**PROCEDURE: Coro Molecular Microbiology Cobas 6800 HIV-1**

1. **PRINCIPLE**
	1. Summary and Background
		1. Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). After seroconversion, infected individuals typically enter a clinically stable, relatively asymptomatic phase that can last for years.
		2. The asymptomatic period is characterized by persistent plasma viremia at set points determined by host genetics and a gradual depletion of CD4+ T lymphocytes. Although virus levels in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which high rates of virus production and infection of CD4+ cells are balanced by high rates of virus clearance, death of infected cells and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viremia and CD4+ cells for approximately 8 years in the average person living with HIV.
		3. Quantitative measurements of HIV viremia in the plasma have shown that higher virus levels are correlated with more rapid clinical progression of HIV disease. Furthermore, nearly two decades of clinical research have established that reductions in plasma virus levels with the use of antiretroviral therapy (ART) significantly decrease the risk of clinical progression, including death, development of AIDS, opportunistic infections, and HIV-associated morbidity. HIV viral load is also predictive of the risk of the transmission of HIV, and randomized controlled clinical trials have established that early initiation of ART with suppression of the viral load reduces HIV transmission by 96%.
		4. The cobas® HIV-1 is a quantitative test performed on the cobas® 6800 System. The cobas® HIV-1 enables the detection and quantitation of HIV-1 RNA in EDTA plasma of infected patients. Two probes are used to detect and quantify, but not discriminate group M, N and O subtypes. The viral load is quantified against a non-HIV-1 armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample processing. The RNA-QS functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.
	2. Intended Use
		1. The cobas® HIV-1 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) in EDTA plasma of HIV-1-infected individuals using the automated cobas® 6800 Systems for specimen processing, amplification and detection. The test can quantitate HIV-1 RNA over the range of 20-10,000,000 copies/mL (33 to 1.67 x 107 International Units/mL).
		2. This test is intended for use in conjunction with clinical presentation and other laboratory markers for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.
		3. Cobas® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
	3. Sample Preparation
		1. The cobas® HIV-1 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800 System consists of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800 software which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HIV-1 RNA detected, a value in the linear range. Results can be reviewed directly on the system screen, exported, or printed as a report.
		2. Nucleic acid from patient samples, external controls and added armored RNA (RNA-QS) molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.
	4. Selective Amplification
		1. Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HIV-1 genome. The HIV-1 gag gene and the HIV-1 LTR region (dual target) are amplified by **cobas®** HIV-1. Selective amplification of RNA QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.
		2. The cobas® HIV-1 master mix contains two detection probes specific for the HIV-1 target sequences and one for the RNA-QS. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target and RNA-QS in two different target channels. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS, respectively.
2. **AVAILABILITLY**
	1. Specimens may be submitted 7 days a week/ 24 hour a day.
	2. Testing is performed Monday thru Thursday
3. **TEST CODE**
	1. HIVL2
4. **SPECIAL REQUIREMENTS**
	1. Specimen Collection

**CAUTION:** Handle all specimens as if they are capable of transmitting infectious

agents.

**NOTE:** Store all samples at specified temperatures.

**NOTE:** Sample stability is affected by elevated temperatures.

**NOTE:** If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed. If necessary, centrifuge tubes to collect all sample volume at the bottom of the tube.

**NOTE:** This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing of specimen collected with other anticoagulants may result in inaccurate results.

* + 1. Whole blood should be collected in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturerinstructions.
		2. Ensure sufficient whole blood collection to allow usage of the processing volume for EDTA plasma of 500 µL (for a total minimum sample requirement of 650 µL).
			1. Collect one 6 ml EDTA tube (3ml for pediatrics) using standard venipuncture techniques.
			2. Pediatric patients <=16
				1. A second label will print with test name HIVLX. A second 3ml tube will be drawn and processed when possible to prevent QNS samples.
	1. Specimen Transport
		1. Whole blood must be transported at 2-25°C
		2. Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x G for 20 minutes at room temperature.

