

BioMerieux NucliSens™ HIV-1 QT System

1. INTENDED USE

The NucliSens® HIV-1 QT is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The test can quantitate HIV-1 RNA over the range of 176 to 3.47×10^6 copies/mL.

The test is intended for the use in conjunction with clinical presentation and other laboratory markers of disease progression for prognostic assessment of HIV-1 infected patients, and for monitoring the effects of antiretroviral therapy by serial measurements of plasma HIV-1 RNA for pediatric and adult patients with baseline viral loads greater than 93,000 and 28,000 copies of HIV-1 viral RNA/mL respectively.

The NucliSens® HIV-1 QT assay is not intended to be used as a screening test for HIV-1 nor is it to be used as a diagnostic test to confirm the presence of HIV-1 infection.

2. SUMMARY AND EXPLANATION OF THE TEST

Acquired Immuno Deficiency Syndrome (AIDS) is an immunosuppressive disorder characterized by depletion of the CD4+T cell population. A progressive, severe immunodeficient state is accompanied by a broad variety of clinical manifestations including opportunistic infections, an array of malignancies, and the frequent presence of neurological disorders..

The etiologic agent of AIDS is the Human Immunodeficiency Virus (HIV). It is transmitted by sexual contact, through contaminated injection needles or through administration of contaminated blood or blood products. HIV is also capable of passing through the placenta. So far, two types of HIV have been found to cause AIDS: HIV1, first isolated in 1983, and HIV2, a second distinct but related type, first isolated in 1985.

The conventional method for the detection of HIV infection is through serologic identification of an immunologic response to HIV, e.g. by ELISA, and confirmation of the results by Western Blot.

Unlike these indirect methods, nucleic acid amplification techniques such as Reverse Transcriptase PCR and NASBA do not depend on the development of an immune response to HIV, which can take 6 months or more from the time of infection to occur. They directly test for the presence of HIV RNA, The advantage of NASBA over RT PCR is that it requires neither separate reverse transcriptase step nor, as amplification is isothermal, any thermocycler equipment.

Nucleic acid amplification is suitable for quantitation of HIV1 RNA in plasma samples. Determination of the viral load appears to be a valuable marker for the prediction of disease progression and for monitoring the efficacy of anti-viral therapy, especially in early stages of the disease when conventional markers are often negative.

Quantitation with NucliSens HIV1 QT is based on co-amplification of HIV1 sample RNA together with internal calibrators, a technique that has proved to be superior to other quantitation methods. The quantity of amplified RNA is measured by means of electrochemiluminescence (ECL).

3. **PRINCIPLE:** The NucliSens HIV-1 QT assay comprises five separate stages as described below:

3.1 Nucleic Acid Release

The sample is added to NucliSens Lysis Buffer containing guanidine thiocyanate and Triton X-100 that causes the lysis of viral particles and cells in the sample and the inactivation of RNases and DNases. Nucleic acid is released.

3.2 Nucleic Acid Isolation

Three synthetic RNA's (Qa, Qb, Qc) of known high, medium and low concentration, respectively, are added to the Lysis Buffer containing the released nucleic acid. These RNA's serve as internal calibrators, each differing from the HIV-1 wild-type (WT) RNA by only a short length of sequence. Under high salt conditions, all nucleic acids in the buffer, including the calibrators, bind to silicon dioxide particles. These particles, acting as the solid phase, are washed several times. Finally, the nucleic acid is eluted from the solid phase.

3.3 Nucleic Acid Amplification

Any wild-type HIV-1 RNA present in the eluted nucleic acid is co-amplified with the three internal calibrators. Amplification is based on repeated transcription, that is, multiple copies of each wild-type and calibrator RNA target sequence are synthesized by T7-RNA polymerase by means of an intermediate DNA molecule which contains the double-stranded T7-RNA polymerase promoter. Each transcribed RNA copy enters a new amplification cycle.

The DNA intermediate is generated by binding a primer to the RNA template, extending the primer by AMV-RT (Avian Myeloblastosis Virus Reverse Transcriptase) to form an RNA-DNA duplex, degrading the RNA strand of the duplex by RNase H, binding a second primer to the remaining DNA needed for transcription. Once transcription has started, the RNA transcripts which are 'negatives' of the original RNA present in the sample will be subjected to the same process, only in this case extension is not restricted to the second primer, since the extension product of the first primer will be extended, too.

