Module 4: Improving the Quality and Efficiency of the Viral Load Testing Phase

Learning objectives:

By the end of the module, participants will be able to:

- Compare and contrast the different viral load technologies commercially available
- Operate an efficient viral load testing laboratory that produces timely and accurate viral load results
  - Proper infrastructure and physical layout
  - Optimal testing workflow
  - Monitoring of key quality indicators
  - Appropriate troubleshooting and corrective action when problems arise
  - Timely result reporting
  - Competent laboratory staff
  - Complete laboratory documentation

Target audiences: Laboratorians

Pre-requisites: Modules 1 and 3

Participant handouts: 4-1, 4-2, 4-3, 4-4, 4-5, 4-6, 4-7, 4-8, 4-9

Special preparations before facilitating: None

Icon	Meaning
	Refer to Handout
	Customize the slide for local context

Module-at-a-glance

Segment		What you do	Time	Handouts
Module opening			**0:15**	
	Slides 1-2	State the module objective.		
	Slides 3-4	Explain the slides according to the content notes.		
1. Viral Load Testing Technology			**0:30**	
	Slides 6-20	Explain the slides according to the content notes.		**4-1**
	Slides 21-22	Gauge participants' knowledge with the Knowledge Check questions.		
2. Viral Load Testing Infrastructure and Workflow			**0:30**	
	Slides 24-29	Explain the slides according to the content notes.		
	Slides 30	Gauge participants' knowledge with the Knowledge Check question.		
3. Sample Receipt, Rejection, Check-in and Storage until Testing			**0:15**	
	Slides 32-34	Explain the slides according to the content notes.		**4-2, 3-10, 3-11, 4-3, 3-5**
4. Preventing and Troubleshooting Problems			**0:45**	
	Slides 36-37	Explain the slides according to the content notes.		**4-4**
	Slides 38-39	Conduct Activity 4A: Troubleshooting and Corrective Action according to content notes.		**4-4, 4-5**
5. Ensuring the Quality of the Testing Phase			**0:45**	
	Slides 41-45	Explain the slides according to the content notes.		**4-6, 4-7, 4-8**
6. 6. Result Recording and Reporting			**0:05**	
	Slides 47-48	Explain the slides according to the content notes.		**4-9**
Module closing			**0:05**	
	Slides 49-50	Invite participants to supply words to complete each key message		
		TOTAL MODULE DURATION:	**3:10**	

Slide Number	Content Notes for PowerPoint Slides
3	**Heading – The Testing Phase** There are five steps in the testing phase, each of which is crucial for achieving quality results for viral load (VL) testing. 1. Specimen receipt - The laboratory inspects the specimens delivered to ensure they meet the VL testing requirements (e.g. shipping temperature, specimen volume, specimen labeling, etc.). 2. Specimen check-in - The laboratory labels the specimens with the unique laboratory identification (ID) that can be linked back to the original specimen ID, and correctly accessions them into the laboratory database, preferably an electronic database. Two people should work together to verify that the specimens are checked in correctly to avoid specimen mix-up. 3. VL testing – This is where specimens are prepared, loaded onto and processed in the instrument. One potential problem that could occur here is specimen mix-up. This can be avoided by generating a specimen testing list prior to testing and labeling the testing tubes in the same order as the specimen testing list. To ensure the quality of testing results, laboratorians should strictly follow the standard operation procedure (SOP). 4. Results validation/recording - Results generated from the testing instrument should be validated before recording. If QC is not acceptable, document occurrence and perform corrective action. If a sample is rejected, the clinic should be notified immediately. If electronic importing system is not available, manually recorded results should be verified by a second staff member. 5. Result reporting - The result report should always be checked and signed by the laboratory supervisor or manager before release.
4	**Heading - Keep Your Turn Around Time Short** Turnaround time (TAT) is defined as the time period from when a specimen is collected from a patient to when results are returned to that patient. The period spans across the pre-testing, testing and post-testing phases, and is touched by various health cadres. If not managed appropriately, the TAT can become prolonged leaving patients in a non-optimal ARV treatment or non-adhering stage longer, which may increase their chances to develop drug resistance to the treatment. The total TAT time can be broken into 5 parts, each of which should be tracked closely by management of responsible parties. ▪ TAT1 is the time from specimen collection to specimen pick-up for transport. Specimen collection sites should ensure specimens are picked up within defined timeframe to safeguard specimen quality. ▪ TAT2 is the total transport time from specimen pickup at a collection site to arrival at the testing laboratory. The specimen may be transported from the collection site to a testing laboratory directly, or very often to a transport/collection hub first, and then to the testing laboratory along with other specimens collected from other collections sites. Management must ensure appropriate specimen delivery schedules, optimal transport conditions, and efficient network to protect specimen integrity. ▪ TAT3 is the time from when a specimen arrives in the testing laboratory to when the specimen result is released back to the clinic. ▪ TAT4 is the time from when a clinic receives the results to when the clinic sends the results to the clinicians.

