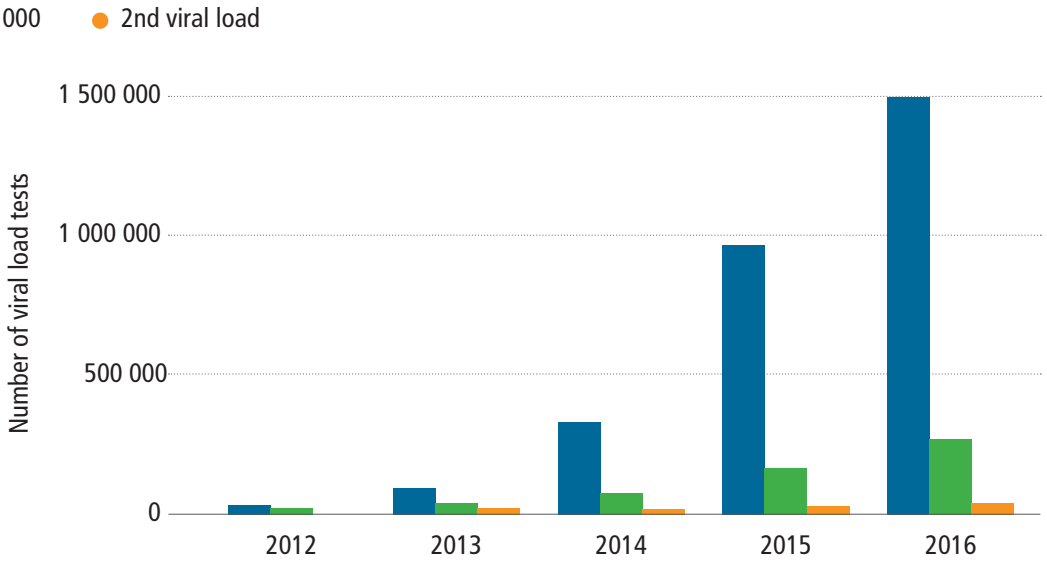


an increase in viral load testing coverage and encouraging viral suppression proportions, less than 10% of the people with an elevated first viral load went through the viral load algorithm to receive a second follow-up viral load test to determine the need for switching to a second-line regimen (Fig. 6). This trend has remained consistent across years.

Key considerations

Diagnostic tests are not of significant value unless the test results are used clinically. The laboratory–clinical interface may be the most difficult yet the most critical and rewarding mechanism for improving patient management. To create effective health services that provide optimal care and treatment to people living with HIV, programmes must revitalize and invest in the laboratory–clinical interface and ensure that the right training, tools and environment are available to improve the uptake and use of all diagnostic results in a timely manner. Several tools currently exist to better support the clinical uptake of viral load test results and could be adapted and adopted by national programmes *(17–19)*. Scaling up successful viral load programmes requires using all test results and integrating them into clinical services to optimize patient care and programmatic success.

Fig. 6. Viral load tests conducted in three countries in eastern Africa, 2012–2016



3. ESTIMATED REACH OF AND ACCESS TO VIRAL LOAD TESTING USING TRADITIONAL PLASMA SPECIMENS

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend viral load as the preferred monitoring approach to diagnose and confirm treatment failure and plasma specimens as the preferred specimen type for viral load testing. Although significant scale-up has occurred across resource-limited countries with a high burden of HIV infection since the initial recommendation in 2013, several significant barriers have limited full access. In particular, the use of liquid plasma using EDTA tubes (see Section 4) can be limited because of strict specimen storage stability times and temperatures, within which the specimens would need to be transported to the testing laboratory or an intermediary hub for processing. Further, there is limited cold-chain availability between many health-care facilities and testing sites in resource-limited settings.

However, traditional EDTA plasma specimens have significant potential for viral load testing. Even within the specimen storage stability times and temperatures, many people still have access to viral load using this type of specimen.

An analysis was recently conducted to better understand the radius around testing laboratories or intermediary hubs within which people may access to viral load testing using traditional EDTA plasma specimens. The analysis was conducted across four countries: Eswatini, Nigeria, Rwanda and Zimbabwe.

Several assumptions were included, such as:

- vehicles travelling at 50 km/h;
- straight-line measurements from the health-care facility to the testing laboratory or intermediary hub plus a 17% circuity factor;
- testing laboratories or intermediary hubs considered as the final point for separating plasma;
- the last specimen collected each day had a maximum wait of two hours at the health-care facility before pick-up and transport to reach the testing laboratory or intermediary hub within the stipulated manufacturer storage stability time; and
- this analysis does not incorporate alternative plasma specimen types or consider on-site centrifugation and associated specimen storage and transport.

Illustrative plasma radius analysis

Across the four countries, just under half of all health-care facilities are near enough to the testing laboratory or intermediary hub to transport traditional EDTA plasma specimens within the time stipulated by the manufacturer. This translates to more than 50% or nearly 1 million people

Table 1. Access to viral load testing using traditional EDTA plasma

Country	Access to viral load testing using traditional EDTA plasma	
	Facilities	People
Eswatini	260/350 (74%)	250 000/320 000 (78%)
Nigeria	750/2 600 (29%)	450 000/1 200 000 (38%)
Rwanda	505/550 (92%)	148 000/165 000 (90%)
Zimbabwe	700/1 500 (47%)	120 000/190 000 (63%)
Total	**2 215/5 000 (44%)**	**968 000/1 875 000 (52%)**

across the four countries analysed having access to viral load testing using traditional EDTA plasma.

Even in such a geographically large country as Nigeria, nearly 40% of the people receiving antiretroviral therapy and needing viral load testing would have access using traditional EDTA plasma. Geographically smaller countries, such as Eswatini and Rwanda, may have fewer laboratory facilities but can provide access to viral load testing using traditional EDTA plasma specimens to nearly 80% or more of the people accessing antiretroviral therapy.

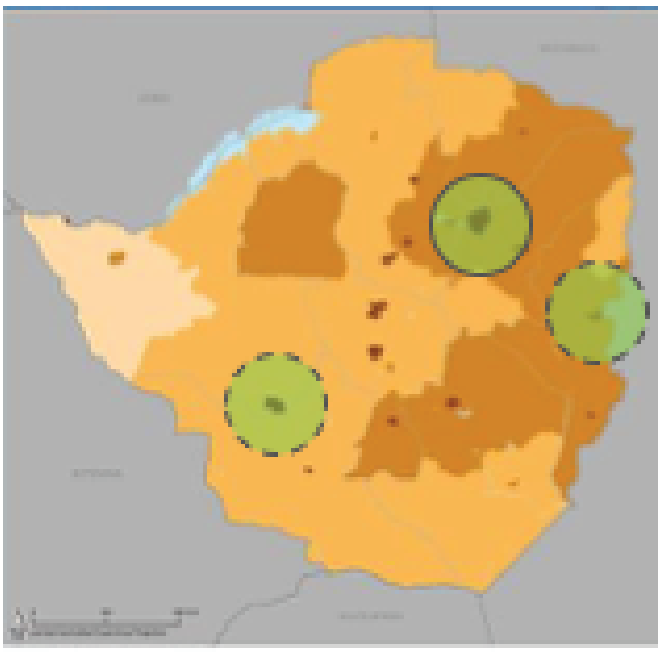
The high access of viral load testing using traditional EDTA plasma is likely because most viral load testing laboratories are in major urban centres. Likewise, the largest antiretroviral therapy centres where people seek care are often also located in major urban centres. This analysis highlights the link in which more than 50% of the people receiving antiretroviral therapy who need viral load testing are within a specimen transport time of a few hours from the testing laboratory or intermediary hub (Fig. 7).

Conclusions

This illustrative analysis provides a snapshot highlighting the potential access to viral load testing using the preferred plasma specimen. The proportion of people who can access viral load testing using traditional EDTA plasma specimens can vary across settings, depending on several factors including the number of laboratories, road infrastructure and size of the country. Efforts should be made and maximized to ensure access to viral load testing using the preferred plasma specimen.

It might be helpful to conduct similar in-depth analyses across countries to determine the facilities and people who may be able to access viral load testing using traditional plasma specimens. Current infrastructure may not always enable the use of traditional EDTA plasma in many settings because of poor roads and infrastructure, large distances, ad hoc specimen transport, etc. Therefore, for the facilities and people without access to viral load testing using traditional EDTA plasma, alternatives could be considered to ensure viral load access, including improved infrastructure, specimen transport networks and alternative specimen types and technologies. This molecular diagnostics toolkit will provide background information and data on several of these alternative strategies to ensure a complementary approach to expanding access.

Fig. 7. Illustrative example of plasma access radius around testing laboratories in Zimbabwe



4. SPECIMEN STABILITY FOR HIV VIRAL LOAD TESTING

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend viral load as the preferred monitoring approach to diagnose and confirm treatment failure and plasma specimens as the preferred specimen type for viral load testing. Although significant scale-up has occurred across resource-limited countries with a high burden of HIV infection since the initial recommendation in 2013, several significant barriers have limited full access to viral load testing. In particular, using liquid plasma and using ethylenediaminetetraacetic acid (EDTA) or plasma preparation tubes (see subsection 6.1) can be limited because of strict specimen storage stability times, within which the specimens would need to be transported to the testing facility or an intermediary hub for processing. Highlighted in Table 2, these are the maximum times according to the storage temperature stipulated by the manufacturers from whole-blood specimen collection to plasma separation. Extending storage times before processing beyond these recommendations could affect performance and risks providing incorrect results to clinicians and patients.

Table 2. Manufacturer-stated whole-blood stability details

Assay	Maximum time from whole-blood specimen collection to plasma separation	
	Room temperature (temperature)	Refrigeration (temperature)
Abbott RealTime HIV-1 *(20,21)*	24 hours (15–30°C)	48 hours (2–8°C)
Abbott m-PIMA HIV-1/2 VL *(22,23)*	48 hours (18–28°C)	NR
Biocentric Generic HIV Charge Virale *(24)*	24 hours (2–25°C)	24 hours (2–25°C)
bioMérieux NucliSENS EasyQ® HIV-1 *(25,26)*	NR	24 hours (2–8°C)
Cavidi ExaVir Load *(27)*	4–6 hours (no temperature specified)	
Cepheid Xpert HIV-1 Viral Load *(28,29)*	8 hours (15–30°C)	72 hours (2–8°C)
Hologic Aptima HIV-1 Quant Dx *(30,31)*	24 hours (2–30°C)	24 hours (2–30°C)
Qiagen artus HI Virus-1 RG *(32)*	6 hours (no temperature specified)	
Qiagen artus HI Virus-1 QS-RGQ *(33)*	6 hours (no temperature specified)	
Roche COBAS TaqMan HIV-1 *(34,35)*	24 hours (2–25°C)	24 hours (2–25°C)
Roche cobas HIV-1 for cobas 4800 System *(36)*	24 hours (2–25°C)	24 hours (2–25°C)
Roche cobas HIV-1 for cobas 6800/8800 Systems *(37)*	24 hours (2–25°C)	24 hours (2–25°C)
Sacace HIV Real-TM Quant Dx *(38)*	NR	12 hours (2–8°C)
Siemens VERSANT HIV-1 RNA 1.5 *(39)*	6 hours (15–25°C)	24 hours (2–8°C)

NR: not reported.

Once whole-blood specimens are separated into plasma, they can be frozen for long periods of time before testing. However, health-care facilities may lack centrifuges, freezers and/or the associated necessary skills to fully leverage these extended stability times after plasma separation.