The primers (one of which contains the sequence of the T7-RNA-polymerase promoter) are complementary to two different parts of the HIV-1 RNA. Together they define the sequence within the HIV-1 gag region that is amplified. Since the NASBA process requires no strand separation, amplification is isothermal and continuous.

3.4 Nucleic Acid Detection

The quantitation of HIV-1 RNA in a sample is based on the measurement of electrochemiluminescence (ECL) with the NucliSens Reader. To differentiate among the amplicons (WT, Qa, Qb, Qc), aliquots of the amplified sample are added to four hybridization solutions, each specific for one type of amplicon. Here, the respective amplicons are hybridized with a bead-oligo (i.e. oligo bound to streptavidin coated paramagnetic beads acting as the solid phase) and a ruthenium-labeled probe. The paramagnetic beads carrying the hybridized amplicon/probe complex are captured on the surface of an electrode by means of a magnet. Voltage applied to this electrode triggers the (ECL) reaction. The light emitted by the hybridized ruthenium-labeled probes is proportional to the amount of amplicons, which in turn are proportional to the HIV-RNA in the input samples. Calculation based on the relative amounts of the four amplicons gives an estimation of the amounts of HIV-1 RNA in the sample.

3.5 Quantitation of HIV-1 RNA

The NucliSens HIV-1 QT system uses three internal calibrator RNA's for quantitation that are identical to the wild-type sequence except for a 20 base sequence recognized specifically by the detector probes. The calibrator RNA's are amplified with the same kinetics as the wild-type target, each reaction product is captured onto the magnetic bead with the same efficiency, and each calibrator reaction product can be distinguished from the other and from the wild-type product by the detector probes with distinct sequences.

The internal calibrators for quantitation are introduced into the specimen immediately after the initial lysis step. The three calibrators are included at known copy numbers. They are co-extracted and co-amplified with the wild-type nucleic acid in a single tube. The reaction product is then divided into four independent detection assays, each with one of the calibrator detector probes or with the wild-type detector probe. At the completion of the hybridization reactions, the four products are loaded into the NucliSens Reader. The ECL signal for each of the hybridization reactions is determined and the input copy number for the wild-type HIV-1 RNA, relative to the input quantities of calibrator RNA's is determined by calculating the ratio of ECL signals for WT to Qa, Qb, Qc.

4. SPECIMEN COLLECTION AND STORAGE

- 4.1 Blood should be collected in sterile tubes using EDTA, citrate or heparin as an anticoagulant.
- 4.2 Whole blood specimens collected using EDTA as an anticoagulant can be stored at room temperature (15-30°C) for up to 24 hours before processing without detectable loss of HIV-1 RNA. EDTA plasma can be stored at 2-8°C for up to 14 days.
- 4.3 Preferred storage of plasma: -70°C or colder.
- 4.4 Plasma collected using EDTA, citrate or heparin is stable for ≥ 1 year at -70°C and can be frozen and thawed up to three times with no significant loss of HIV-1 RNA reported by the NucliSens HIV-1 RNA QT system. Specimens repeatedly

frozen and thawed or those containing particulate matter may give erroneous results.

- 4.5 In Lysis Buffer, EDTA plasma specimens can be stored:
 - 4.5.1 up to one year at -70°C or
 - 4.5.2 for a maximum of 14 days at 2-8°C or
 - 4.5.3 for a maximum of 24 hours at room temperature.

NOTE: Do not store specimens in Lysis Buffer at -20°C.
- 4.6 Purified RNA eluate (post Isolation) can be stored at 12 months at -20 or -70°C or 14 days at 2-8°C.
- 4.7 Amplified material may be stored for 12 months at -20°C.
- 4.8 If the shipment can be made within 24 hours of collection, the specimen may be shipped at room temperature. Frozen specimens should be transported on dry ice.

Note: Specimens in Lysis Buffer should be shipped at a temperature \leq -30°C (on dry ice).