Slide Number	Content Notes for PowerPoint Slides
	▪ TAT 5 is the time when the clinician receives a result to the time when he/she sends the result to the patient and uses it for patient care. The total time for the first 3 TAT should be within 4 weeks. The first 2 TATs should be finished within 2 weeks so that the laboratory will have 2 weeks to test the specimens and return the VL results back to clinic. Once the clinic and clinician receive the results from the laboratory, they should contact the patient as soon as possible.
1.	**Viral Load Testing Technology**
6	**Heading – Viral Load Testing** VL test is a quantitative test as the results are expressed as number of copies/mL or in log10/mL. The majority VL tests are molecular biology tests using real-time PCR technology.
7	**Heading - Technologies and Assays Available for Viral Load Testing** There are four major technologies available for VL testing: ▪ Real-Time PCR, e.g. the Roche COBAS Ampliprep/COBAS TaqMan, 2.0, and the Abbott m2000 real-time HIV test. Both are FDA cleared tests. ▪ NASBA, e.g. EasyMag/Easy Q from the bioMerieux, a CE marked VL test. ▪ bDNA, e.g. Simons, a CE marked VL test ▪ Reverse Transcriptase activity measurement, e.g. Cavidi, an ELISA based test which converts the enzyme activity (reverse transcriptase) to a VL result. The first three are collectively referred to as Nucleic Acid Amplification Technology (NAT).
8	**Heading – Commercially Available Viral Load Platforms** Here are the commercially available VL testing platforms. ▪ Roche - Two instruments are shown here: a lower throughput COBAS Ampliprep/COBAS TaqMan 48 (left) and a full automated higher throughput COBAS Ampliprep/COBAS TaqMan 96 (right). COBAS Ampliprep/COBAS TaqMan 96 has an identical throughput to the Abbott m2000 system (about 110 specimens in an 8-hour shift). Recently, Roche has rolled out a fully automated, COBAS Ampliprep/COBAS TaqMan 8800, with even higher throughput. The Roche system is a closed system, which minimizes the chance of specimen cross-contamination. ▪ Abbott – Shown here is the Abbott m2000sp and rt. The Abbott is not a closed system like the Roche COBAS and needs more human intervention to set up and has a break point between the nucleic acid (NA) extraction and Real-time PCR. Failed specimens during the extraction phase may be able to be retrieved for retest. ▪ Siemens - Another well designed CE marked VL testing system using NAT technology. ▪ Biocentric – Another VL test using NAT technology. ▪ bioMerieux - The EasyMag/easyQ is a semi-automated testing system, CE marked, using the NASBA technology. ▪ Cavidi – Uses ELISA format of test. It measures the reverse transcriptase activity and converts it to VL results. All these VL tests are sophisticated, lab based, well validated for plasma VL, and require well trained lab staff to operate. However, they vary by their throughput, automation level, price, and may not be compatible with the DBS (e.g. Cavidi). The Roche and Abbott are high throughput platforms or tests but require strict laboratory conditions (e.g. dedicated electric power, deionized water source, air conditioned environment) and highly trained laboratory staff to operate.

Slide Number	Content Notes for PowerPoint Slides
9	**Heading – Extraction** The three major VL testing methods, Roche, Abbott, and bioMerieux, share similar technology for nucleic acid extraction, using the magnetic particles to capture target nucleic acid (NA), and purify the NA through several washing steps. The purified NA will be released from the magnetic particles by heating, and then the NA eluate will be separated from the magnetic particles by the magnetic device.
10	**Heading – Amplification** The three major VL testing methods, Roche, Abbott, and bioMerieux, use different technology for amplication - bioMerieux uses Real-Time NASBA while Roche and Abbott use Real-time RT-PCR Technology.
11	**Heading – NASBA® Amplification (bioMérieux System)** bioMerieux VL test uses NASBA technology, which amplifies the viral RNA only using isothermal thermal conditions, a constant temperature throughout the amplification process. Because of this design, the test does not generates false positive results caused by amplifying genomic dsDNA as in the case of RT-PCR. This test is especially ideal for DBS VL testing since the DBS contains both viral RNA and provirus DNA.
12	**Heading – bioMérieux System: NucliSens miniMAG/easyMAG and easyQ** ▪ The device on the left is the miniMag device for manual NA extraction ▪ The instrument on the right is the EasyMag for semi-automated NA extraction. ▪ The instrument on the bottom is the EasyQ device for quantification of VL from the extracted NA.
13	**Heading – bioMérieux System: NucliSENS EasyQ HIV-1 V2.0** This table lists the features of of NucliSENS EasyQ HIV-1 V2.0, including test sensitivity, VL testing range, subtypes detected and HIV testing region, instrument throughput and device required.
14	**Heading – Real-time RT-PCR Amplification (Roche and Abbott)** Real-time Reverse Transcription Polymerase Chain Reaction (Real-time RT-PCR) amplifies viral RNA by reverse transcribing the extracted RNA to cDNA, which is then amplified by conventional PCR, all in the same tube. Detection occurs in real-time, and is monitored via fluorescently labelled oligonucleotide probes specific to target regions. The probes only fluoresce when specifically bound to their respective targets. Quantification is accomplished by comparing fluorescent signals from various probes. Before extraction, a Quantification Standard (QS) is added to all samples, including controls. This is a known quantity of copies of an unrelated RNA sequence. The fluorescent signals from the QS and HIV-1 RNA signals are compared and the difference between them allows the calculation of the starting viral RNA titre in the sample.
15	**Heading – Roche Systems: COBAS® Ampliprep/ COBAS® TaqMan ® Analyzers** Roche offers a couple of analyzers with varying testing capacities. The extraction and amplification analyzers can be purchased separately, and in this case, two separate rooms are required for testing. The combined system for a fully automated workflow (docking station) requires only one room for operation.