Whole-blood stability for HIV viral load systematic review

A systematic review of nine studies entitled "Expanding access to HIV viral load testing: RNA stability in EDTA tubes and plasma preparation tubes beyond current time and temperature thresholds" was published in 2014 *(40)*.The systematic review highlighted three key findings.

- Whole blood and plasma were stable up to 168 hours after specimen collection when refrigerated.
- Whole blood was stable up to 72 hours after specimen collection when stored at 25°C.
- Plasma was stable up to 48 hours after specimen collection (plasma preparation tubes) or plasma separation (EDTA) when stored at 25°C.

Some important limitations to be considered, however, are that all studies included laboratory analyses rather than active realistic storage and transport times and temperatures and all were conducted in the United States or Europe. Further, only a few relevant studies were available for inclusion, and most had small sample sizes.

In addition, few studies included samples that had suppressed viral loads (<1000 copies/mL), making the results difficult to interpret within this range. However, a recently published study observed elevated viral load results of undetectable viral load specimens when plasma was stored beyond 72 hours *(41)*. Interestingly, 20% of the undetectable viral load results became low-level viraemic at any room temperature or refrigeration. Further, 51% of undetectable specimens became low-level viraemic if the plasma was not centrifuged again before testing after 48 hours of storage.

Conclusions

Fortunately, since the systematic review was published, several manufacturers have now lengthened their room temperature stability intended claims to allow for 24 hours from whole-blood specimen collection to plasma separation. Although the systematic review suggests that specimens are stable beyond the manufacturer intended claims, countries and laboratories would be responsible for viral load test results under such off-label conditions.

Additional research and manufacturer support to extending whole-blood stability intended use claims should be encouraged since broader specimen stability would support the expansion of viral load access using the preferred specimen type: plasma.

5. TECHNICAL BACKGROUND: NUCLEIC ACID–BASED TESTING

What is viral load testing?

HIV viral load testing is a way to measure the number of viruses present in a blood sample. Whole blood consists of cellular components (white blood cells, red blood cells and platelets) and cell-free plasma. Nucleic acid–based testing is done using a nucleic acid amplification test, which determines the number of copies of HIV per millilitre of plasma. Nucleic acid amplification tests work by amplifying either HIV genetic material or a probe that binds to HIV *(42)*. The test then uses a chemical reaction to measure the amount of amplification seen during the test, which corresponds to the quantity of HIV present in the sample. The most common type of viral load test is a quantitative polymerase chain reaction (qPCR). Other types of viral load testing include transcription-mediated amplification and branched DNA testing *(2)*.

Usefulness of viral load testing

- HIV viral load monitoring is important to ensure successful antiretroviral therapy. Viral load monitoring is the preferred approach to diagnose and confirm treatment failure *(2)*.
- Viral load testing provides clients with knowledge, control and motivation to understand their HIV infection and adhere to their treatment *(43)*.
- Having low or undetectable viral loads reduce the risk of disease progression and HIV transmission *(44,45)*.

HIV DNA versus HIV RNA

HIV is an RNA virus comprising RNA and proteins. During its replication cycle, the genetic material of HIV exists in both RNA and DNA forms.

HIV DNA is the genetic material of HIV that is found inside cells of the body infected by HIV. In whole blood, HIV DNA is mostly found inside white blood cells called CD4 cells, which are an important part of the immune system. HIV integrates its DNA into the DNA of the CD4 cells so it can use the cells to make more copies of itself. In this form, it is known as HIV proviral DNA *(46–48)*.

HIV RNA is most commonly found in plasma, which is the part of whole blood after removing all of the cells. Whole blood is typically separated into plasma and its cellular components by centrifuging the blood. HIV exists as an RNA virus in plasma before it infects cells, as intracellular RNA inside cells as copies of the virus are being made and in plasma again once these viral copies are released *(6–8)*.

When HIV is suppressed by antiretroviral therapy, HIV DNA remains present inside cells and occasionally as intracellular RNA, but little to no HIV RNA can be detected in plasma since the medicines prevent viral replication. However, when HIV is not suppressed, most of the HIV nucleic acid is typically present as HIV RNA in plasma, with additional intracellular RNA from active viral replication, and a smaller proportion present as HIV DNA inside cells *(7)*.

How does viral load testing work?

Nucleic acid testing for HIV can detect both HIV DNA and RNA that are present in a sample. Some assays have been designed to preferentially detect DNA or RNA, but since HIV DNA and RNA are copies of the same genetic material, they can also be hard to distinguish. However, viral load testing is designed to measure the amount of HIV RNA in plasma. Plasma is therefore the preferred sample type for viral load testing; however, alternative specimen types and technologies exist to support expanded access to viral load testing, including dried blood spots prepared using whole blood *(2,49)*. Dried blood spot specimens can allow for longer transport and storage times; however, using whole blood results often in detecting HIV proviral DNA, intracellular RNA and cell-free RNA. Together, this can result in excessive quantification of viral load results. Table 3 explains the differences between the two major components of whole blood (DNA and RNA) in HIV viral load testing.

Table 3. HIV DNA and RNA in whole blood

	Blood: cellular portion	Blood: plasma portion
HIV DNA and RNA	White blood cells (such as CD4 cells): HIV DNA is contained inside the cells along with copies of HIV that contain HIV RNA during HIV replication. HIV has also been found to be associated with platelets, most likely on the cell surface, although the virus is not found inside platelets or red blood cells.	HIV RNA is found in free virus in plasma. HIV DNA should not be present in any significant proportions, although small amounts of DNA may be found in plasma from cells that have broken open or if cells have carried over into the plasma from insufficient separation of whole blood.
Sample type	Whole blood contains both the cellular component of blood as well as the plasma. Whole blood contains both HIV DNA, intracellular RNA and cell-free RNA and has been used for early infant diagnosis of HIV and HIV drug resistance testing.	Plasma is the preferred sample type for viral load testing, which aims to detect the number of copies of HIV RNA per millilitre of plasma. Plasma can also be used for HIV drug resistance testing if adequate HIV RNA (>400 copies/mL) is present.
Testing methods	Whole blood is tested either in liquid form or from a dried blood spot. Viral load testing using whole blood may be inaccurate if significant quantities of HIV DNA and/or intracellular RNA are detected by the assay in addition to the cell-free (plasma) RNA the assay is designed to detect.	Plasma is typically tested in liquid form but can also be tested from a dried plasma spot.

Box 2. Timing versus technologies for diagnosing HIV among infants

The nucleic acid–based technique (qPCR) used for viral load testing is very similar, often the same, for infant testing or qualitative assays. HIV "DNA PCR" is a commonly used synonym for HIV infant diagnosis testing. However, it is important to distinguish between the technology used for testing (such as PCR versus an HIV antibody test) and the time frame of testing. Early infant diagnosis specifically refers to nucleic acid–based testing at birth or in the first two months of life, whereas infant diagnosis refers to testing during the exposure period including the nine-month nucleic acid test.

Infant diagnosis is often done on whole blood, either in liquid form or on a dried blood spot. These assays can detect HIV DNA, intracellular RNA and cell-free RNA. This is not a problem and will even improve the sensitivity of the assay, since the presence of any HIV genetic material in the blood can indicate HIV infection. Since both HIV DNA and RNA are present, virological testing or HIV nucleic acid amplification testing are more accurate terms for infant PCR testing than HIV DNA PCR.

Box 3. Key viral load terms

Suppressed viral load: viral load measurements below 1000 copies/mL. An unsuppressed or elevated viral load is a measurement above 1000 copies/mL *(2)*.

Undetectable viral load: the absence of any HIV found in a blood specimen by viral load testing. Table 4 shows the detection limits of commercially available viral load assays.

Table 4. Summary of HIV viral load assays

Manufacturer and test name	Sample type	Limit of detection (copies/mL)	Maximum time from whole-blood specimen collection to plasma separation	Regulatory approval	Early infant diagnosis testing
Abbott: RealTime HIV-1 *(20,21)*[1,2]	Plasma DBS	40 839	24 h at 15–30°C, 48 h at 2–8°C	CE, FDA, WHO CE, WHO	Available, separate test
m-PIMATM HIV-1/2 VL *(22,23)*	Plasma	800	48 h at 18–28°C	CE, WHO	Available, separate test
Biocentric GENERIC HIV Charge Virale *(24)*	Plasma	390	24 h at 2–25°C	CE	Available, separate test
bioMérieux NucliSENS EasyQ® HIV-1 v2.0 *(25,26)*	Plasma DBS	25 802	24 h at 2–8°C	CE, WHO CE, WHO	N/A
Cavidi ExaVirTM Load *(27)*	Plasma	200	4–6 h, no temperature specified	CE	N/A
Cepheid Xpert® HIV-1 Viral Load *(28,29)*	Plasma	40	8 h at 15–30°C, 24 h at 15–25°C, 72 h at 2–8°C	CE, WHO	Available, separate test
Hologic Aptima™ HIV-1 Quant Dx *(30,31)*	Plasma	30	24 h at 2–30°C	CE, FDA, WHO	Same Test
Qiagen: *artus*® HI Virus-1 RG *(32)*	Plasma	60	6 h, no temperature specified	CE	N/A
artus® HI Virus-1 QS-RGQ *(33)*	Plasma	45	6 h, no temperature specified	CE	N/A
Roche: COBAS® AmpliPREP/COBAS® TaqMan® HIV-1 Test, v2.0 *(34,35)*	Plasma PSC	20 738	24 h at 2–25°C	CE, FDA, WHO CE, WHO	Available, Separate Test
cobas® HIV-1 for cobas® 4800 System *(36)*	Plasma PSC	20 599	24 h at 2–25°C	CE	Same Test
cobas® HIV-1 for cobas® 6800/8800 Systems *(37)*	Plasma PSC	13.2 790	24h at 2-25°C	CE, FDA CE	Available, Separate Test
Sacace HIV Real-TM Quant DX *(38)*	Plasma	48 IU/mL	12 h at 2–8°C	CE	N/A
Siemens VERSANT® HIV-1 RNA 1.5 *(39)*	Plasma	37	6 h at 15–25°C, 24 h at 2–8°C	CE	N/A

h: hours; CE: Conformité Européenne, conforming to European Union regulations; FDA: United States Food and Drug Administration approval; WHO: WHO prequalification of in vitro diagnostics; DBS: dried blood spot; PSC: dried plasma spot from a plasma separation card; N/A: not currently available; IU: international units.

[1] Abbott Laboratories (2014). Abbott RealTi*me* HIV-1 Instructions for Use.

2 WHO Prequalification of Diagnostics Programme (2016). Public Report: Abbott RealTi*me* HIV-1. Available at https://www.who.int/diagnostics_laboratory/evaluations/pq-list/hiv-vrl/180423_amended_final_pqpr_0145_027_00_v11.pdf?ua=1

6. ALTERNATIVE SPECIMEN TYPES AND TECHNOLOGIES FOR CONSIDERATION WHEN LIQUID PLASMA CANNOT BE USED WIDELY FOR VIRAL LOAD TESTING BECAUSE OF INFRASTRUCTURE, TRANSPORT OR OTHER CONSTRAINTS

6.1 ALTERNATIVE SPECIMEN TYPES AND TECHNOLOGIES: DRIED BLOOD SPOT SPECIMENS FOR HIV VIRAL LOAD TESTING

Although plasma specimens are the standard for viral load testing, their use is restricted by the limited ambient temperature stability of viral biomarkers in whole blood and plasma during storage and transport and the limited cold-chain availability between many health-care facilities in resource-limited settings. Dried blood spot specimens for HIV testing are well established in resource-limited settings and have been routinely used for collecting and shipping infant HIV diagnosis specimens for testing by PCR in centralized laboratories. They are beneficial since they do not require centrifuges, refrigerators or freezers at the specimen collection site, can be stored and transported for weeks at ambient temperature and require a simple finger-prick or heel-stick blood specimen that can be prepared by lower cadres of health-care facility staff. Similar benefits could be achieved by using dried blood spot specimens for viral load testing programmes in resource-limited settings. The required storage and shipping conditions may differ when dried blood spot specimens are used for drug resistance testing.