**Note**: Per PI: Whole blood collected in EDTA tubes may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation.

* 1. Specimen Storage
		1. Upon separation EDTA plasma samples may be stored for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. For long-term storage up to 6 months, temperatures at ≤ -60°C are recommended.
		2. Per Lab Protocol:
			1. RIH: Freeze plasma samples at -80°C immediately after centrifugation and transport to Coro Molecular Microbiology Lab
			2. TMH: Store plasma at 2-8°C and send to RIH Micro. RIH Micro will freeze plasma at -80°C and transport to Coro Molecular Microbiology lab.
			3. Coro Molecular Micro lab will store all plasma samples at -80°C
		3. Plasma samples are stable for up to four freeze/thaw cycles when stored frozen at ≤ -18°C.
1. **MATERIALS AND REAGENTS**
	1. Reagents
		1. The cobas® HIV-1 includes the following components:

|  |
| --- |
| **cobas**® **HIV-1 reagents and controls** |
| **cobas**® **HIV-1 96T Cassette** |
| **Proteinase Solution**  | **PASE** | Tris buffer< 0.05% EDTACalcium chlorideCalcium acetate8% (w/v) proteinase |
| **RNA Quantitation Standard**  | **RNA-QS** | Tris buffer< 0.05% EDTA< 0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage) < 0.1% sodium azide |
| **Elution Buffer** | **EB** | Tris buffer0.2% methyl-4 hydroxybenzoate |
| **Master Mix Reagent 1** | **MMX-R1** | Manganese acetatePotassium hydroxide < 0.1% sodium azide |
| **HIV-1 Master Mix Reagent 2** | **HIV-1 MMX-R2** | Tricine bufferPotassium acetate18% dimethyl sulfoxide, glycerol< 0.1% Tween 20, EDTA< 0.12% dATP, dCTP, dGTP, dUTPs< 0.01% upstream and downstream HIV primers< 0.01% Quantitation Standard forward and reverse primers< 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the HIV Quantitation Standard< 0.01% oligonucleotide aptamer< 0.1% Z05D DNA polymerase< 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial)< 0.1% sodium azide |
| **cobas**® **HBV/HCV/HIV-1 Control Kit** |
| **HBV/HCV/HIV-1****Low Positive Control**  | **HBV/HCV/HIV-1 L(+)C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)< 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein< 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat proteinNormal human plasma non-reactive by licensed tests for antibody to HCV antibody to HIV-1/2, HBsAg, antibody to HBc, HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods0.1% ProClin® 300 preservative |
| **HBV/HCV/HIV-1****High Positive Control** | **HBV/HCV/HIV-1 H(+)C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)< 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein< 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat proteinNormal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.0.1% ProClin® 300 preservative |
| **cobas**® **NHP Negative Control Kit** |
| **Normal Human Plasma Negative Control**  | **NHP-NC** | Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.< 0.1% ProClin® 300 preservative |
| **cobas**® **omni reagents for sample preparation** |
| **cobas omni MGP Reagent** | **MGP** | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Specimen Diluent** | **SPEC DIL** | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Lysis Reagent** | **LYS** | 42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate |
| **cobas omni Wash Reagent** | **WASH** | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate |

* + 1. The following supplies are needed but not supplied in the cobas® HIV Test Kit.

|  |  |
| --- | --- |
| **Instrumentation and Software required** |  |
|  | **cobas**®6800 System (Fix) (P/N 05524245001 and 06379664001) |  |
|  | Sample Supply Mode (P/N 06301037001) |  |
| **Materials and consumables for use on the cobas**® **6800 Systems\*** |  |
|  | **cobas** omni Processing Plate (P/N 05534917001) |  |
|  | **cobas** omni Amplification Plate (P/N 05534941001) |  |
|  | **cobas** omni Pipette Tips (P/N 05534925001) |  |
|  | **cobas** omni Liquid Waste Container (P/N 07094388001) |  |
|  | **cobas** omni Lysis Reagent (P/N 06997538190) |  |
|  | **cobas** omni MGP Reagent (P/N 06997546190) |  |
|  | **cobas** omni Specimen Diluent (P/N 06997511190) |  |
|  | **cobas** omni Wash Reagent (P/N 06997503190) |  |
|  | Solid Waste Bag (P/N 07435967001) |  |
|  | Solid Waste Container (P/N 07094361001) |  |