Slide Number	Content Notes for PowerPoint Slides
16	**Heading – PCR: Roche Viral Load Assays - COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1** This table lists the features of COBAS® AmpliPrep/ COBAS® TaqMan® HIV-1, 2.0, including test sensitivity, testing range, subtypes detected and HIV testing region (GAG), instrument throughput, etc. The price of the instrument is around $200K. The price of each test is $9 for low income countries.
17	**Heading –Abbott m2000sp and m2000rt System** The ABBOT *m2000 system* is comprised of the ABBOTT *m2000sp for extraction (middle) and the ABBOTT m2000rt for amplification (right).* ▪ The ABBOTT *m2000sp is an automated system for* performing sample preparation for nucleic acid testing. ▪ The ABBOTT *m2000rt is an automated system for* performing fluorescence-based PCR to provide quantitative and qualitative detection of nucleic acid sequences.
18	**Heading – PCR: Abbott Viral Load Assay RealTime HIV-1** This table lists the features of Abbott Viral Load Assay RealTime HIV-1, including test sensitivity, testing range, subtypes detected and HIV testing region (POL), instrument throughput and device required. The price of the instrument is around $160K. The price of each test has also been reduced to $10 per test for the low income countries.
19 **Handout 4-1**	**Heading – Summary of Commercially Available Platforms** Refer participants to Handout 4-1 and walk them through the table.
20	**Heading – Selecting Viral Load Technologies for your Setting** Facilitate a group discussion to answer the question, "What should a country consider, besides costs, when selecting the viral load technologies and platforms?" before unveiling the answers (box).
21--22	**Heading - Knowledge Check** Gauge participants' knowledge with the Knowledge Check questions.
2. **Viral Load Testing Infrastructure and Workflow**	
24	**Heading – Viral Load Testing Laboratory Infrastructure** The general principle of setting up a VL testing laboratory is the same as setting up a PCR laboratory. A VL testing laboratory should have 2 to 3 rooms. The rooms should be arranged in a unidirectional manner so that the work flow starts from the specimen prep/extraction room (cleanest), to the master mix room, followed by the amplification room (dirtiest). This unidirectional work flow will minimize potential specimen contamination from PCR products, which causes false positive results. If a laboratory only run highly automated instrument like the Roche COBAS or Abbott m2000, the master mix room or reagent room can be omitted since the reagents are packaged by the Roche and Abbott and ready to use.

Slide Number	Content Notes for PowerPoint Slides
	In the specimen extraction room, it is highly recommended that specimen processing be done in a biosafety cabinet (BSC) to avoid potential biosafety issues to the operator and to keep the specimens in a dust-free environment.
25	**Heading – Viral Load Testing Laboratory Physical Set-up** ▪ Bench space - In an ideal situation, each room should have 3 – 4 meters bench space if the size of a room allows. ▪ Reagent storage appliance - fridge and freezer are required for reagents storage at 2-8^{0}C or -10^{0}C. ▪ Electricity – dedicated power is required for the sophisticated instrument like the Roche COBAS and the Abbott m2000, and uninterruptible power supply (UPS) unit is needed for automated extraction and amplification machines. ▪ Biohazard waste disposal facilities - biohazard bags, bins, autoclave and incinerator must be available in the VL testing laboratory.
26	**Heading – Suggested Laboratory Layout - Specimen Extraction and Master-Mix Prep. Rooms (1 & 2)** Here is an example layout for the master mix room and specimen processing room with dimensions. ▪ For the master mix room, the main item is the dead air box if the room is too small to hold anything else. ▪ For the specimen extraction area, it is ideal to have a fridge and a freezer in the room or nearby the BSC or the automated extraction instrument so that the reagents can be easily accessed. It is also advantageous if the specimen extraction room is near the specimen receiving and specimen storage area. Each room should have its own designated laboratory coats, gloves, and wastes disposal bins.
27	**Heading – PCR: Suggested Laboratory Layout - Specimen Amplification and Detection Room (3)** The Amplification and Detection room houses the Amplification and Detection instrument and result validation and documentation area. Result validation and documentation can also be located in a separate room. Designated laboratory coats, gloves, biohazard bags and bins should be available in this room for waste disposal.
28	**Heading – Maximizing your Viral Load Testing Throughput** Ask participants to compare their current level of throughput to the most optimal level. Conduct a brain storm session on how their VL testing throughput could be improved. Write their responses on the flipchart.
29	**Heading – Tips for Maximizing Workflow Efficiency** Walk participants through the list of tips for maximizing efficiency. Provide additional detail for the last bullet point for how to maximize the instrument usage or minimize the instrument down time. For example, if you laboratory has 2 or more Abbott m2000 sp & rt, you can streamline the work in an overlay fashion using teams/shifts. Depending on the number of instrument you are running, teams of 2 people can perform the testing in parallel or consecutive 8-hour shifts around the clock throughout the day. Additionally, instead of everyone running the same procedures from extraction to