Dried blood spot specimens for viral load testing using nucleic acid–based detection methods use whole blood as the input specimen, which can result in extraction and

detection of proviral DNA and intracellular RNA in addition to the primary biomarker target of free viral RNA circulating in the plasma. Together, this may result in excessive quantification of the viral load result.

Limited progress has been made in ensuring the quality of using dried blood spot specimens for HIV viral load testing through international regulatory approval.

Dried blood spot specimen regulatory approvals and technical evaluations (countries often consider these approvals when procuring or selecting diagnostic technologies):

- **CE-IVD (Conformité Européenne in vitro diagnostics):** two technologies have received CE-IVD for using dried blood spot specimens for viral load testing: Abbott RealTime HIV-1 and bioMérieux NucliSENS EasyQ® HIV-1; and
- **WHO prequalification:** two technologies have met WHO requirements: bioMérieux NucliSENS EasyQ® HIV-1 in January 2017 and Abbott RealTime HIV-1 *(21)* on 24 August 2017.

The limit of detection of the Abbott RealTime HIV-1 assay using dried blood spot specimens is 839 copies/mL *(21)*.

Independent technical evaluations: the results from 40 technical evaluations of dried blood spot specimens across over 25 countries examining six commercially available viral load testing technologies were included in a comprehensive clinical meta-analysis, which resulted in more than 10 000 paired dried blood spot–plasma data points (Table 5) *(50)*.

WHO recommendations

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend that dried blood spot specimens using venous or capillary whole blood can be used to determine the HIV viral load. A threshold of 1000 copies/mL should be used to determine treatment failure when using dried blood spot specimens, as defined for testing in plasma. Although plasma specimens are preferred for viral load testing, dried blood spot specimens are recommended for use in settings where logistical, infrastructural or operational barriers prevent routine viral load monitoring using plasma specimens.

Current use

Dried blood spot specimens provide a way to improve the coverage and reach of viral load testing where the preparation and transport of plasma specimens may be limited by cold-chain requirements or transport challenges. Several countries are currently implementing dried blood spot specimens to support viral load access and scale-up. In 2018, more than 2 million viral load tests were run using dried blood spot specimens across six countries with a high burden of HIV infection. Further, some countries have begun implementing the use of DBS specimens for viral load testing using protocols recommended by manufacturers despite its off-label use.

Table 5. Summarized results from technical evaluation meta-analysis

Assay	Sample size	Sensitivity (95% CI)[a]	Specificity (95% CI)[a]
Abbott RealTime HIV-1, one-spot[b]	700	88.26% (49.64–98.28)	99.07% (68.38–99.98)
Abbott RealTime HIV-1, two-spot	2004	93.13% (83.72–97.27)	91.11% (82.35–95.75)
Biocentric Generic HIV Charge Virale	531	94.86% (71.14–99.28)	55.16% (35.01–73.75)
bioMérieux NucliSENS EasyQ® HIV-1	1062	82.95% (78.38–86.71)	95.06% (89.29–97.80)
Hologic Aptima	382	87.52% (77.93–93.30)	87.18% (59.01–96.98)
Roche COBAS TaqMan HIV-1 Free Virus Elution	3076	94.77% (84.59–98.36)	93.93% (71.95–98.94)
Roche COBAS TaqMan HIV-1 SPEX	3190	98.23% (95.85–99.26)	48.49% (22.63–75.18)
Siemens VERSANT HIV-1 RNA	144	90.97% (69.20–97.83)	87.76% (75.28–94.41)

[a] Sensitivity and specificity using a treatment failure threshold of 1000 copies/mL.
[b] As a change notification, a laboratory evaluation of dried blood spot specimens using the CE-marked protocol was not conducted within WHO prequalification review.

Conclusions

Sufficient evidence has been generated on the performance of dried blood spot specimens for viral load testing to support rapid national regulatory approval and initiation of scale-up. Further technical evaluations of these technologies are unlikely to add value but may instead delay implementation and timely treatment monitoring. However, it is essential that suppliers seek regulatory approval and WHO prequalification of such alternative specimen types to support country scale-up and access to viral load testing.

6.2 ALTERNATIVE SPECIMEN TYPES AND TECHNOLOGIES: DRIED PLASMA SPOT SPECIMENS FOR HIV VIRAL LOAD TESTING

An additional alternative to using liquid plasma for viral load testing is dried plasma spot specimens. These specimens use the same or similar filter paper as dried blood spot specimens for viral load or infant diagnosis; however, with the application of plasma instead of whole blood. Plasma separation cards and simple devices are also currently in development or recently available on the market to support the expansion of viral load testing using plasma specimens.

Dried plasma spot specimens for HIV testing are an alternative specimen type developed similarly to the well-established dried blood spot specimens (subsection 6.1) that have been routinely used for collecting and shipping infant HIV diagnosis specimens for testing by PCR in centralized laboratories. Although they require centrifugation or collection of plasma for spotting on the card, they can be stored and transported for weeks at ambient temperature.

An advantage of dried plasma spot specimens is that plasma separation and use removes the detection and quantification of intracellular RNA and proviral DNA often observed with whole-blood specimens; however, the smaller input specimen volume may limit the perfect comparability with liquid plasma specimens.

Typically, plasma prepared for dried plasma spot specimens or plasma separation cards or devices is derived from whole blood taken in EDTA tubes or plasma preparation tubes (see subsection 6.3). Manufacturers should, therefore, include one or both tubes types in their intended use claims and regulatory approval documentation. Most viral load assays currently on the market include one or both tube types.

Independent technical evaluations: the results from 17 technical evaluations across 12 countries and looking at four commercially available technologies were included in a comprehensive meta-analysis, which resulted in nearly 2000 paired dried plasma spot–plasma data points (Table 6) *(50)*.

The performance of dried plasma spot specimens across all technologies was comparable to using traditional liquid plasma. As expected, since the input specimen type, plasma, was used, limited upward and downward misclassification was observed.

WHO recommendations

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend viral load as the preferred monitoring approach to diagnose and confirm treatment failure and prefer plasma specimens for viral load testing. A threshold of 1000 copies/mL can be used to determine treatment failure when using any specimens, including dried plasma spot specimens, as defined for testing in plasma.

Current use

Dried plasma spot specimens provide a way to improve the coverage and reach of viral load testing, where storage and transport of liquid plasma specimens may be limited by cold-chain requirements or transport challenges. However, preparation of dried plasma spot specimens requires centrifugation to separate plasma from whole blood. This can be done either at the point of specimen collection, if feasible, or within a few hours of specimen collection, depending on manufacturer guidelines, by a hub or regional laboratory.

Table 6. Summarized results from technical evaluation meta-analysis

Assay	Sample size	Sensitivity (95% CI)[a]	Specificity (95% CI)[a]
All technologies	1872	92.54% (87.85–95.52%)	95.15% (87.41–98.23%)
Abbott RealTi*m*e HIV-1	245	99.39% (95.78–99.91%)	85.37% (75.97–91.50%)
Biocentric Generic HIV Charge Virale	148	98.12% (56.78–99.95%)	75.00% (46.90–91.06%)
bioMérieux NucliSENS EasyQ® HIV-1	173	77.78% (53.53–91.40%)	99.35% (95.57–99.91%)
Roche COBAS TaqMan HIV-1	1077	93.05% (87.75–96.16%)	94.90% (78.59–98.95%)

[a] Sensitivity and specificity using a treatment failure threshold of 1000 copies/mL.

Conclusions

Sufficient evidence has been generated on the performance of dried plasma spot specimens for viral load testing to support the initiation of scale-up, if desired within national operational plans to support country scale-up and access to viral load testing. Further technical evaluations of these technologies are unlikely to add value but may instead delay implementation and timely treatment monitoring. However, information focusing on the feasibility and operational best practices of using dried plasma spot specimens within viral load scale-up plans has been limited.

6.3 ALTERNATIVE SPECIMEN TYPES AND TECHNOLOGIES: PLASMA PREPARATION TUBES FOR HIV VIRAL LOAD TESTING

The gold standard plasma specimen for viral load testing is generally collected using whole blood in an EDTA (ethylenediaminetetraacetic acid anti-coagulant) tube (purple or lavender cap). As highlighted in Section 4, whole blood in EDTA tubes must be transported and plasma separated within 6–24 hours, depending on the manufacturer. This can be restrictive for many countries and health-care facilities. However, some alternative plasma specimens can be considered. Plasma preparation tubes as well as plasma collected on cards, such as dried plasma spots (subsection 6.2) and plasma separation cards can also be considered to support scale-up.

Unlike standard EDTA blood collection tubes, plasma preparation tubes can facilitate simpler handling and storage of plasma for nucleic acid–based testing. Plasma preparation tubes use the same EDTA anticoagulant but contain a gel that separates the plasma from blood cells after centrifugation. After the blood is collected, the plasma preparation tube is spun in a centrifuge within 24 hours and a gel barrier inside the plasma preparation tube separates the plasma from the rest of the whole blood so the plasma can be used for HIV viral load testing. The same plasma specimen volume is used for the viral load assay; therefore, limits of detection are generally synonymous with EDTA plasma.

Plasma preparation tube regulatory approvals:

- **CE-IVD (Conformité Européenne in vitro diagnostics):** seven technologies have received CE-IVD for using plasma preparation tubes for viral load testing: Abbott RealTi*m*e HIV-1, Cepheid Xpert® HIV-1 Viral Load, Hologic AptimaTM HIV-1 Quant Dx, Roche COBAS® AmpliPREP/COBAS® TaqMan® HIV-1 Test, v2.0, Roche cobas® HIV-1 for cobas® 4800, Roche cobas® HIV-1 for cobas® 6800/8800 and Siemens VERSANT® HIV-1 RNA 1.5.
- **WHO prequalification:** four technologies have met WHO requirements: Abbott RealTi*m*e HIV-1, Cepheid Xpert® HIV-1 Viral Load, Hologic AptimaTM HIV-1 Quant Dx and Roche COBAS® AmpliPREP/COBAS® TaqMan® HIV-1 Test, v2.0.
- **FDA (United States Food and Drug Administration):** four technologies have received FDA approval for using plasma preparation tubes for viral load testing: Abbott RealTi*m*e HIV-1, Hologic AptimaTM HIV-1 Quant Dx, Roche COBAS® AmpliPREP/COBAS® TaqMan® HIV-1 Test, v2.0 and Roche cobas® HIV-1 for cobas® 6800/8800.

Systematic review and best practices: a systematic review was conducted to examine the accuracy of plasma preparation tubes for HIV viral load testing. The review identified 16 peer-reviewed published studies from 1995 to 2014 that compared plasma preparation tubes to standard EDTA blood collection tubes on HIV viral load assays approved by a stringent regulatory authority.