5 REAGENTS: Four separate modules are required to be able to perform 50 complete NucliSens HIV-1 QT tests. Isolation reagents, amplification reagents, and detection reagent kits contain reagents sufficient for five 10-test batches.

5.1	NucliSens Lysis Buffer (9mL/tube)	Product Number 84047
5.2	NucliSens Isolation Reagents	Product Number 84160
5.3	NucliSens HIV-1 QT Amplification Reagents	Product Number 84152
5.4	NucliSens HIV-1 QT Detection Reagents	Product Number 84043
5.5	NucliSens HIV-1 RNA Controls	Product Number 84010

Note: For technical assistance, contact bioMerieux, Inc. at 1-800-682-2666.

5.7 Nucleic acid release

Store NucliSens Lysis Buffer at 2-8°C in an area intended for the preparation of specimens for nucleic acid release. Remove reagents from the refrigerator only in quantities needed for the number of tests to be performed. Protect from excess heat or light. Lysis buffer is supplied in 500-mL bottles from which aliquots are made.

- 5.7.1 Lysis Buffer: 50 tubes (9mL/tube)
5 mol/L guanidine thiocyanate, TritonX-100, TRIS/HCL

5.8 Nucleic acid isolation

Store the NucliSens Isolation Reagents at 2-8°C in the laboratory area intended for isolation of nucleic acid. Protect from excess heat and light. **Note: When performing a NucliSens HIV-1 QT test use only amplification and detection reagents from modules of which the first six digits of each of the lot numbers printed on the box label correspond.**

- 5.8.1 Wash Buffer: 50 tubes (9mL/tube)
5 mol/l guanidine thiocyanate, TRIS-HCL
- 5.8.2 Silica: 5 tubes (0.8mL/tube)
acid-activated silicon dioxide particles
- 5.8.3 Elution Buffer: 5 tubes (1.5mL/tube)
TRIS/HCL

5.9 Nucleic Acid Amplification

Store Amplification reagents at 2-8°C in the laboratory area intended for amplification. Remove reagents from the freezer only in quantities needed for the number of tests to be performed. Protect from excess heat or light.

- 5.9.1 Calibrators: 5 x 6mg
Lyophilized synthetic RNA (Qa, Qb, Qc) sphere, each tube contained in a foil pack with desiccant. **Color code: Yellow capped tube.**
- 5.9.2 Enzymes: 5 x 6.5mg
Lyophilized sphere containing AMV-RT, RNase H, T7-RNA polymerase and BSA, each tube contained in a foil pack with desiccant. **Color code: Red capped tube.**
- 5.9.3 Enzyme Diluent: 5 x 0.5mL
TRIS/HCL. **Color code: Red capped tube.**
- 5.9.4 Primers: 5 x 10mg
Lyophilized sphere with synthetic primers, nucleotides, dithiothreitol, KCL and MgCL₂; each tube contained in a foil pack with desiccant. **Color code: Blue capped tube.**
- 5.9.5 Primer Diluent: 5 x 0.5mL
TRIS/HCL, 30% DMSO. **Color code: Blue capped tube.**

5.10 Nucleic Acid Detection

Store Detection reagents at 2-8°C in the laboratory area intended for detection of amplified nucleic acids. Protect from excess heat and light.

- 5.10.1 Bead-Oligo: 2 x 1.68mL
DNA oligo bound to streptavidin-coated paramagnetic beads with preservative. Pink capped tube.
- 5.10.2 Wild-Type probe: 1 x 0.84mL
Ruthenium-labeled DNA oligo with preservative. White capped tube.
- 5.10.3 Qa probe: 1 x 0.84mL
Ruthenium-labeled DNA oligo with preservative. Red capped tube.
- 5.10.4 Qb probe: 1 x 0.84mL
Ruthenium-labeled DNA oligo with preservative. Yellow capped tube.
- 5.10.5 Qc probe: 1 x 0.84mL
Ruthenium-labeled DNA oligo with preservative. Blue capped tube.
- 5.10.6 Detection diluent: 2 x 15mL
TRIS/HCL with preservative.
- 5.10.7 Instrument Reference Solution: 1 x 1.7mL
Streptavidin-coated paramagnetic beads.