Slide Number	Content Notes for PowerPoint Slides
	amplification together, one person can perform instrument set up (maintenance, extraction ordering) for all instrument and the other people can label specimen tubes and process specimens for loading, to minimize the instrument down time.
30	**Heading - Knowledge Check** Gauge participants' knowledge with the Knowledge Check question.
3.	**Sample Receipt, Rejection, Check-in and Storage until Testing**
32 **Handout 4-2** **3-10**	**Heading – SOP for Specimen Receipt at Testing Sites** Carefully following the SOP for specimen receipt at the testing sites is critical to ensuring quality VL testing results. Refer participants to the SOP (Handout 4-2). Emphasize the importance of each item. ▪ Integrity of the shipping container is the first important thing to check for specimen quality. Is the container broken? Dented? Wet? ▪ Recording the specimen arrival time helps track TAT2. It also helps the laboratory determine if a specimens meets acceptance criteria. ▪ Leakage indicates the specimen integrity may be compromised. ▪ Shipping temperature is another important indicator of the specimen quality or integrity. Are cold packs cool or frozen upon arrival? ▪ Checking the specimens against the transport log helps avoid specimen mix-up or loss. ▪ Rejecting specimens that do not meet the testing requirement is another step to ensure quality testing. It is important to check specimen type (whole blood, plasma) and condition of tubes (labeled properly, broken, clotted, etc). ▪ When a specimen is rejected, an occurrence form (OM) should be filed, transport log should be filled out and clinic that sent samples should be notified immediately.
33 **Handout 3-11**	**Heading – Specimen Rejection Criteria** Refer participants to Handout 3-11. Provide additional comments below: ▪ Whole blood - Correct volume is critical for getting enough plasma for testing. Hemolyzed specimens inhibit the PCR reaction during testing and affect the results. EDTA specimens arrive after 24 hours cannot provide accurate results based on the performance data from the manufacture. ▪ DBS - Normally VL testing requires one good spot per test. However, human error or instrument failure may occur during testing. When this happens, additional spot is needed for repeat testing. Clotted DBS cannot be lysed completely during nucleic acid extraction and will cause inaccurate VL results. ▪ Plasma –1.2 mL of plasma is enough for one test. However, that amount does not allow for repeat testing in case of instrument failure or operator errors. Ideally, 5 ml of EDTA blood should be collected to obtain around 2.4 mL plasma for two tests.
34 **Handout 4-3** **3-5**	**Heading – Guidelines for Specimen Check-in and Storage Until Testing** Once specimens are deemed acceptable for testing, they are checked in and stored until testing. Refer participants to Handout 4-3 for the guidelines. ▪ Emphasize the importance of specimen check-in and correct storage. It is a critical step to avoid specimen mix-up and obtain quality VL testing results. ▪ Special attention should be paid for each specimen type when specimens are received since each requires different storage temperature per length of storage. Refer to Handout 3-5 for required storage conditions.

Slide Number	Content Notes for PowerPoint Slides
4.	**Preventing and Troubleshooting Problems**
36	**Heading – Common Errors – How to Prevent them?** Ask participants what errors they most often encounter during viral load testing and the possible causes for those errors before unveiling the examples (left box) on the slide. Facilitate a discussion on how to prevent those errors from happening before unveiling the answers (right box).
37  **Handout 4-4**	**Heading – When Errors Occur: Troubleshooting Guide** The troubleshooting guide groups errors into 7 categories and assists laboratory personnel in pinpointing sources of errors. Refer participants to Handout 4-4 and walk them through the troubleshooting guide.
38-39  **Handout 4-4** **4-5**	**Heading – Activity 4A: Troubleshooting and Corrective Action** Read the activity slide (#38). Allow participants 10 minutes to write down their responses on the flipchart. Allow each team's spokesperson 2 minutes to present their responses. Facilitate a group discussion after each presentation. Debrief the activity by referring participants to Handout 4-5. Walk participants through the handout.
5.	**Ensuring the Quality of the Testing Phase**
41  **Handout 4-6**	**Heading - Viral Load Laboratory Readiness Assessment Checklist** Refer participants to Handout 4-6. Walk them through the checklist so they become familiar with the requirements for quality viral load testing as specified in the checklist items. Consider asking participants to review the checklist as overnight homework prior to teaching this module.
42	**Heading – Quality Indicators** Facilitate a group discussion for each quality indicator: ▪ What is it? What does the indicator measure? ▪ Why is it important to track it? ▪ How to monitor it?
43	**Heading - Tracking Specimen Rejection by Criteria and by Clinic Pinpoints the Source of the Problem** Here is an example of specimen rejection summary report – it summarizes the number of rejections by reasons and by specimen collection site in a given time period. The report helps laboratory management pinpoint the nature of the specimen problems and the site where the problems come from; improvement projects can be then initiated targeting the right site with the right interventions to reduce specimen rejection rate.
44 	**Heading - Ensuring the Competency of Testing Personnel** Emphasize the importance of proper training and regular competency assessment of those who perform viral load testing.

Slide Number	Content Notes for PowerPoint Slides
Handout 4-7	Refer participants to Handout 4-7. Walk them through the competency assessment tools: Competency Assessment Form and Unknown (sample) Assessment Form.
45  **Handout 4-8**	**Heading - Documentation and Records for Viral Load Testing** Documents include written policies, processes and procedures, and provide a framework for the quality system. They need to be updated and maintained. Records include information captured in the process of performing and reporting a laboratory test. This information is permanent and does not require updating. Refer participants Handout 4-8 for a list of recommended documents.
6. **Result Recording and Reporting**	
47-48  **Handout 4-9**	**Heading – Guidelines for Result Reporting** Refer participants to Handout 4-9. Walk them through the Guidelines for Result Reporting and the flowchart.
49-50	**Heading – Module 6: Key messages** Invite participants to supply words to complete each key message.