Although the earliest studies demonstrated that plasma preparation tubes could be used with no significant differences in viral load results *(51–54)*, later studies demonstrated elevated viral loads from plasma preparation tubes, especially at viral loads less than 5000 copies/mL *(55–57)*. The increase in viral load results likely resulted from the leakage of HIV nucleic acids, such as proviral HIV DNA and intracellular RNA present in the cellular component of whole blood, which moved back through the gel barrier into the plasma. Additional studies found that this issue could be resolved by either aliquoting the plasma into a second tube quickly after the initial centrifugation *(58–60)* or repeating centrifugation after transport of the plasma preparation tubes to the laboratory before aliquoting and testing *(61,62)*.

Four published studies evaluated plasma preparation tubes on currently available viral load assays (Abbott RealTi*m*e HIV-1 and Roche COBAS AmpliPREP/COBAS TaqMan HIV-1 Test, v2.0) *(63–66)*. The three studies using the Abbott viral load assay showed no significant change in viral load results regardless of whether the plasma preparation tubes were frozen and thawed or transported after initial centrifugation and before testing. The three studies using a Roche assay found elevated viral load results if the plasma preparation tubes were frozen or transported without a second centrifugation before testing. These viral load results were found to be between zero and several thousand copies/mL higher than plasma prepared from a standard EDTA collection tube, with the difference being most noticeable for plasma viral loads less than 1000 copies/mL. Consequently, manufacturer instructions recommend an additional centrifugation step before testing using the Roche assay. For both the Abbott and Roche assays, aliquoting the plasma into a secondary tube after initial centrifugation also ensured accurate viral load results (Table 7).

Table 7. Published handling methods for commercially available plasma preparation tubes and viral load assays

Product	Published plasma preparation tube handling methods providing accurate viral load results
Abbott RealTime HIV-1 *(63–65)*	• Aliquoting plasma into new tube after initial centrifugation • Freezing plasma preparation tubes at –20°C after initial centrifugation and thawing before testing, without the necessity for another centrifugation step • Transporting plasma preparation tubes after initial centrifugation between sites before testing, without the necessity for another centrifugation step
Roche COBAS® AmpliPREP/COBAS® TaqMan® HIV-1 Test, v2.0 *(64–66)*	• Aliquoting plasma into new tube after initial centrifugation • Repeat centrifugation after transport or freezing of plasma preparation tubes to ensure complete the separation of the cellular and plasma components of blood before testing Note: In the absence of repeat centrifugation after freezing and thawing of plasma preparation tubes or after transport of plasma preparation tubes, some viral load results were observed to be erroneously high. Repeat centrifugation is not necessary if the plasma has already been aliquoted into a new tube before freezing or transport.
BD Vacutainer® PPTTM *(67)*	• Centrifuge for at least 10 minutes at 1100 × g at room temperature, within 6 hours of collecting whole blood to prepare plasma. • Follow assay manufacturer instructions for storage and transport: typically plasma preparation tubes can be stored at ambient temperature for one day or refrigerated at 4°C for up to five days; if longer storage is desired, the plasma should be frozen.

All studies evaluated only BD Vacutainer® PPTTM. Additional plasma preparation tubes exist (also referred to as EDTA with gel separate tubes: Grenier *(68)* or TUD *(69)*); however, no studies have been published. Further, other regulatory-approved and/or WHO-prequalified viral load assays (such as the Cepheid Xpert HIV-1 and Hologic Aptima HIV-1 Quant Dx assay) that include plasma preparation tubes in their instructions for use do not provide any further specific guidance on how they should be used (Table 8).

Conclusions

Plasma preparation tubes allow plasma to be prepared, stored and transported in the same tube used to collect venous whole blood. Plasma preparation tubes provide equivalent viral load results to plasma from standard EDTA tubes if their proper handling is followed according to manufacturer instructions and guidance from independently published studies. Centrifugation of plasma preparation tubes and/or aliquoting of plasma into a separate tube before viral load testing has been shown to prevent spuriously elevated viral load results. However, not all viral load assays have clear instructions or peer-reviewed evaluations published on their use of plasma preparation tubes, and centrifuges (and the associated skills) are necessary at the point of specimen collection.

Plasma preparation tubes may be worth considering in settings in which simpler plasma preparation, reduced cross-contamination risk, and the need for longer sample transport times can facilitate the scaling up of viral load testing.

Table 8. Advantages and challenges associated with plasma preparation tubes

Advantages	Challenges
• Fewer manual sampling handling steps than standard EDTA tubes • Reduced risk of sample contamination and laboratory errors if plasma is not aliquoted into a new tube • Ability to store plasma for longer periods of time than uncentrifuged whole blood, which can facilitate longer transport times to the laboratory	• Higher cost of plasma preparation tubes than standard EDTA tubes • Programmatic complexities involving supply chain logistics, staff training and proper implementation of plasma preparation tubes • Centrifuges are required on-site for immediate plasma separation • Primary tube sampling is not always possible • Inaccurate viral load results may be seen if manufacturer-specific instructions are not followed: for example, a repeat centrifugation step may be necessary before testing • Sample bundling currently unavailable

6.4 ALTERNATIVE SPECIMEN TYPES AND TECHNOLOGIES: POINT-OF-CARE AND NEAR-POINT-OF-CARE TOOLS FOR HIV VIRAL LOAD TESTING

Technologies developed for use at or near the point of care may also be considered for viral load testing. These technologies can be decentralized and used at the point of care. Point-of-care technologies do not require consistent electricity, temperature-controlled rooms or routine calibration, are relatively easy to use, are automated, have no or minimal third-party commodity requirements and can be operated by non-laboratory professionals. Near-point-of-care technologies are similar but may require the use of consistent electricity and/or temperature-controlled rooms. Further, most technologies currently available require plasma specimens.

Significant progress has been made in ensuring the quality of new point-of-care viral load technologies.

Point-of-care and near-point-of-care viral load regulatory approvals and technical evaluations (countries often consider these approvals when procuring or selecting diagnostic technologies):

- **CE-IVD (Conformité Européenne in vitro diagnostics):** four technologies have received CE-IVD: Abbott™ m-PIMA HIV-1/2 VL, Cepheid Xpert® HIV-1 Viral Load and Diagnostics for the Real World's SAMBA I HIV-1 Semi-Quantitative Plasma Test and SAMBA II HIV-1 Semi-Quantitative Plasma Test; and
- **WHO prequalification:** two technologies have met WHO requirements: Abbott™ m-PIMA HIV-1/2 VL *(23)* and Cepheid Xpert® HIV-1 Viral Load *(29)*[1] received WHO prequalification on 8 April 2019 and 20 July 2017, respectively.

The Abbott™ m-PIMA HIV-1/2 VL assay requires 50 µl of venous EDTA plasma and can detect HIV-1 groups M, N, and O and HIV-2. The limit of detection is 800 copies/mL *(23)*.

The Cepheid Xpert® HIV-1 Viral Load assay requires 1 mL of plasma (can be derived from ACD, EDTA or PPT-EDTA blood specimen tubes) and can detect HIV-1 groups M, N, and O. The limit of detection is 40 copies/mL *(29)*.

Additional specifications of these and products in development are available *(10,70)*.

Independent technical evaluations: the results from 13 technical field evaluations of the Cepheid Xpert® HIV-1 Viral Load assay were consolidated across 11 countries into a meta-analysis (Table 9) *(71)*.

[1] The Cepheid Xpert® HIV-1 Viral Load assay can be used with a variety of Xpert devices at or near the point of care, from the 1-module EDGE to the 16-module Xpert.

Table 9. Summarized results from WHO prequalification and independent technical evaluations

Assay	Evaluator	Sample type	Sample size	Sensitivity (95% CI)[a]	Specificity (95% CI)[a]
Abbott™ m-PIMA HIV-1/2 VL[b]	WHO prequalification/ United States Centers for Disease Control and Prevention	Plasma	421	95.1% (91.7–97.5%) *(23)*	99.4% (96.8–99.9%) *(23)*
Cepheid Xpert® HIV-1 Viral Load	WHO prequalification/ United States Centers for Disease Control and Prevention	Plasma	439	94.14% (90.37–96.76%) *(29)*	98.50% (95.68–99.69%) *(29)*
	Meta-analysis	Plasma	3790	96.47% (95.10–97.47%) *(72)*	96.59% (92.90–98.39%) *(72)*

[a] Sensitivity and specificity using a treatment failure threshold of 1000 copies/mL.
[b] No meta-analysis has yet been prepared because of a lack of published independent technical evaluations.

Considerations

As of 2019, WHO does not have a recommendation for the consideration of point-of-care viral load technologies; however, this will be reviewed in 2020. Considering some of the challenges in scaling up viral load testing, both clinically and logistically, point-of-care viral load testing may support broader access to viral load, deliver results to clinicians and patients more quickly and accelerate decision-making through same-day testing.

Box 4. Setting priorities for viral load testing

Several population groups could be considered and given priority for point-of-care viral load testing when overall volumes may overwhelm such technologies.

- Pregnant and breastfeeding women, especially around the time of delivery, may benefit from faster result delivery and clinical decision-making to prevent mother-to-child transmission.
- Infants and other children living with HIV, who typically are at higher risk of treatment failure and drug resistance because of exposure to maternal antiretroviral therapy and postnatal prophylaxis, may benefit from more rapid delivery of results and more attentive treatment monitoring.
- Further, people re-entering care, those who for whom treatment failure is suspected and those with advanced HIV disease may benefit from more rapid delivery of results and clinical decision-making.

In addition, several point-of-care technologies are also polyvalent or multi-disease technologies capable of testing different conditions using disease-specific tests on the same platform. Significant existing device footprint may allow for programmatic and diagnostic integration to expand access to viral load testing *(73).*

Conclusions

Sufficient evidence has been generated on the performance of some point-of-care viral load assays to support rapid national regulatory approval and the initiation of scale-up. Further technical evaluations of these technologies are unlikely to add value but may instead delay implementation.

Studies of the impact on patient management and care, operational feasibility, acceptability and cost–effectiveness are ongoing. However, countries need to individually determine the contextual importance, utility and range of point-of-care viral load assays within their patient care and diagnostic networks.

7. OPERATIONAL INTERVENTIONS AND CONSIDERATIONS IN SCALING UP VIRAL LOAD TESTING AND INFANT DIAGNOSIS

7.1 OPTIONS FOR TRANSPORTING SPECIMENS FOR NUCLEIC ACID–BASED DIAGNOSTICS

Laboratories and testing capacity within a diagnostics network are not present onsite at every health-care facility patients attend. The testing and analysis usually offered at centralized laboratories are critical for managing people living with HIV, such as providing viral load testing and infant diagnosis of HIV, but accessing these services can be a challenge. Testing at or closer to the point of care is one solution to address the limitations of the laboratory network (see subsection 6.4), including providing same-day results. However, point-of-care testing is not available at all facilities or may not be cost-effective at health-care facilities with low patient volumes.

When testing is not available on site, specimen referral systems can provide access to the diagnostics network by moving specimens from the collection facility (also known as the referring facility) to a facility with the necessary capacity (the testing or referral laboratory). Alternative specimen types, such as dried blood spot specimens, can also be used to further increase access. Moving the specimen removes the burden of people living with HIV having to travel to the laboratory for testing. In this way, the specimen referral network extends the reach and coverage of the diagnostics network. The same system for referring specimens is also often used for returning paper results, which may be sent even if electronic results are available.