*\*The 1G server is provided with the system.*

* 1. Reagent Precautions
		1. Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
		2. Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
		3. Cobas omni Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
		4. cobas® HIV-1 kits, cobas omni MGP Reagent, and cobas omni Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
		5. Do not allow cobas omni Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
		6. Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
	2. Reagent Storage and Handling
		1. Do not freeze reagents or controls.
		2. When reagents are not loaded on the cobas® 6800/8800 Systems, store them at the corresponding temperature specified below:
			1. Store the cobas® HIV-1, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni Lysis Reagent, cobas omni MGP Reagent, and cobas omni Specimen Diluent at 2-8 °C.
			2. Store the cobas omni Wash Reagent at 15-25 °C.
		3. Reagents loaded onto the cobas® 6800 System are stored at appropriate temperatures and their expiration is monitored by the system. The cobas® 6800 System allows reagents to be used only if all of the conditions shown in Table below are met. The system automatically prevents use of expired reagents. Table below allows the user to understand the reagent handling conditions enforced by the cobas® 6800 System.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability** |
| **cobas®** HIV-1 | 30 days from first usage | Max 10 runs | Max 8 hours |
| **cobas®** HBV/HCV/HIV-1 Control Kit | N/A | N/A | Max 8 hours |
| **cobas®** NHP Negative Control Kit | N/A | N/A | Max 10 hours |
| **cobas omni** Lysis Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** MGP Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Specimen Diluent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Wash Reagent | 30 days from loading\* | N/A | N/A |

*\*Time is measured from the first time that reagent is loaded onto the* ***cobas®*** *6800/8800 Systems.*

* + 1. Do not use reagents after their expiration dates.
		2. Do not pool reagents. Gloves must be worn and must be changed between handling specimens and cobas® 6800 reagents to prevent contamination. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
		3. Handle all reagents with caution and avoid contact with skin, eyes, or mouth. Refer to the package insert for any known toxicity.
			1. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
			2. If spills occur on the cobas® 6800 System, follow the instructions in the appropriate cobas® 6800 System – System Manual to clean.
			3. Reagents required to perform this assay are located:

Equipment Room Refrigerator MM R 6 and MM R7.

1. **QUALITY CONTROL**
	1. Quality Control Information
		1. One negative control (–) C and two positive controls, a low positive control HIV-1 L(+)C and a high positive control HIV-1 H(+)C, are processed with each batch.
		2. Store controls at 2-8°C in refrigerator MM R6 and MM R7. Controls are stable until the expiration date indicated.
		3. Record QC results on the sheets provided. Include date of testing, kit lot #, control lot #s, expiration dates, and results.
		4. Batch validity is checked within the cobas® 6800 software (monitor) and is printed with the run report.
		5. The batch is valid if no flags appear for all three controls, which include one negative control and two positive controls.
		6. Validation of results is performed automatically by the cobas® 6800 software based on negative and positive control results.
		7. New lot numbers/shipments of HIV kits are QC’d using control kits that have passed QC.
		8. New lot numbers/shipments of Positive and Negative Control Kits are run using HIV kits that have passed QC.
		9. Verification of Performance is run on new lot numbers of HIV-1 kits and/or every 6 months, after Software Upgrades, and after major System Upgrades.
			1. A purchased Verification Panel is run which tests the linear range of the assay.
			2. Results are plotted and the R² value is determined.
			3. An unacceptable result would be an R² value <0.9900 or 2 panel members with a >0.5 Log difference from the expected result and would warrant an investigation and repeat.
		10. Environmental testing is performed monthly.
			1. The hood, instruments, and bench space is swabbed and placed in an aliquot of plasma dilution matrix and placed on the run of patient samples.
			2. For any positive pooled result, clean all areas and retest.
	2. Acceptable Limits
		1. The HIV L (+) and HIV H (+) Control results must be ‘Valid.’ The (–) Control result must be ‘Valid.’
		2. For any run, valid results must be obtained for both the Positive and Negative Control for the cobas® 6800 Software to display the reportable cobas® HIV-1 results from that run.
		3. QC statistics are calculated monthly to define analytic imprecision and to monitor trends over time.
2. Refer to Viral Load Monitoring Statistics on the M drive Molecular folder.
	1. Corrective Actions
		1. Assay will require repeating if either positive or negative controls are not valid.
		2. If the HIV L (+) and HIV H (+) Control or the (–) Control results are consistently invalid, contact your local Roche Customer Support Center for technical assistance.
	2. Control Flags
		1. Control flags for negative and positive controls:

|  |  |  |  |
| --- | --- | --- | --- |
| **Negative Control** | **Flag** | **Result** | **Interpretation** |
| (–) C | Q02(Control batch failed) | Invalid | An invalid result or the calculated titer result for the negative control is not negative. |
| **Positive Control** | **Flag** | **Result** | **Interpretation** |
| HxV L(+)C | Q02(Control batch failed) | Invalid | An invalid result or the calculated titer result for the low positive control is not within the assigned range. |
| HxV H(+)C | Q02(Control batch failed) | Invalid | An invalid result or the calculated titer result for the high positive control is not within the assigned range. |

*If the batch is invalid, repeat testing of the entire batch including samples and controls.*

*HxV L(+)C stands for cobas® HBV/HCV/HIV-1 low positive control and HxV H(+)C stands for cobas® HBV/HCV/HIV-1 high positive control in the cobas® 6800/8800 software.*

1. **TEST PROCEDURE**
	1. Procedure Notes
		1. Do not use cobas® HIV-1 reagents, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, or cobas omni reagents after their expiry dates.
		2. Do not reuse consumables. They are for one-time use only.
		3. Refer to the cobas® 6800/8800 Systems Operator’s Manual for proper maintenance of instruments.
	2. Running the test

**Note**: Refer to Cobas 6800 Systems Operator Manual on M drive/Molecular Folder/ Roche 6800.

**Note**: Refer to Coro Molecular Micro Cobas 6800 Operating Procedure for detailed procedure

**Note**: Refer to Appendix A of Cobas Operating Procedure for Cobas 6800 Quick Start Guide

**Note**: Refer to Appendix B of Cobas Operating Procedure for Cobas 6800 Maintenance

**Note**: Clean benchtops with 10% bleach followed by 70% alcohol pre and post running the assay.

* + 1. Remove frozen plasma samples from freezer to thaw and come to room temperature. They may be placed in DI H20.
		2. Required sample volume is 650 ul.
		3. Go to the 6800 **Monitor Tab** and check the taskbar and messages at the top left on the monitor screen.
			1. Address any issues or maintenance due.
		4. Refill reagents and consumables as prompted by the system:
			1. Load wash reagent, lysis reagent and diluent.
			2. Load tip racks, processing plates and amplification plates.
			3. Load Magnetic Glass Particles.
			4. Load test specific reagents.
			5. Load control cassettes.
			6. Replace rack for clotted tips.
		5. Set the system to “Ready”.
			1. In the task overview, ensure that there is no maintenance overdue.
			2. On the **Monitoring** tab, Choose the **Start** button.
				1. The system changes to **Preparing** status.
			3. Wait for the system to change to **Ready** status before you start loading. This may take 15 minutes.
		6. Organize the Viral Load runs for the day and make Tasklists.
		7. Load sample racks onto rack trays.
			1. Cobas 6800 has LIS Order Download
			2. For any samples without LIS barcode use Rack Based Ordering, ie. environmental samples.
				1. Designated HIV racks have Blue labels on them
				2. Sample ID must be entered in Manual Barcode Entry tab
		8. Bring racks and samples to hood for loading racks with samples.
			1. HIV-1 and HCV viral loads can be performed at the same time on the same processing plates.
			2. Vortex samples and discard caps
			3. Check for bubbles and remove if needed
			4. Samples can be centrifuged to collect all sample volume at bottom of tube if needed
			5. Add sample plasma tubes to sample racks
		9. Load trays with sample racks onto the Sample Supply module and go to the “Batches” tab.
			1. Monitor the “error lane” for any problems
			2. After the sample barcodes are read it will make the “batch” and list the number of HIVs or HIVs and HCVs in the batch.
				1. **Make sure that number matches the expected number of tests**.
				2. Resolve any discrepancies
		10. Hit the “**Start Manually**” button to begin processing.
			1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
		11. Monitor the instrument during processing in the Transfer Module.
			1. Address any errors or issues.