Handout 4-1

Summary of Commercially Available Platforms

	Abbott	Biocentric	bioMérieux S.A.	Roche	Siemens Healthcare Diagnostics
Assay name	Abbott RealTi*m*e HIV-1	Generic HIV viral load (research use only)	NucliSens EasyQ® HIV-1 v2.0	COBAS®AmpliPrep/ COBAS® TaqMan® (CAP/ CTM) HIV-1 Test v2.0	VERSANT® HIV RNA 1.0 (kPCR)
Type of assay	Real-time reverse-transcriptase polymerase chain reaction (RT-PCR), quantitative	Real-time PCR	Real-time nucleic acid sequence–based amplification (NASBA)	Real-time RT-PCR, quantitative	Real-time RT-PCR, quantitative
Dynamic range (copies/ml)	40–10 000 000	100–50 000 000	25–10 000 000	20–10 000 000	37–11 000 000
Specimen type	EDTA and ACD Plasma, dried blood spot (research use only)	Plasma, dried blood spots	Plasma, dried blood spots	Plasma or dried blood spot (research use only)	Plasma, serum, dried blood spots (research use only)
Specimen volume required	200 – 500 – 600 – 1000 µl	Regular protocol: 200–500 µl Ultra-sensitive protocol: 400–1200 µl	100, 500 and 1000 µl of plasma Two dried blood spots (2 x 50 µl)	1 ml of plasma 60–70 µl of dried blood spot (research use only)	500 µl (plasma, serum) One dried blood spot (50–100 µl) (research use only)
Area of HIV genome amplified	Pol/INT	Long terminal repeat genes	Gag	Gag and long terminal repeat	Pol (Integrase)
HIV-1 subtypes amplified	Group M (subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G and H), Group O and Group N	B and non-B subtypes including circular recombinant form (CRF) (ANRS and WHO panels)	A, B, C, D, CRF01-AE, F, G, CRF02-AG, H and J	Group M, subtypes A–H Group O	Group M (A–H, CRF01-AE and CRF02-AG); Group O
Time to result	5 hours	4 hours including RNA isolation	2.5–3 hours including extraction	5–6 hours	5–6 hours
Cost per test (US dollars)[1]	US$ 12.50–70	US$ 14	US$ 18	US$ 20–30 per test US$ 35–90 per test	US$ 20–75 (includes all consumables)
Number of specimens per run	21–93 patient specimens (plus 3 external controls)	1–96	8–48 per run	21–63 for batch loading (168 per 8-hour day continuous loading)	89 (plus 7 calibrators and controls)
Extraction method available	Manual and automated	Manual only	Semi-manual and automated	Automated	Automated only
Equipment required[2]	Extraction: M2000sp or M24sp Amplification: M2000rt: US$ 50 250	Real-time thermocycler and small consumables	NucliSens miniMAG system or NucliSens easyMAG system, NucliSens EasyQ Analyser and Strip centrifuge	COBAS® AmpliPrep with COBAS® TaqMan® 96 (docked or undocked) or COBAS® TaqMan® 48	Main system: VERSANT™ kPCR Molecular System

1 Prices vary considerably with quantities and special negotiations.

2 All assays require pipettes, vortex mixers and refrigerators.

Handout 4-1 (Cont'd)

Equipment cost (US dollars)	M2000sp: US$ 100 000 or M24sp: US$ 90 000 M2000rt: US$ 50 250	US$ 40 000	miniMAG: US$ 12 900 EasyQ: US$ 57 400 easyMAG: US$ 114 100	COBAS® TaqMan® 48/96: US$ 45 000–100 000/US$ 80– 150 000 AmpliPrep: US$ 80–150 000	VERSANT™ kPCR Molecular System: US$ 180 000
Infrastructure required	Three dedicated areas for performing the assay with the Abbott *m*1000sp/ m24sp Systems and manual specimen preparation, Abbott *m*Sample Preparation System and Abbott *m*2000*rt*. Two dedicated areas, specimen preparation area and amplification area, for the Abbott *m*2000*sp* and Abbott *m*2000*rt*	None stated, but given the manual nature of the assay, three dedicated areas should be recommended	Three dedicated areas	Two or three dedicated areas are required depending on the workflow chosen	**Deep-freezing** Three dedicated areas
Storage conditions	mSample Preparation System RNA (4 x 24 preps) must be stored at 15–30°C The Abbott RealTime HIV-1 Calibrator A and Calibrator B must be stored at –10°C or colder. The Abbott RealTime HIV-1 Negative and Positive Controls must be stored at –10°C. The Abbott RealTime HIV-1 Amplification Reagent Pack and Internal Control vials must be stored at –10°C or colder when not in use	–20°C	2–8°C for amplification reagents For extraction reagents: buffers 1, 2 and lysis buffer: 2–30°C Buffer 3 and magnetic silica: 2–8°C	2–8°C for all reagents	VERSANT™ HIV-1 RNA (kPCR) Kit, IVDD Box 1: –30°C to –10°C VERSANT™ HIV-1 RNA (kPCR) IVDD Box 2: –90°C to –60°C VERSANT™ Sample Preparation 1.0 Reagents Box 1: 15–30°C VERSANT™ Sample Preparation 1.0 Reagents Box 2: 2–8°C
Regulatory status	WHO prequalification, Therapeutic Goods Administration, CE-IVD, US-IVD, Canada-IVD, Japan-IVD for plasma	None	WHO prequalification, CE-IVD (plasma and EDTA dried blood spot)	WHO prequalification, CE-IVD, US-IVD, Japan-IVD, Canada-IVD for plasma	WHO prequalification, CE-IVD for plasma