Various specimen referral systems can be found at different levels of a tiered health system, in different regions of a country and across disease programmes. Together, these systems should be harmonized, connected and efficiently coordinated to form the overall specimen referral network, which, in turn, is a vital part of a diagnostics network. A specimen referral system or network has five main goals (Box 5).

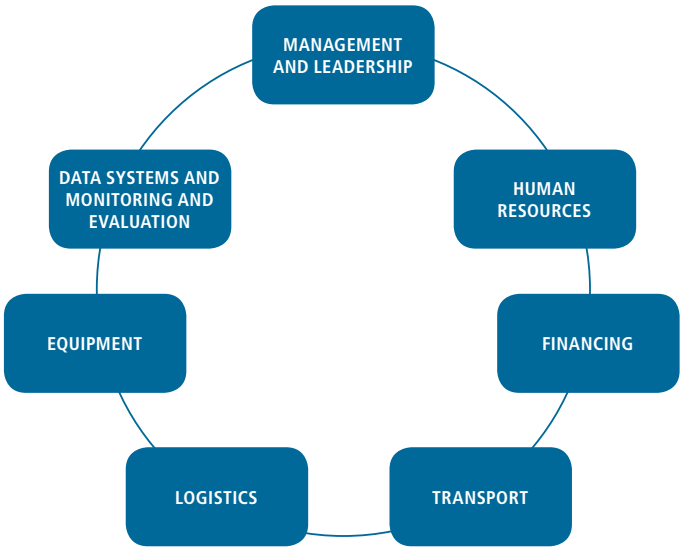
A specimen referral system comprises various components that are critical to ensure a successful and efficient system (Fig. 8):

- management and leadership – ideally, someone in the health ministry should supervise the overall referral

Box 5. Five main goals of a specimen referral system

- Contribute to increased access to diagnostics where on-site services do not exist by referring the specimen to the testing laboratory
- Maintain and improve the quality of specimens delivered to the testing laboratory by proper specimen management in transit, including cold-chain requirements
- Ensure the safety and security of all individuals and the environment involved with specimen referrals by proper management in transit, including packaging and handling
- Meet the timeliness requirements of the specimen reaching the testing laboratory and the paper result reaching the facility, clinician, patient and necessary files
- Enhance the cost-efficiency of the diagnostic network through harmonization and coordination

Fig. 8. Components to ensure a successful and efficient specimen referral system



network, ensure that it is supporting the needs of the diagnostics network and advocate for necessary resources throughout the network;

- human resources – these are the personnel at the referring facilities, referral laboratories, regional health teams, transporters, etc. that are involved in the entire referral process and returning results;

- financing – the funding that is necessary for the overall referral network, incorporating all aspects of the network as well as specimen types and disease areas;

- transport – this includes the type of vehicle (motorcycle or four-wheeled vehicle) and service provider (professional courier or clinical implementing partner) and ideal combinations thereof to service all facilities as necessary;

- logistics – this includes the overall logistical system, such as scheduling and routing and depends on many factors, such as timeliness requirements for managing specimens and returning the results;

- equipment – such as necessary packaging materials, contributes to specimen quality and biosafety during referrals; and

- data systems and monitoring and evaluation – the system to collect data, analyse them and use the analysis for decision-making and continual quality improvement.

Three key components of the specimen referral system can be challenging and may require additional consideration and focus: transport, logistics and data systems and monitoring and evaluation.

Design of transport and logistics systems is closely related and may or may not be managed by the same organization or company. Although these components are only two in the overall system, they require additional technical expertise that is usually not a core capacity of health-care facility or laboratory staff. Three considerations for these important components include:

- **Type of vehicle.** The type of vehicles used depends on resources, distances, terrain and carrying capacity. If the transport and logistics systems are outsourced, the service provider will likely decide the vehicle type. Examples include (listed by highest to lowest prevalence): motorcycles, four-wheeled vehicles, bicycles, boats, horses, on foot, airplanes and unmanned aerial vehicles (also known as drones). Key considerations when choosing a vehicle type include:
  - specimen types (dried blood spot specimens, whole blood, plasma, etc.) and requirements per test (cold chain or specimens tested for highly contagious diseases may require additional packaging, which may not fit on or be suitable for certain vehicle types such as drones);
  - level or tier of system used, distance travelled and type of terrain covered; and
  - demand and volumes at the referring facilities to understand the carrying capacity required.

- **Transport service provider.** The service provider, which could be the health ministry, an implementing partner or a private company, is the one who operates the transport and usually employs the vehicle operators. When choosing a service provider, key considerations include:
  - paying the provider and the sustainability of the system;
  - the availability of local private sector transporters or third-party logistics providers, such as Riders for Health, DHL, G4Sor the national postal service, and the ability to contract with a third party logistics provider;
  - which entity will manage, own and operate the vehicles: health-care facility ownership versus provided to the facility through the health ministry or vehicles belonging to the government, partners or a private company;
  - which entity will manage and employ the vehicle operators (rider, driver, etc.);
  - specimens accompanies by a person during transit; and
  - dedicating the system solely to transporting specimens and results: vehicles such as ambulances, whose primary purpose is not specimen transport, should not be the only mode of transport of specimens available and used.

- **Logistics, scheduling and routing.** At its core, a specimen referral system is a logistics system. Specimens need to be moved physically from collection points to first-line diagnostic testing sites or hubs and then possibly specialized testing sites and the results returned in reverse. Key logistics considerations include the following.
  - Health facilities, collection points, hubs and referral laboratories should be mapped using geocodes. Then current referral needs, links and pathways for each specimen type should be mapped out, based on testing algorithms and capacity and between each relevant level or tier of the health system, including the community or health post level, if considered. The mapping exercise should be redone when the overall diagnostics network changes, such as further decentralization of equipment or integration.
  - Consider whether to have a fixed schedule for pick-ups and returning results versus on-demand services.
  - Frequencies of pick-up should be based on patient volumes and need, specimen collection, specimen type, specimen stability and machine capability at the testing laboratory. For example, whole-blood and plasma specimens require same-day, rapid transport and storage at the correct temperature.

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Box 6. Considerations for data systems and monitoring and evaluation

This is a critical component to the systems and network but often weak and overlooked. A standardized monitoring and evaluation framework for specimen transport is required to assess and compare the performance of the often fragmented systems. Key considerations for this component include the following.

- The monitoring and evaluation framework and standardized indicators should be based on the five goals of a specimen referral system (Box 5) and included in the national specimen referral guidelines.
- Data collection tools should be in place or introduced, including registers and logbooks, chain-of-custody forms (showing every time a specimen or result changes hands), transport logs, reporting forms, questions within supervisory checklists, etc.
- Indicators may be aspirational, but once the specimen transport network and necessary data systems are in place, the feasibility of collecting each should be assessed.
- Reporting processes should be outlined and feedback mechanisms used.
- Detailed turnaround time is important to collect, including each step between the collection of the specimen from the patient to the time the result is filed in the patient's records.
- Continual quality improvement should be emphasized, including corrective actions.

  - In a hub-and-spoke system, the hub may be a testing facility and/or a facility to consolidate and store specimens on the way to a higher level versus point to point, in which the specimen goes directly from the referring facility to the testing laboratory without consolidation at a hub on the way. For viral load testing using plasma specimens, a hub system should be set up in which each hub is equipped with refrigerators, freezers and centrifuges to process the specimens and ensure the integrity of the specimens.
  - Consider the ability of the referring facility or hub to prepare and store specimens.
  - Consider the ability to reconsider administrative boundaries if it is more logistically efficient: whether a specimen can be referred to a laboratory in a different administrative region if it is closer than the pre-assigned laboratory.
  - Can the system be integrated with other types of specimens?
  - Is the delivery of paper results included, if necessary?
  - What are the cut-off times for specimen reception (time by which specimens need to reach the hub for further processing or storage or laboratory) and the earliest arrival and pick-up time at the referring facilities?
  - For shared (non-dedicated) vehicles, schedules need to be carefully planned to not disrupt other activities.

Best practices. Although there are many ways to design, implement and monitor a specimen referral system, countries are currently adopting key best practices, including the following.

- Management: the health ministry must lead, coordinate and supervise the overall specimen referral network, regardless of the transport mechanism used or funding.
- National guidelines are developed for specimen referrals as well as a laboratory handbook, which describes individual procedures for collection, packaging, storage and transport depending on the specimen type and test requested.
- Monitoring: a robust monitoring and evaluation framework should include standardized indicators.
- Network approach: the design of the specimen referral network must work within the diagnostics network and be optimized periodically to improve efficiency and costs.
- Specimen types should be integrated with disease programme activities, where this is possible and logistically efficient.
- Transport and referral procedures must be well documented for each specimen type and all personnel at all levels properly trained, including: specimen collection, storage, documentation, packaging and dispatch, transport, specimen receipt, results dispatch and results receipt.
- Biosafety and quality: provide appropriate personal protective equipment, spill kits and proper packaging materials, including safe and secure shipping containers as necessary for each specimen type.

Integration. The diagnostics network can be integrated to use a specimen referral system and network for multiple specimen types or disease programmes. In this case, usually integration is easiest and most efficient from the referring facility to the first hub or first-line diagnostic location. The overall national specimen referral network should always be fully integrated, meaning that it should cater for all specimen types and diseases. However, the transport and logistics necessary to achieve this integration may require incorporating some separate systems for certain tests or specimens based on routing and/or laboratory locations and specimen management requirements. For example, the nucleic acid–based laboratory for HIV testing may differ from the tuberculosis culture laboratory, and so separate routes and logistics may be necessary for specific aspects of the specimen transport networks. Further, depending on the specimen type and other factors, the transport mechanisms used on each route may differ. For example, for specimens that require very timely, same-day transport, or require temperature controls, public transport with no control over timeliness or temperatures may not be appropriate. If there is an outbreak investigation, the specimens may not be able to wait for and use the routine transport mechanism.

Transport and logistics service provider options. The national specimen referral network will likely include a combination of the options listed in Table 10 depending on the level of the health system and local geography of a region.

Box 7. Links to tools and resources for specimen referrals

- Global Laboratory Initiative (GLI) Specimen Referral Toolkit. Geneva: Stop TB Partnership; 2019 (http://www.stoptb.org/wg/gli/srt.asp).
- GLI guide to TB specimen referral systems and integrated networks. Geneva: Stop TB Partnership; 2019 (http://www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf).
- Guidance for developing a specimen transport and referral system for viral load and infant virologic HIV diagnosis testing networks. Addis Ababa: African Society for Laboratory Medicine; 2015 (http://www.aslm.org/?wpdmdl=18275).