**Note: Do not walk away from instrument until samples are moved to the Processing module**

* + 1. Unload racks and samples when finished pipetting
			1. Recap tubes in the hood with new caps and store in freezer boxes
		2. Unload consumables at the end of processing:
			1. Remove amplification plates from the analytic module.
			2. Unload empty control cassettes.
			3. Empty solid waste.
			4. Empty liquid waste.
1. **RESULTS INTERPRETATION**
	1. Results
		1. From the **Routine Tab > Control Batch**
		2. Choose the control batch for your run
		3. From the drop-down list choose Print from both right and left side of screen.
			1. The printout from the left has lot #s of reagents and controls
			2. The printout from the right has the actual results of controls.
			3. View results
	2. Reporting Results- Refer to Appendix A for LIS resulting instructions.

**NOTE:** All assay and run validation is performed by the cobas® 6800 Software.

**NOTE:** A valid run may include both valid and invalid specimen results.

* + 1. The cobas® 6800 Systems automatically determines the HIV-1 RNA concentration for the samples and controls. The HIV-1 RNA concentration is expressed in copies per milliliter (cp/mL).
		2. For a valid batch, check each individual sample for flags in the cobas® 6800 software and/or report. The result interpretation should be as follows:

|  |  |
| --- | --- |
| **Results** | **Interpretation** |
| Target Not Detected | HIV-1 RNA not detected.Report results as “Not Detected.” |
| < Titer Min | Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “<20 Copies/mLTiter min = 20 cp/mL  |
| Titer | Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max.Report results as “(Titer) Copies/mL”. |
| > Titer Maxa | Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “>10,000,000 Copies/mLTiter max = 10,000,000 Copies/mL |
| a*Sample result > Titer Max refers to HIV-1 positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with HIV-1 negative EDTA plasma, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.* |

* + 1. Release Results
			1. Select all test results to be released
			2. Choose the **Release button**
				1. It is possible to release an Invalid result so use caution when releasing.
			3. Test results are sent to the SOFT Instrument Menu for posting.
		2. Invalid patient Results
			1. Retest the sample on the next run. If it repeats as invalid report as Indeterminate- Suggest repeat.
		3. Assay is not FDA approved for diagnosis of acute HIV in patients that are

HIV antibody negative. On physician request test is performed with approval from Medical Director. A disclaimer is added to lab report and results and

 statistics are reviewed by Lab Director.

* + 1. Assay will be performed on pediatric patients <2 years as the HIV Ab/Ag test cannot be performed. A disclaimer is added to lab report and results and

 statistics are reviewed by Lab Director.