From WHO Technical and Operational Considerations for Implementing HIV Viral Load Testing, July 2014.

Handout 4-2

SOP for Specimen Receipt at Testing Sites

The laboratory scientist who receives the cooler box must:

1. Check ***the container*** to ensure there are no issues.
2. Record ***time of arrival*** on the *Transport Log*. Document delays in the log and the *Occurrence Form*.
3. Check for ***spillage*** inside the cooler box. Document the spill on an *Occurrence Form*. Inform laboratory manager and/or safety officer.
4. Record ***temperature on arrival*** on all specimen tracking forms.
5. Count the ***number of specimens*** (EDTA tubes, plasma tubes and DBS). Cross reference with the total on the *Transport Log*.
6. If there is a discrepancy in specimen totals,
    - Identify ***missing*** specimens by matching each unique identifier on a specimen with the unique identifier on the *Transport Log*.
    - Fill out an *Occurrence Form* and comment on the *Transport Log*.
    - Immediately contact the laboratory supervisor or QA officer who will follow-up with the clinics/health centers to locate the specimen.
7. Apply specimen rejection criteria to accept or reject specimens
8. Note all rejections on the specimen transport log and specimen rejection log (by rejection reasons and by sites)
9. Discard all specimens without any labelling
10. Immediately notify the collection sites about those rejected specimens citing both specimen identifications and reasons for rejection

Adapted from Appendix I from Guidance for Developing a Specimen Transport and Referral System for Viral Load and Infant Virologic HIV Diagnosis Testing Networks

Handout 4-3

Guidelines for Specimen Check-in and Storage Until Testing

Before specimen check-in is initiated

- Whole blood and plasma should be kept refrigerated between 2 and 8°C.
- Whole blood should be spun down only after check-in has been completed. If this is not possible, maintain the whole blood EDTA tube refrigerated between 2 and 8°C for no more than 24 hours.
- DBS cards can be held at ambient temperature until check-in is complete.

Specimen check-in

- Affix each specimen received at the laboratory with a unique identifier. When possible, use barcodes as the unique identifier so that barcode readers can be utilized to reduce human error at input.
- Log each specimen into a database or on a receiving log with their original specimen ID and the unique identifier assigned.
- Whole blood for DBS should be spotted as soon as possible or within 24 hours of collection. Whole blood for plasma should be centrifuged and the plasma removed and placed into fresh tubes within 6 hours of collection for viral load testing.

Specimen Storage until Testing

- DBS can be maintained at ambient temperature for up to two weeks. If testing is not possible within two weeks, DBS must be placed at -70°C for long term storage.
- Whole blood cannot be used more than 24 hrs post collection.
- Plasma aliquots can be kept at -70°C until tested.

Adapted from Appendix I from Guidance for Developing a Specimen Transport and Referral System for Viral Load and Infant Virologic HIV Diagnosis Testing Networks

Handout 4-4

When Errors Occur - Troubleshooting Guide

Problem	Question
Clerical	Were specimens mixed up?
	Check the original data. Were the results recorded properly?
	Were the data reported in the correct units?
	Did a second person verify the results entry form for accuracy and correctness prior to submission?
Environmental	Have the specimens or instrument undergone any large temperature changes?
	Have the specimens or instrument undergone any significant humidity changes?
	Have the specimens or instrument been exposed to any abnormal vibrations or lighting?
	Were your testing instruments located in a properly ventilated room?
	Is your instrument on a stable and reliable power supply?
Procedural	Is your laboratory's procedure congruent with the manufacturer's package insert?
	Was manufacturer's most current procedure followed?
	Have personnel been properly trained on the test procedure?
	Is your procedure easy to understand?
Competency	Was the procedure followed correctly by laboratory staff?
	Have the laboratory staff been thoroughly and regularly trained?
	Are the laboratory staff ever observed?
	Has a different laboratory staff member tested the same specimens with different results?
Equipment	Was your instrument recently calibrated or due for calibration?
	Was maintenance performed recently and properly?
	Is there documentation of all maintenance events?
	Has your instrument been properly/regularly serviced?
	Did your instrument controls fail?
Supply	Have your reagents been stored properly?
	Are reagents within expiration dates?
	Were kit components substituted from other kits?
	Was a new lot # of reagent or calibrators recently introduced?
Specimen	Did the specimens arrive in good condition?
	Were the specimens stored properly?
	Were specimens handled with care to prevent contamination?
	Do you suspect the specimens may have been contaminated in your lab?
	Were gloves changed if contamination of gloves was even suspected?
	Are lab benches, working areas, equipment, and biosafety cabinets decontaminated between use?
	Is there documentation of every decontamination event?
	Are sterile gloves, tools, and supplies being used while handling the specimens?