Table 10. Options for selecting transport and logistics service providers

	Type or example	Benefits	Challenges	Best-use case
Self-run – operated by the health ministry directly or a clinical implementing partner; all can easily carry results or other supplies for no additional cost	Dedicated health ministry courier system	Likely share existing health ministry resources, such as staffing, to run and manage the system to save on the overall costs required	Transport and logistics expertise is generally not a core competency within the health ministry	Use in countries with high referral volumes where outsourcing is difficult and health ministry capacity to manage a complex transport and logistics network is high
	Dedicated partner-run courier system	Will share some existing partner resources, such as staffing, to run and manage the system to save on the overall costs required	Transport and logistics expertise is generally not a core competency – to run these systems, additional staff must be hired just for this one system, which is not cost-effective	Use in countries with high referral volumes where outsourcing is difficult and health ministry capacity to manage a complex transport and logistics network is low
	Hand-carried by facility staff	Often carried out by laboratory staff so biosafety and quality control are well understood	Takes limited staff out of the health facility and away from their main responsibilities; more expensive than sending a package on its own	Use where specimen referral volumes are very low and erratic
	Use of non-dedicated health ministry vehicles	Used by programme officials to conduct supervisory visits and to deliver supplies and commodities. Some programmes have also used the vehicles to transport specimens and results	Often do not visit the collection sites frequently enough for timely transport; with shared priorities, specimens are not always transported in a timely and quality-controlled manner; the use of ambulances is not recommended, since this form of transport is unpredictable and interferes with regular duties	Use for health posts or facilities that only collect specimens when an outreach health team is visiting, since they can bring back the specimens with them to the laboratory
	Use of public transport, not accompanied (such as buses, trains, boat and aircraft)	Play a major role in both rural and urban transport with extensive nationwide access and coverage; used by private courier companies and national postal systems to send letters, packages and money; less expensive to send a package unaccompanied than with a facility staff member	Usually have to bring packages to depot; special permission may be needed to transport potentially infectious material; schedules may not be adhered to strictly; specimens and test results may not be properly handled due to lack of training, limited personnel and lack of clear roles and responsibilities; may not have a system in place to track specimens	Use where there are reputable bus companies with regular schedules, professional staff and a central depot where health facility staff can collect and dispatch specimens
Outsourced – all have logistics expertise and will manage transport	Dedicated professional courier (NGO, social enterprise, private), such as Riders for Health	Ability to design a dedicated system including in hard-to-reach or underserved areas; result return or carrying other supplies on scheduled routes at no additional charge	Total costs may appear to be higher since the system is all-inclusive (includes vehicles, transport, drivers and riders, operating costs, etc.) and run by a third-party (resources, such as health ministry or partner staffing, will not be shared but will be at an additional cost)	Use in countries with limited or undeveloped road infrastructure and transport providers
	Non-dedicated private professional courier, such as FedEx or DHL	Specialize in collecting and delivering packages, on-demand or regularly scheduled pick-ups, documentation and tracking of shipments	Not all are able or willing to transport potentially infectious biological specimens; costs may be higher; coverage and flexibility may be limited; may not be a cost-effective way to return results	Use where speed, security, documentation, tracking, name and signature of receiving person, specialization and individualization of express services are sufficiently important to warrant the extra cost; best coverage in major cities
	National postal service, non-dedicated courier (public or semi-private)	Usually a parastatal entity, which may be easier for the health ministry to contract with than a private courier; mandate to be present across an entire country; typically on a predictable schedule	Availability of and accessibility to local post offices; adherence to schedules; specimens requiring strict transit time or careful temperature control may be challenging unless a guaranteed service is offered (such as express mail)	Use where the national postal system is strong and has good coverage; otherwise, use only for less stringent and longer shelf-life specimens such as dried blood spot specimens

7.2 INFANT DIAGNOSIS AND VIRAL LOAD SPECIMEN COLLECTION BUNDLES

More than 10 individual commodities are required for collecting whole-blood specimens for plasma separation or dried blood spot specimens from patients for nucleic acid–based testing (either diagnosis or viral load). Some of these items, such as the dried blood spot filter paper collection cards used to collect blood specimens, are specialized and only recommended from specific suppliers. Other items, such as gauze and alcohol swabs, are generic. In the early stages of establishing infant diagnosis testing programmes, countries needed to procure these items individually, which made ordering and facility distribution a complex endeavour. Further, stock-outs of any single item could compromise the quality of specimens or prevent the collection and/or processing of blood specimens altogether.

Drawing from the experience with infant diagnosis, for ease of procurement and distribution and to ensure the quality of commodities, suppliers have developed plasma and dried blood spot specimen collection bundles for viral load testing as well. Plasma and dried blood spot specimen collection bundles contain individual and single-use collection kits that include all required items and commodities to draw, dry (for dried blood spot specimens) and transport a specimen from the facility to the laboratory.

Contents of specimen collection bundles

Table 11 lists the items included in single-use EDTA blood collection kits (100 tests per bundle) to obtain a plasma specimen for viral load testing using venepuncture. These specimens can then either (1) be shipped directly to the testing laboratory for processing (centrifugation) into plasma and tested or (2) be separated into plasma by centrifugation at the health-care facility and transferred to another tube, which is then sent to the laboratory under appropriate storage conditions for testing.

Table 12 lists the items included in single-use DBS specimen collection kits with perforated dried blood spot cards (20 or 50 tests per bundle) that can be used for both infant diagnosis and viral load testing.

Table 11. Whole-blood and plasma specimen collection bundles

No.	Item	Quantity	Specifications
1	Swab alcohol, 70% isopropyl	1	Swab alcohol WBCL
2	Swab gauze 8 ply non-sterile 10 × 10cm	1	Swab gauze 8 ply non-sterile 100 × 100mm
3	Gloves examination latex powder free	2	Gloves examination latex, powder free, medium
4	Bandage fabric	1	Bandage fabric
5	Bag autoclave clear biohazard 415 × 600 mm (1 per bundle)	1	Bag autoclavable clear print biohazard 415 × 600 mm
6	5 mL EDTA-treated evacuated tube	1	Tube 5 mL K2EDTA lavender 13 × 100 mm
7	Vacuum tube needle holder	1	Speedy quick release holder
8	Vacuum tube needle, 20G	1	Vacuum multiple use draw needle 21G × 1.5" 38 × 0.8 mm Green Sterile
9	Tourniquet (one per bundle)	1	Tourniquet disposable without clip, latex-free, synthetic rubber band, non-sterile
10	Packing box	1	Box plain white with liner 385 × 310 × 145mm
11	Pasteur transfer pipette (optional)a	1	Pasteur transfer pipette 1 mL fine tip, individual sterile pack (can be requested at additional cost)

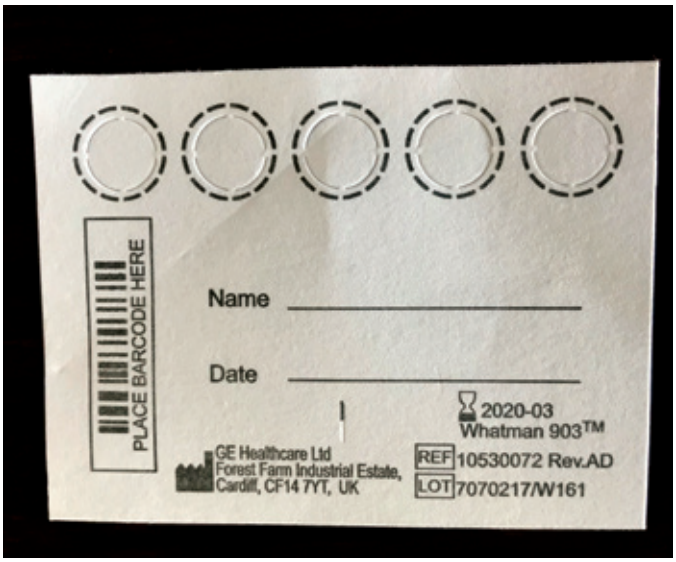
[a] Used to transfer the plasma aliquot after centrifugation and before transport to the laboratory.

Table 12. DBS specimen collection bundles

No.	Item	Quantity	Infant diagnosis	Viral load	Specifications
1	DBS collection instructions	1	✓	✓	DBS collection instructions card
2	Powder-free gloves	2	✓	✓	Examination gloves powder free latex – medium
3	Alcohol gauze pad	2	✓	✓	Single-use individually wrapped alcohol-impregnated medical gauze swab
4	Lancets	1	✓	✓	Single-use retractable lancet with non-adjustable 2 mm penetration depth blade (not needle-type)
5	Gauze swab	1	✓	✓	Swab gauze non-sterile 8 ply 50 mm × 50 mm
6	EDTA capillary tube	1	✗	✓	100 µl EDTA capillary tube, plastic, with 70 µl markings
7	DBS Filter paper S&S 903	1	✓	✓	Whatman 903 card or Munktell TNF, perforated
8	Drying rack for DBS card	1	✓	✓	Drying rack for Whatman 903 card
9	Silica desiccant pack	3	✓	✓	Indicating silica gel 1-gram sachet
10	Plastic bags	1	✓	✓	Low gas permeable double Ziploc bag (150 mm × 180 mm) with white write-on area
11	Packing and repacking	1	✓	✓	Packing and repacking: five pieces per bundle
12	Packing box	1	✓	✓	Buff board box to contain all bundle contents, with tuck-in lid
13	Lab requisition form (optional)	1	✓	✓	Customized early infant diagnosis/viral load requisition form in a pad of 50s or 100s in duplicate copies
14	Barcode stickers (optional)	1	✓	✓	Customized early infant diagnosis/viral load barcode stickers

Source: M. Rioja, Clinton Health Access Initiative.

A specific dried blood spot specimen collection bundle for viral load testing contains similar items, with the addition of an EDTA microcapillary tube added (Fig. 8). The EDTA microcapillary tube serves to ensure that the required volume per spot is accurately collected. Viral load testing is a quantitative measure that heavily relies on the quantity of blood inputted into the assay, regardless of specimen type. Limited evidence suggests that free drops of blood applied directly to a dried blood spot card can produce accurate test results compared with plasma specimens. Therefore, using a fixed volume or graduated measuring microcapillary tube or pipette may support increased accuracy of dried blood spot specimen preparation. Health-care workers should be appropriately trained on the differences in the specimen collection technique and process for infant diagnosis and viral load dried blood spots.



Source: M. Rioja, Clinton Health Access Initiative.

What are the expected benefits of using sample collection bundles?

The following are benefits of specimen collection bundles for a national programme:

- simpler and more standardized forecasting, procurement and supply chain management (instead of ordering individual items from various manufacturers);
- ensured availability of necessary items in the correct proportions and reduced waste;
- simpler and more rapid scaling up of testing services at new sites since all materials to train and test are packaged together; and
- bundles result in lower costs for all components compared with individual itemized procurement.

The following are benefits of specimen collection bundles for health facilities:

- quality assurance of items in bundles if the supplier has a proven track record, which is especially critical for certain items that must adhere strictly to quality standards (such as lancets and powder-free gloves) to ensure that proper care is delivered to the patient and that the specimen is prepared correctly (thus mitigating the risk of the specimen being rejected at the laboratory) and the safety of the end-user ensured;
- reduced risk or repurposing of items such as gloves for other services, thereby reducing stock-outs and waste of individual items;
- easier sharing or distribution of individually packed single-use bundles to satellite sites with lower patient testing demand;
- simplified stock audit, monitoring, inventory and distribution of supplies; and
- simplified workflow in the clinic because of individually packed single-use bundles, enabling health-care workers to reach into the box and grab one bag that has everything they need to collect a specimen.

Which suppliers offer specimen collection bundles?

Such bundles are readily available for procurement through at least two suppliers that source items and components directly from individual manufacturers:

- LASEC (https://www.lasec.com/diagnostics); and
- LabMate (https://www.labmate.co.za).