5.11 Additional materials required

- 5.11.1 Centrifuge capable of 1,500 x g for 9mL Lysis Buffer tubes and centrifuge capable of 10,000 x g for 1.5mL test tubes.
- 5.11.2 Calibrated micropipets with variable settings for 5 through 1,000 uL delivery volumes.
- 5.11.3 Sterile-packaged, disposable, aerosol-resistant tips.
- 5.11.4 1% sodium hypochlorite solution (a freshly prepared 1:5 dilution of household bleach).
- 5.11.5 Disposable powder free gloves.
- 5.11.6 70% (v/v) ethanol prepared from 100% ethanol, molecular grade. Use nuclease-free water for dilution.

- 5.11.7 Disposable transfer pipettes or aspirating mechanism.
- 5.11.8 Heat block capable of heating 1.5mL tubes to $56 \pm 1^{\circ}\text{C}$, $41 \pm 0.5^{\circ}\text{C}$, and $65 \pm 1^{\circ}\text{C}$.
- 5.11.9 Acetone (analytical grade).
- 5.11.10 RNase free 1.5mL and 10.0mL screw cap test tubes.
- 5.11.11 Timer.
- 5.11.12 Vortex.
- 5.11.13 RNase free 0.5mL flip-cap tubes for amplification.
- 5.11.14 Thermal cycler programmed for 41 degrees and 65 degrees.
- 5.11.15 Polypropylene tubes (5mL) for hybridization and test tube racks.
- 5.11.16 Water bath to heat 5mL tubes to $41 \pm 0.5^{\circ}\text{C}$.
- 5.11.17 Repeater pipette and tips.
- 5.11.18 Plate sealers or tape to cover hybridization tubes.
- 5.11.19 NucliSens Reader with Personal Computer.

6 CALIBRATION

A calibration curve is performed with each sample and control tested.

7 QUALITY CONTROL

The integrity of each individual result can be monitored by reference to the performance of the three internal calibrators. However, it is recommended that a high Positive Control, low Positive Control and a Negative Control be included with each run. If using the NucliSens HIV RNA Controls, the expected range for the Positive Controls is stated in the package insert for the controls. The copy number per mL for each Positive Control should fall within the range indicated in the package insert. The Negative Control should give a less than lower detection limit result. If controls are not as expected, the run should be repeated. Four VQA HIV RNA Copy Controls could also be included in each run. A run is deemed valid if all four controls fall within the ranges provided by the VQA for that particular lot. VQA HIV RNA Copy Controls may be obtained from the VQA website (<http://aactg.s-3.com/vqaform.htm>).

- 7.1 VQA RNA Copy Controls: RNA Standard Assay 1.25 mL (includes 0, 1,500, 15,000 and 150,000 cp/mL controls)

VQA HIV RNA Copy Controls are intended to be single use only and should be maintained at -70°C or colder until used, and should not undergo multiple freeze/thaw cycles. For more information please contact the VQA (VQA@rush.edu).

- 7.2 Proposal for Basic Criteria to define a valid run- any failure of the control criteria results in an invalid run. All samples and controls need to be re-extracted, re-amplified, and re-detected.

Table 1:
 VQA Copy Control **
 Ranges

VQA CC	Assay	Assay SD	-3SD	-2SD	-1SD	Mean	+1SD	+2SD	+3SD
log 3	NucliSens	0.15	532	752	1062	1500	2118	2992	4227
log 4	NucliSens	0.15	5321	7516	10616	15000	21184	29923	42267
log 5	NucliSens	0.15	53211	75162	106169	150000	211836	299226	422669

**Means will be established with each copy control production, but SD will remain constant. If any copy control falls outside the 3SD range, then the entire run is invalid and all specimens and controls will need to be re-extracted, amplified and detected.

- 7.2.1. All copy control results must be valid
- 7.2.2. $Q_a > Q_b > Q_c$ (Q_c dropout is acceptable)
- 7.2.3. Initial failure can be re-detected
- 7.2.4. Re-detections should not be less than 1:2 dilution
- 7.2.5. VQA Control results must demonstrate the following pattern
 $\log_3 < \log_4 < \log_5$
- 7.2.6. VQA copy control result must be within range (Table 1 shows example of VQA control range sheet)
- 7.2.7. 0 copy control must be undetectable (<LDL)

7.3 **Proposal for Basic Criteria to define a sample repeat-** if these criteria are observed, the sample must be re-extracted, re-amplified and/or re-detected as defined.