Handout 4-5

Problem	Suggested Corrective Action
Clerical	Always double check data and other information have been copied to forms correctly. Develop your own systems to keep specimens organized. Have specimens clearly labeled or color coded to ensure they never get mixed up. If your specimens were mixed up, repeat testing correctly and resubmit your results. If there was any sort of typo, resubmit your results form after correcting it.
Environmental	Be sure your instrument and specimens are in a consistent environment that aligns with manufacturer recommendations. Ensure your instrument and specimen storage utilities are connected to a back-up power supply in case of power failure.
Procedural	Thoroughly review the manufacturer's package insert and align the manufacturer's procedure with your laboratory's procedure. Manufacturers often make small corrections to their package inserts over time. Therefore, it is very important to review the package insert every time a new package insert is received and every time a new testing kit is opened. Your laboratory procedure should be detailed and easy to understand. If any changes are made to your laboratory procedure, or if any changes are noticed in the manufacturer's package insert, be sure all laboratory staff are fully aware of the changes. Have all procedure updates approved by a supervisor.
Competency	Ensure all laboratory staff are performing the procedure uniformly and correctly. All laboratory staff should be required to pass competency assessment on a regular basis. All laboratory staff should perform testing regularly to keep their skills current. Provide retraining to laboratory staff if necessary. Laboratory staff should be observed periodically to ensure the assay is being conducted correctly.
Equipment	Recalibrate your instrument if calibration is due, near due, or if mis-calibration is suspected. Perform all manufacturer recommended maintenance procedures. Document any time maintenance is performed in a single location that all laboratory staff are aware of. Have a supervisor periodically check the maintenance documentation to ensure it is being conducted properly. Provided maintenance retraining to laboratory staff. Ensure your instrument has been properly and regularly serviced by the manufacturer. If your instrument controls failed, repeat testing. If controls continue to fail, contact the manufacturer with details.
Supply	Never use expired reagents. Never mix testing kits unless the manufacturer has explicitly stated it is acceptable. Repeat testing with a different lot of reagents, calibrators, and controls. Contact the manufacturer if you suspect faulty reagents, calibrators, or controls.
Specimen	If the specimens do not arrive in good condition and have to be rejected, notify the clinic immediately with reason for rejection. Ensure all specimens are stored properly according to manufacturers recommendations. Handle all specimens with extreme care to prevent contamination using only clean gloves and sterile tools and supplies. If gloves are suspected of touching any specimen directly, change gloves immediately. Clean and decontaminate lab benches, working areas, equipment, and biosafety cabinets after every use. Document each time an area is cleaned. If there is no documentation of a cleaning event, clean the area. Have a supervisor check the documentation of cleaning events periodically.

Handout 4-7

Center for Global Health Division of Global HIV/AIDS

International Laboratory Branch

ILB160F04D Competency Assessment (LTR13266)
Written By: Mary Garcia
Revised By: Monte Martin and Carole Moore
Approved By: Turgeon, David (2/23/2016 4:27:37 PM)
Cross , David (2/20/2016 4:07:52 AM)
Moore , Carole (2/19/2016 8:23:26 AM)
Martin, Monte (2/19/2016 7:27:50 AM)
Westerman, Larry (2/18/2016 4:49:03 PM)
Effective Date: 2/23/2016 4:27:37 PM Revision: .50

Testing Personnel Name / UserID:		**Test System:**	
[1]**Assessment Type:**	**New Testing Personnel (1st year)**		**Established Testing Personnel (2nd year and beyond)**	
	☐ Initial Competency	☐ 6 Month Competency	☐ Annual competency	☐ Corrective Action

	Specific Skills/Task/Knowledge Assessed[2]	**Successful Yes / No[3]**	**Observed by /Date**
Direct Observation: Testing • Specimen Handling and Processing • Test Performance	**Describe what testing was observed:**		
Direct Observation: Instrument/ Equipment • Instrument/Equipment Maintenance • Instrument/Equipment Function Checks	**Describe what instrument/equipment was observed:**		
Review • Recording and Reporting Results	**List what reports/results were monitored:**		
Monitor • Intermediate test results or worksheets • Quality Control • Preventive Maintenance Records • Proficiency Testing Results	**List what records were reviewed:**		

Handout 4-7 (Cont'd)

CGH/DGHT – International Laboratory Branch – Atlanta, GA

Competency Assessment Form

Assess • Test performance using previously analyzed specimens, internal blind testing samples, or external proficiency testing (PT) samples[4]	**Describe what was used to assess test performance:**		
Evaluate • Problem solving skills	**Describe how problem solving skills were assessed :**		

[1]See ILB100F23N Employee Records Checklist for date individual was authorized to begin testing.
[2]Enter details of what was observed, monitored, reviewed or assessed and attach supporting documentation.
[3]Remedial action required.
[4]Only one person may use PT for competency **during** the PT event. After the PT event closing, the PT samples may be used to assess test performance of others.