1. **PROCEDURAL NOTES**
	1. Warnings and Precautions
		1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination (Refer to CLSI guideline, MM19-A10).
			1. cobas® HIV-1 is only intended for quantitation of HIV-1 viral load and is not intended for initial clinical diagnosis of HIV-1 infection.
			2. cobas® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
			3. For in vitro diagnostic use only.
			4. All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.11,12 Only personnel proficient in handling infectious materials and the use of cobas® HIV-1 and cobas® 6800 System should perform this procedure.
			5. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10).
			6. Cobas® HBV/HCV/HIV-1 Control Kit and cobas® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
			7. Do not freeze whole blood or any samples stored in primary tubes.
			8. Use only supplied or specified required consumables to ensure optimal test performance.
			9. Safety Data Sheets (SDS) are available on request from your local Roche representative.
			10. Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
			11. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
	2. Limitations
		1. The cobas® HIV-1 has been evaluated only for use in combination with the cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 6800 System.
		2. Reliable results depend on proper sample collection, storage and handling procedures.
		3. Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods.
		4. Though rare, mutations within the highly conserved regions of a viral genome covered by cobas® HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
		5. The detection rate of HIV-1 group O at 20 cp/mL (claimed LLoQ for the cobas® HIV- 1) was observed to be 90.5% which is less than what was determined for all other genotypes.
		6. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
		7. cobas® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma, or as a diagnostic test to confirm the presence of HIV-1 infection.
		8. Samples from subjects under 19 years of age were not evaluated.
	3. Interferences
		1. The analytical specificity of cobas® HIV-1 was evaluated by testing a panel of microorganisms prepared in HIV RNA negative EDTA plasma. Potential interference was evaluated by testing the same organisms in EDTA plasma containing low levels of HIV-1 RNA. None of the non-HIV pathogens interfered with test performance. Negative results were obtained with cobas® HIV-1 for all microorganism samples without HIV-1 target and positive results were obtained on all of the microorganism samples with HIV-1 target. The mean log10 titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.3 log10 of the mean log10 titer of the respective positive spike control.

|  |  |  |
| --- | --- | --- |
| **Viruses** | **Bacteria** | **Yeast** |
| Adenovirus type 5CytomegalovirusEpstein-Barr VirusHepatitis A VirusHepatitis B VirusHepatitis C VirusHepatitis D VirusHuman T-Cell Lymphotropic Virus types 1 and 2Human Herpes Virus Type-6Herpes Simplex Virus Type 1 and 2 | Varicella-Zoster VirusWest Nile VirusSt. Louis encephalitis VirusMurray Valley encephalitis VirusDengue virus types 1, 2, 3, and 4TBE Virus (strain HYPR)Influenza Virus AZika VirusHuman PapillomavirusYellow Fever Virus | Propionibacterium acnesStaphylococcus aureus | Candida albicans |

* 1. Potentially Interfering Endogenous and Exogenous Substances
		1. Elevated levels of triglycerides (up to 34.5 g/L), conjugated bilirubin (0.252 g/L), unconjugated bilirubin (0.253 g/L), albumin (58.7 g/L), hemoglobin (up to 2.85 g/L) and human DNA (2 mg/L) in samples as well as the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA) have been tested in presence and absence of HIV-1 RNA.
		2. In addition, the drug compounds listed in the following table were tested at three times the Cmax in presence and absence of HIV-1 RNA.
		3. All potentially interfering substances show no interference with the test performance.
			1. Negative results were obtained with cobas® HIV-1 for all samples without HIV target and positive results were obtained on all of the samples with HIV-1 target. The mean log10 titer of each of the positive HIV-1 samples containing potentially interfering substances was within ± 0.3 log10 of the mean log10 titer of the respective positive spike control.

|  |  |
| --- | --- |
| **Class of drug** | **Generic drug name** |
| Immune Modulators | Peginterferon α-2aPeginterferon α-2b | Ribavirin |
| HIV Entry Inhibitor | Maraviroc |  |
| HIV Integrase Inhibitors | Elvitegravir/Cobicistat | Raltegravir |
| Non-nucleoside HIV Reverse Transcriptase Inhibitors | EfavirenzEtravirine | NevirapineRilpivirine |
| HIV Protease Inhibitors | AtazanavirTipranavirDarunavirFosamprenavir | LopinavirNelfinavirRitonavirSaquinavir |
| HCV Protease Inhibitors | BoceprevirSimeprevir | Telaprevir |
| Reverse Transcriptase or DNA Polymerase Inhibitors | AbacavirEmtricitabineEntecavirFoscarnetCidofovirLamivudineGanciclovir | TenofovirAdefovir dipivoxilTelbivudineZidovudineAcyclovirValganciclovirSofosbuvir |
| Compounds for Treatment of Opportunistic Infections | AzithromycinClarithromycinEthambutolFluconazoleIsoniazid | PyrazinamideRifabutinRifampicinSulfamethoxazoleTrimethoprim |