Conclusion

By ensuring that all items needed are available to health-care workers or laboratory technicians in a single kit or box, bundled products for nucleic acid–based test sampling have simplified and standardized the supply chain for such commodities and reduced the occurrence of testing delays resulting from the stock-out or misappropriation of a single item. Many countries have become experienced in using these bundles and, as a result, have less waste and order lower buffer stock. Although the bundles themselves are a cost-effective alternative to bulk individual commodity purchasing, the significant reductions in waste contribute to additional cost savings for countries. Further, the use of bundled specimen collection products has contributed significantly to the scaling up of infant diagnosis and viral load testing services in several resource-limited countries.

7.3 OPERATIONAL INTERVENTIONS: USING VIRAL LOAD TESTING TO DIAGNOSE INFANTS

Infant diagnosis testing has expanded considerably in the past decade in low- and middle-income settings, but access remains limited. In 2017, only 51% of HIV-exposed infants received an early infant diagnostic test within the first two months of life *(11),* as recommended by WHO *(2)*. Several challenges have limited the scaling up of this critical test for a highly vulnerable population. Infant diagnosis has primarily been offered at centralized testing laboratories, requiring transport of dried blood spot specimens that can often take weeks and often months before the results are returned to clinicians and caregivers for clinical action. The delays can be caused by several issues, including:

- the need to batch infant specimens until a full run can be performed, to ensure that testing is cost-effective and cost-saving;
- low infant diagnosis volumes limit the number of devices and laboratories capable of testing, which can be far from health-care facilities, and create a challenging procurement environment that has often lead to stock-outs of reagents in the laboratories;
- in the past, and sometimes still, infant diagnosis reagents can be more expensive than other HIV nucleic acid–based tests, such as viral load; and
- since this type of specimen, dried blood spots, is also often used for viral load testing and infant volumes are as low as a few needed tests per month, specimen collection materials have and can be reappropriated for viral load specimen collection, occasionally resulting in stock-outs when an infant specimen may be needed.

Qualitative infant diagnosis assays have primarily been used to diagnose HIV among HIV-exposed infants in resource-limited settings. The nucleic acid–based technique (quantitative PCR) used for viral load testing is very similar, often the same, for infant testing or qualitative assays. HIV DNA PCR is a commonly used synonym for HIV infant diagnosis testing; however, several technologies currently on the market do not specifically target HIV DNA. The primary specimen type for nucleic acid–based infant diagnosis is whole blood, which can contain proviral DNA, intracellular RNA and extracellular RNA. Similarly to when using whole-blood dried blood spot specimens for viral load testing (see subsection 6.1), whole blood for qualitative infant diagnosis assays generally results in the detection of the variety of HIV nucleic acids. Since both HIV DNA and RNA are present, virological testing or HIV nucleic acid amplification testing are now more accurate terms for infant PCR testing than HIV DNA PCR.

Current considerations

The 2010 WHO recommendations on the diagnosis of HIV infection in infants and children *(74)* and 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend that virological testing to diagnose HIV infection among infants be performed using HIV DNA PCR on whole-blood specimens or dried blood spot specimens, HIV RNA PCR on plasma or dried blood spot or ultrasensitive p24 antigen on plasma or dried blood spot. Further, guidelines in high-income countries, including guidelines in the United States of America *(75)*, recommend HIV RNA testing to diagnose HIV infection among infants.

Existing research has suggested that HIV RNA (often quantitative) testing may yield comparable results to assays specifically detecting DNA *(76–79).* However, questions remain about the technical and clinical feasibility of using RNA and/or quantitative testing for diagnosis given the increased maternal and infant exposure to antiretroviral therapy through programmes to prevent the mother-to-child transmission of HIV, option B+ and "treat all" policies, since all previous studies were conducted before 2003 and the option B+ era.

Updated data

Two studies have recently been conducted to better understand the performance and potential role of using HIV quantitative (viral load) assays using dried blood spot specimens for diagnosing HIV among infants younger than 18 months *(80,81)*. These studies were conducted in current settings with high rates of maternal and infant exposure to drugs. In Mozambique, 95% of mothers and infants were on antiretroviral therapy or receiving antiretroviral prophylaxis, respectively. While in Uganda, 75% of mothers were receiving antiretroviral therapy and 65% of infants were receiving antiretroviral prophylaxis.

In the study conducted in Mozambique, the sensitivity and specificity of using the viral load assay to detect infection were 100.0% and 99.9%, respectively. The positive and negative predictive values were 99% (95% CI: 94.3–100.0%) and 100% (95% CI: 99.6–100.0%). In the study conducted in Uganda, the sensitivity and specificity of using the viral load assay to detect infection were 98.9% and 98.8%, respectively.

One key consideration in both studies was that the dried blood spot specimens were prepared using the buffer and specimen preparation techniques traditionally used to prepare infant diagnosis specimens.

Current WHO recommendations and these data further indicate that viral load can be used as a diagnostic assay for infants. In fact, some technologies specifically aim to

Table 13. Sensitivity and specificity of viral load assays in Mozambique

Study setting	Sample type	Sample size	Sensitivity (95% CI)[a]	Specificity (95% CI)[a]
Mozambique *(80)*	Plasma	1021	100% (96.2–100.0%)	99.9% (99.4–100.0%)
Uganda *(81)*	Plasma	520	98.9% (96.7–99.6%)	98.8% (96.6–99.6%)

[a] Sensitivity and specificity using a treatment failure threshold of 1000 copies/mL.

target only HIV RNA and yet have been shown to have high sensitivity and specificity, comparable to gold standard technologies, and achieved WHO prequalification.

Although some manufacturers have already sought dual claims, it would be ideal for manufacturers to seek regulatory approval within their current and/or future intended use claims for their viral load assays, to support implementation of this technique.

Programmatic considerations

There are potentially some advantages to using viral load or dual claim assays as a diagnostic assay for infants, including:

- optimizing laboratory work flow, where infant diagnosis and viral load samples could be batched together, reducing the need to wait for full infant diagnosis batches;
- reducing the risk of giving lower priority to infant diagnosis at the facility and laboratory levels as the viral load programmes scale up;
- streamlining forecasting and quantification for infant diagnosis and viral load testing;
- simplifying procurement, supply chain management and distribution of infant diagnosis and viral load specimen collection commodities;
- saving money resulting from price parity between viral load and infant diagnosis tests and increased efficiency of laboratory operations and procurement processes; and
- improving care, since a viral load result could be provided for an infant living with HIV at the time of diagnosis.

Conclusions

Creating more efficient, streamlined and clinically supportive diagnostic systems is critical to improving care. Using viral load assays with a validated dual intended use claim to also support infant diagnosis should be considered to alleviate some of the current challenges and improve infant diagnosis.

7.4 NOVEL POINT-OF-CARE TOOLS FOR EARLY INFANT DIAGNOSIS OF HIV

A decade of investment in conventional laboratory networks has expanded access to early infant diagnosis testing, but only 51% of HIV-exposed infants were tested for HIV infection before two months of age in 2015 *(82)*.

The advent of point-of-care early infant diagnosis technologies *(10)* is a breakthrough that creates the opportunity to increase coverage of early infant diagnosis testing. It will enable same-day test results, enable treatment to be initiated earlier and address some of the key limitations of conventional early infant diagnosis networks – especially long turnaround times for tests and high rates of loss to follow-up.

Significant progress has been made in ensuring the quality of new point-of-care early infant diagnosis technologies.

The following regulatory approvals and technical evaluations have been made for point-of-care early infant diagnosis (countries often consider these approvals when procuring diagnostic technologies):

- **CE-IVD (Conformité Européene in vitro diagnostics).** Four point-of-care early infant diagnosis technologies have received CE-IVD: AlereTMq HIV-1/2 Detect, Cepheid Xpert®HIV-1 Qual and Diagnostics for the Real World's SAMBA I HIV-1 Qual Test and SAMBA II HIV-1 Qual Whole Blood Test.
- **WHO prequalification:** Two point-of-care early infant diagnosis technologies have met WHO requirements: AlereTMq HIV 1/2 Detect *(83)* and Cepheid Xpert®HIV-1 Qual (84) received WHO prequalification on 13 June 2016.

Independent technical evaluations: the Point-of-care Early Infant Diagnosis Consortium comprised a group of principal investigators across six countries conducting technical

Table 14. Technical evaluations of point-of-care early infant diagnosis technologies

Study setting	Sample type	Sample size	Sensitivity (95% CI)	Specificity (95% CI)
AlereTMq HIV-1/2 Detect	WHO PQ CDC/NHLS	Whole blood	98.67% (95.27–99.84%)	100.00% (97.59–100.00%)
	Early Infant Diagnosis Consortium	Whole blood	99.00% (96.45–99.88%)	99.97% (99.83–100.00%)
Cepheid Xpert® HIV-1 Qual	WHO PQ CDC/NHLS	Whole blood	98.86% (93.83–99.97%)	100.00% (97.55–100.00%)
	Early Infant Diagnosis Consortium	Whole blood	96.79% (92.68–98.95%)	99.91% (99.76–99.97%)
	WHO PQ CDC/NHLS	Dried blood spots	99.34% (96.40–100.00%)	100.00% (97.60–100.00%)

field evaluations of point-of-care early infant diagnosis technologies to expedite the release of independent performance data to accelerate national approval processes and in-country implementation. The results from nine technical field evaluations were consolidated across the six countries (Table 14). A total of 3383 specimens were tested using the AlereTMq HIV-1/2 Detect, and 4401 specimens were tested using the Cepheid Xpert®HIV-1 Qual *(85)*.

WHO recommendations

The 2016 WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection *(2)* recommend that nucleic acid testing technologies that are developed and validated for use at or near the point of care can be used for early infant HIV testing. Point-of-care early infant diagnosis provides the opportunity to reduce test turnaround times, limit patient loss along the HIV testing cascade, reduce infant mortality and enable task shifting to lower cadres of health-care workers at decentralized facilities *(2)*.

Current use

Several countries are implementing point-of-care early infant diagnosis technologies. For example, Malawi, Mozambique and South Africa reported results from point-of-care early infant diagnosis pilot projects in 2016 showing significantly shorter test turnaround times for results and increased rates of initiation of antiretroviral therapy compared with conventional laboratory systems *(86–88)*. Considering the high and early mortality rate of untreated infants living with HIV *(89,90)*, point-of-care early infant diagnosis could also reduce observed infant mortality. Based on the CE-IVD and WHO PQ approvals, robust results from independent technical field evaluations, procurement eligibility, the WHO recommendation for the use of point-of-care early infant diagnosis and initial results on patient impact from implementation pilots, countries should begin planning to implement point-of-care early infant diagnosis by incorporating it into national HIV care and treatment guidelines, national strategic plans, PEPFAR country operational plans, grant applications to the Global Fund to Fight AIDS, Tuberculosis and Malaria and HIV programme budgets.

Conclusions

Sufficient evidence has been generated on the performance of these assays in the intended field settings to support rapid national regulatory approval and initiation of scale-up. Performance was consistent between laboratory and field settings and across countries Further technical evaluations of these technologies are unlikely to add value but may instead delay implementation and timely diagnoses of infants living with HIV, a critical and vulnerable population.

National regulatory agencies are encouraged to not delay adoption by conducting further evaluations but instead adopt a rapid and streamlined registration and national approval process for immediate implementation.