Corrective Action (if required)	
Specific Skills/Task/Knowledge:	
Action Plan:	
Reassessment Satisfactory: ☐ Yes ☐ No (Document Further Action)	**Date Completed:**
Testing Personnel: (Print Name) (Signature)	**Date:**
Assessor: (Print Name) (Signature)	**Date**

Handout 4-7 (Cont'd)

CGH/DGHT – International Laboratory Branch – Atlanta, GA
Competency Assessment Form

Final Review and Approval	
Successful completion of all six assessments demonstrates competency in all skills and knowledge to perform work independently and ensure quality testing and results.	
Testing Personnel: (Print Name) (Signature)	**Date:**
Assessor: (Print Name) (Signature)	**Date:**
Team Leader : (Print Name) (Signature)	**Date:**
Comments:	

Handout 4-7 (Cont'd)

Center for Global Health Division of Global HIV/AIDS

International Laboratory Branch

ILB160F04E Unknown Assessment Form (LTR13200)
Written By: Monte Martin and Carole Moore
Revised By:
Approved By: Turgeon, David (3/7/2016 1:45:56 AM)
Cross , David (3/1/2016 3:13:15 PM)
Moore , Carole (3/1/2016 3:09:29 PM)
Martin, Monte (3/1/2016 8:06:20 AM)
Yang , Chunfu (3/1/2016 7:21:20 AM)
Parekh, Bharat (2/3/2016 9:38:52 PM)
Alexander , Heather (12/15/2015 4:48:28 PM)
Westerman, Larry (12/15/2015 2:57:56 PM)
Effective Date: 3/7/2016 1:45:57 AM Revision: 0

Employee/Trainee Name_____

Instructions to the Employee/Trainee:
Re-analyze previously analyzed samples provided by the assessor and record results on this form.

Instructions to the Assessor:

1. Record previous test results.
2. Compare employee results with previously analyzed test results, if results are correct, mark Satisfactory. If results are not correct, mark Unsatisfactory and describe corrective action necessary to obtain a satisfactory rating.
3. Place your name and date on Assessed By line. Ask employee/trainee being assessed to sign and date form.
4. Attach form to **ILB-160-F04D Competency Assessment Form** and retain in employee records binder.

Assessment of Test Performance

Specimen#	Date Tested	Test Performed	Your Result	Expected Result (provide acceptable range for quantitative results)

Attestation: I certify the samples listed above were tested in compliance with approved laboratory procedures and Clinical Laboratory Improvement Amendments requirements specified in 42 CFR Part 493, section 801.

Handout 4-7 (Cont'd)

ILB160F04E Unknown Assessment Form (LTR13200) Revision: 0

CGH/DGHA – International Laboratory Branch – Atlanta, GA

Unknown Assessment Form

Employee/Trainee:_____ Date:_____

Assessment of Test Performance: ____ Satisfactory ____Unsatisfactory*

*If unsatisfactory complete Corrective Action section on **ILB-160-F04D Competency Assessment Form**

Assessor Comments:_____

Assessed By:_____ Date: _____

Employee/Trainee Comments:_____

Handout 4-8

Documentation and Records for Viral Load Testing

1. Quality assurance:
    - Proficiency, internal control and EQAS results logging in a quality data folder
    - Equipment daily etc. maintenance logging and service
    - QC documentation
    - Stock inventory
    - Fridge and freezer and room temperature logging sheets
    - Calibration logs (pipettes and plate washer)
2. Specimen documentation:
    - Specimens documents/log sheets
    - Chain of custody forms
    - Viral load lab request form
    - Results reporting document
3. Results reporting form:
    - Results received confirmation (email, SMS, fax, logbook, etc)
4. SOPs:
    - Testing Procedures
    - Specimen Management
    - Biohazard waste disposal
    - Blood handling safety
5. Safety documentation:
    - Training logs
        - MSDS
        - Risk Assessments
    - Incident reports
6. Management:
    - Staff training and performance management
    - Quotes, ordering, receipt of supplies, entry into inventory
    - Quarterly reports for funding bodies
    - Internal Audit forms

Handout 4-9

Guidelines for Protecting the Confidentiality of the Results

- Staff (clinical, laboratory, and transport) should read and sign a confidentiality statement.
- Patient results should be stored in a secure area. Only authorized and trained personnel can access or collect the results.
- Paper results should be delivered in a sealed envelope. A signature can be made on the seal for inspection on delivery.
- Broken signature seals should be reported, investigated and the appropriate disciplinary action taken.
- Digital or SMS results should be securely transmitted.
- Only authorized personnel can access the SMS devices and results.

Guidelines for Release of Results

- Release the results to the clinic as soon as they have been generated.
  - Alert (by calling) the clinics as soon as possible for results >1000 copies/ml.
  - Use e-mail for results reporting if internet is available.
  - If internet is not available, transmit results via SMS, fax, a courier service or the specimen transport drivers.
- If sent by courier or specimen transport drivers, place results in a sealed envelope accompanied by a transport log, signed and dated by the driver when they take custody of the report.
- Employees who send final reports must verify they are received by appropriate authorized individuals.
- Retain copies of reports at the testing laboratory for at least one year.
  - Keep hard copies in a locked cabinet.
  - Keep electronic copies in an access-restricted drive. Result folder access is limited to the laboratory manager, their designee(s), quality assurance officer, and laboratory.

Based on Guidance for Developing a Specimen Transport and Referral System for Viral Load and Infant Virologic HIV Diagnosis Testing Networks

Handout 4-9 (Cont'd)