1. **TECHNICAL ASSISTANCE**
	1. Roche Support Network Customer Support Center at 1-800-526-1247.
2. **REFERENCES**
	1. Roche Cobas HIV-1 Package Insert Rev 3
	2. Roche Cobas 6800 Operating Manual
	3. Cobas® HIV-1 Laboratory Procedure Manual 7202-00-1216, Version 0
	4. Mellors JW, Muñoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126(12):946-54.
	5. Mellors JW, Margolick JB, Phair JP, et al. Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 Cell count slope for progression to AIDS and death in untreated HIV-1 infection. JAMA, 2007 Jun 6;297(21):2349-50.
	6. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at: http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Updated 2015.
	7. Cohen MS, Chen YQ, McCauley M, et al. HPTN Study Team. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011 Aug 11;365(6):493-505.
	8. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 1990;93(1):125-8.
	9. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature. 1995 Feb 9;373(6514):487-93.
	10. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. Cell. 1995 Mar 24;80(6):869-78.
	11. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY) 1992;10(4):413-7.
	12. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res. 1996 Oct;6(10):986-94.
	13. Clinical and Laboratory Standards Institute (CLSI) MM19-A. Establishing molecular testing in clinical laboratory environments; approved guideline. CLSI Document MM-19A: Wayne, PA; CLSI, 2011.
	14. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
	15. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.
	16. Clinical and Laboratory Standards Institute (CLSI) EP06-A. Evaluation of the linearity of quantitative measurement procedures: A statistical approach. CLSI Document EP06-A:Wayne, PA; CLSI, 2003.
	17. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. HHS Publication No. (CDC) 21-1112. Biosafety in microbiological and biomedical laboratories. 5th edition. Revised December 2009.
	18. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.
	19. CLSI Document GP2-A5; Laboratory Documents: Development and Control; Approved Guideline; Fifth Edition; 2006.

 **Appendix A**

**HIV Viral Load SCC Soft Resulting**

Test ID: **HIVL2**

Template: **HIVLD**

Workstation: **MMOLM**

1. Print a “Resulting Worklist” by Template: HIVLD
	1. Status= Pending and Nonverified
	2. Start date= go back 1 month
	3. End date= current date
	4. Received box- unchecked
	5. Check list for any old outstanding orders- investigate and resolve any issues.
	6. List will be in Order sequence number from low to high. Except:
		1. STATs will go to top of list
		2. Add ons or any order that has been changed will stay at the bottom
		3. Print list:
			1. Click Printer icon
			2. Choose Worklist- Layout Horizontal or Vertical
			3. Print to Local Printer J73
	7. Use this list to check against specimens in the freezer
	8. Use this list to check other lab results, HIV AB, diagnosis, ect.
		1. There is a DX box- check diagnosis
		2. Bridge to Lab Query (magnifier glass) to check other lab results
		3. In pt info box- Click “More” for doctor, location, dx, pt phone #, ect.
2. Create a Tasklist
	1. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST
		1. Template= HIVLD
	2. Number the specimens according to the tasklist beginning with #1 and ending with #93
	3. Print the worklist and check it against the samples to verify both are in the same order.
3. Posting Results using LIS Interface
	1. View and Review results from the cobas 6800
	2. Click on all results to be released and click the **RELEASE** button
	3. Results will transfer to the Soft Instrument Menu
	4. From SoftLab, go to “Interfaces”, and “Instrument Menu”
	5. Select Cobas 6800 from the Instrument Menu
	6. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
	7. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
	8. If any Result Comments, ie. Acute HIV, (@AHIV) need to be added:
		1. Do not Post result
		2. Go to ”Lab Result” tab.
		3. Open “Comment” box for line HIPCR and add comment, ie @AHIV, to the box. OK and Save
		4. Go back to “Instrument” tab and “Post” result.
	9. Check Results
		1. Go to “Resulting Worklist” by Tasklist
		2. Choose Tasklist
		3. Enter Tasklist ID
		4. Review Worklist to verify that all results have posted. They should all have “\*” next to them/
		5. Print a new pending worklist and check on any outstanding orders.