7.5 OPERATIONAL INTERVENTIONS: UPDATED CONSIDERATIONS FOR A COMPREHENSIVE QUALITY MANAGEMENT PACKAGE FOR POINT-OF-CARE TESTING WITHIN NATIONAL HEALTH PROGRAMMES

The introduction and implementation of point-of-care technologies and the ability to decentralize testing has greatly improved access to diagnostic services. Since 2015, new WHO recommendations have been published *(2)*. In 2016, WHO conditionally recommended nucleic acid testing technologies that are developed and validated for use at or near the point of care for early infant HIV testing. Further, CD4 cell count testing at the point of care can be used to give priority for urgent linkage to care and antiretroviral therapy initiation. Finally, several point-of-care or near-

point-of-care technologies have been prequalified since 2015 for early infant diagnosis, CD4, HIV viral load, hepatitis C viral load, cervical cancer screening and HIV and syphilis *(91)*.

Point-of-care testing has been found to facilitate rapid and decentralized delivery of health services. A systematic review of using point-of-care CD4 to support antiretroviral therapy initiation *(92)* showed significantly improved linkage to HIV care and timeliness of antiretroviral therapy initiation. Further, recent published studies in Malawi and Mozambique have shown significantly reduced test turnaround times and increased antiretroviral therapy initiation rates when using point-of-care testing for early infant diagnosis *(93,94)*.

This decentralization of both qualitative and quantitative testing has presented both opportunities and challenges as countries monitor an increasing number of devices and operators across a decentralized testing network. This has required expanding traditional external quality assessment schemes to reach an unprecedented number of health facilities, and in many cases, considering novel mechanisms to support the quality management process.

The principles presented throughout the publication on improving the quality of HIV-related point-of-care testing *(95)* remain highly relevant. However, it is now critical to update considerations for countries and implementing partners, since experiences with point-of-care technologies and quality assurance mechanisms have developed. As more experience has been gained, a more comprehensive approach to quality assuring point-of-care technologies is critical to ensure reliable and accurate testing. Several alternative options to quality assurance that should form a comprehensive package along with traditional proficiency testing include:

- in-training and ongoing competency assessment;
- internal quality controls;
- proficiency testing panels;
- alternative external quality assessment, if traditional proficiency testing panels are not available:
  - paper-based and online
  - duplicate specimen testing/reverse testing;
- data management through connectivity; and
- site training and mentorship

Each quality assurance mechanism may touch on different steps within the testing cascade; however, once consolidated into a package, they provide a comprehensive and inclusive approach.

Importance of a comprehensive quality management package for point-of-care technologies

Quality management of diagnostics is critical to the overall quality of care by ensuring reliable and accurate test results. Pre-market quality assessments of in vitro diagnostics, such as WHO prequalification, provide information on product safety, quality and performance, manufacturing reliability and quality management systems. Further, stringent regulatory authorities aim to assess high-quality products for their intended use. Together, these processes ensure that only high-quality products are eligible for procurement.

However, ongoing quality assurance and quality control are necessary to ensure the accuracy and precision of the results produced by diagnostic testing to prevent misdiagnosis. Internal controls and standards are meant to eliminate differences in random and systematic errors between each specimen and between specimens and known standards. External quality assessment proficiency testing schemes specifically assess the performance of a laboratory or health-care facility in accurately testing stabilized specimens of known value or result. The results of these assessments should alert national programmes to a problem, at which point action can be taken to identify the cause and potential remediations. This is valuable for understanding the performance levels of individual facilities but also in reviewing the overall national laboratory network. Further, data monitoring of the invalid rates, daily controls and utilization patterns of device-based technologies through connectivity can provide critical information on testing quality, recurring device or operator errors and the need for refresher training or specific mentorship.

A comprehensive quality management package can identify gaps and bring them to the attention of laboratory programme managers. Good quality assurance programmes enable testing sites and laboratory programmes to work together to prevent, detect and correct problems throughout the entire testing cascade and to monitor all aspects of a testing programme for continual and high-quality testing services. The comprehensive quality management programme should bring together a series of activities that can together touch on all aspects of testing, including:

- identifying patients;
- collecting specimens;
- handling specimens;
- ensuring specimen and reagent storage conditions and expiry dates;

- applying specimens;
- ensuring the performance of technology;
- applying reagents, if necessary;
- ensuring technical procedures;
- interpreting results; and
- recording results,

Such a comprehensive quality management package for point-of-care testing is meant to complement the suggested and ongoing national laboratory-wide pre- and post-market surveillance activities outlined in other WHO publications *(96–99)*.

Implementation considerations for developing a quality management package

A strong and comprehensive quality management package for point-of-care testing requires quality activities in addition to proficiency testing panels. Establishing a comprehensive package with some of the alternative strategies discussed here will enable coverage of the entire testing cascade and provide more regular monitoring of decentralized testing. At a minimum, national programmes should consider proficiency testing panels, encouraging suppliers to develop robust internal control systems, in-service training and competency assessments, data management through connectivity as well as regular and planned site training and mentorship.

In an effort to provide a comprehensive quality management programme, national health policies must be developed that consider the available resources to ensure sustained adoption for the implementation of quality assurance in each context. In addition to exploring different models, countries must consider the timing and frequency of quality assurance activities, the content of each activity and the subsequent cost of conducting these activities. All these parameters have important quality and cost implications, and using country-specific policies and data to inform these decisions is critical. Some of the parameters to help understand implementation are listed below.

In addition, routine programmatic quality mechanisms are still critical to ensure consistent procurement and introduction of high-quality technologies. Regular lot testing, service and maintenance and post-market surveillance are necessary structures of an overall laboratory quality system that this comprehensive quality management package for point-of-care testing should complement *(98,99)*.

No quality management programme is complete without reviewing data and taking clear and consistent preventive and corrective action when necessary. This is a critical component of the programme that must be clearly planned and determined to ensure that issues are addressed and operators are given the necessary support to continue providing testing and results in a high-quality manner.

8. CONCLUSIONS

Increasing scale-up of treatment monitoring approaches through viral load testing as well as infant diagnosis will be critical to ensure high-quality care and treatment as well as programmatic success. Considering the optimal diagnostic network, specimen types, interventions and strategies in each country and across national, regional and partner stakeholders will support this effort, enhance collaboration and maximize diagnostic investment into clear clinical impact.

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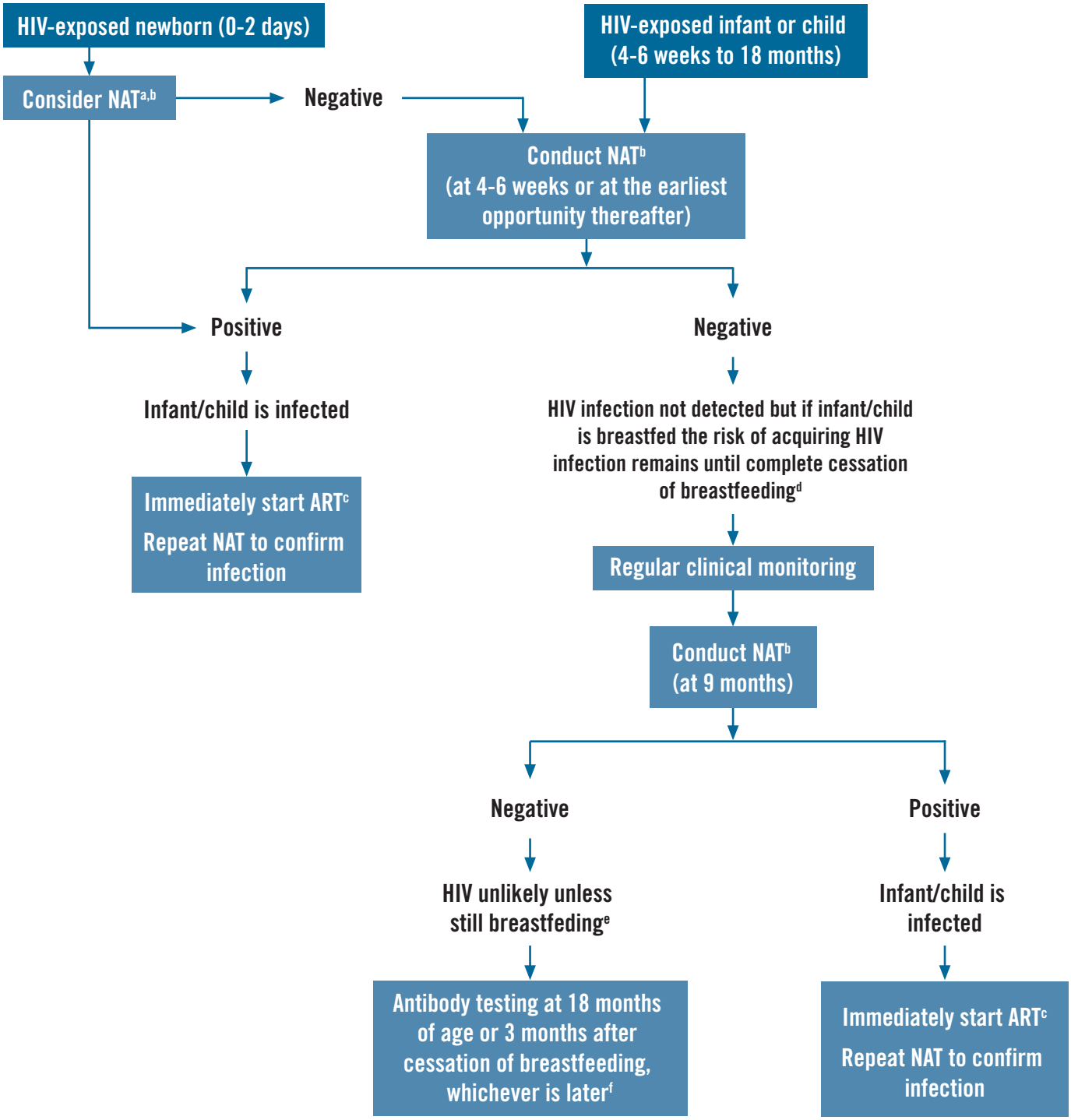
ANNEX 1. INFANT DIAGNOSIS ALGORITHM



[a] Based on *2016 WHO Consolidated ARV Guidelines*, addition of NAT at birth to the existing testing algorithm can be considered.
[b] POC NAT can be used to diagnose HIV infection as well as to confirm positive results.
[c] Start ART without delay. At the same time, retest to confirm infection. As maternal treatment is scaled up and MTCT transmission rates decrease, false-positive results are expected to increase: retesting after a first positive NAT is hence important to avoid unnecessary treatment, particularly in settings with lower transmission rates. If the second test is negative, a third NAT should be performed before interrupting ART.
[d] For children who were never breastfed, additional testing following a negative NAT at 4–6 weeks is included in this algorithm to account for potential false-negative NAT results.
[e] The risk of HIV transmission remains as long as breastfeeding continues. If the 9-month test is conducted earlier than 3 months after cessation of breastfeeding, infection acquired in the last days of breastfeeding may be missed. Retesting at 18 months or 3 months after cessation of breastfeeding (whichever is later) should be carried out for final assessment of HIV status.
[f] If breastfeeding extends beyond 18 months, the final diagnosis of HIV status can only be assessed at the end of breastfeeding. If breastfeeding ends before 18 months, the final diagnosis of HIV status with antibody testing can only be assessed at 18 months. Antibody testing should be undertaken at least 3 months after cessation of breastfeeding (to allow for development of HIV antibodies). For infants younger than 18 months of age NAT should be performed to confirm infection. If the infant is older than 18 months, negative antibody testing confirms that the infant is uninfected; positive antibody testing confirms infant is infected.

Source: HIV diagnosis and ARV use in HIV-exposed infants: a programmatic update *(12)*.

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