- 7.3.1 For any undetectable sample, Q_a signal must be greater than 250,000, **re-detect**
- 7.3.2 Q_c drop-out on an undetectable sample- re-extract, **re-amplify and re-detect**
- 7.3.3 Any sample flagged by the OTC reader as re-detect, must be **re-detected**
- 7.3.4 Any sample flagged by the OTC reader as invalid, **re-extract, re-amplify and re-detect**

8 WARNINGS AND PRECAUTIONS

- 8.1. All materials of human origin should be handled as though they contain potentially infectious agents.
- 8.2. Lysis Buffer and Wash Buffer contain guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled, comes in contact with skin and/or if swallowed. Contact with acid or bleach liberates very toxic gas.
- 8.3. The waste material from the extraction process should be disposed of separately and not mixed with any other laboratory waste.
- 8.4. Prepare Lysis Buffer before starting nucleic acid release. Make sure any crystals in the Lysis Buffer have dissolved.

- 8.5. Make sure reagents and samples are at room temperature (15-30°C) before starting nucleic acid release, isolation, amplification and detection, respectively.
- 8.6. All reagents must be mixed thoroughly before use. **Note: Enzymes must not be vortexed.** Ensure complete reconstitution of the lyophilized spheres by gently flicking the closed tube with your finger after the addition of the Enzyme Diluent. Before opening the tube that contains lyophilized material, make sure the lyophilized material is at the bottom of the tube.
- 8.7. When performing a NucliSens HIV-1 QT test, the first six digits of each of the amplification and detection reagent modules lot numbers printed on the box label should match. The lysis buffer and isolation module used in a test run may be from any lot.
- 8.8. Specimens that have been repeatedly frozen and thawed or that contain particulate matter may yield erroneous results. However, specimens containing particulate matter may be used for testing after centrifugation.
- 8.9. Label tubes with appropriate patient information.
- 8.10. Do not use materials after the expiration date given on the package label. Return unopened isolation reagents, amplification reagents and detection reagents immediately to 2-8°C.
- 8.11. Keep testing areas separate from areas where blood or blood products are stored.
- 8.12. Do not pipette any of the materials by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- 8.13. Do not perform the test in the presence of reactive vapors (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust because the enzymatic activities may be affected.
- 8.14. Use disposable powder free gloves and handle all materials used in the test, including samples, reagents, and pipettes, cautiously as though capable of transmitting infectious agents.
- 8.15. Immediately clean up any spillage containing potentially infectious agents with a 1:10 dilution of 5%(w/v) sodium hypochlorite and rinse well with distilled water.
- 8.16. Dispose of all specimens and materials used to perform the test as if they contain infectious agents.

9 TEST PROCEDURE

- 9.1. NUCLEIC ACID RELEASE AND ISOLATION.
 - 9.1.1. Fill out EXTRACTOR RUN WORKSHEET including Accession #, patient name and volume of sample used: 1000uL for HIVQT. **Note: If 1000 uL is not available, use smaller volume and adjust sensitivity range when reporting results.**
 - 9.1.2. Warm lysis buffer tubes and thaw specimen tubes at 37°C for about 20 min.
 - 9.1.3. Add specimens to lysis buffer. Vortex. Incubate at 37°C for 30 min.
 - 9.1.4. Prepare HIV-1CALIBRATOR by adding 220 uL ELUTION BUFFER to spherule. Vortex. Quick spin.
 - 9.1.5. Add 20uL of calibrator to each lysis tube. Vortex.
 - 9.1.6. Vortex silica solution and add 50uL to each lysis tube. Vortex silica between additions to ensure an even suspension.
 - 9.1.7. Place lysis tubes on rotator for 20 min.

- 9.1.8. Set up the extractor and prepare the extractor cartridges. (Refer to Extractor Procedure).
- 9.1.9. Add contents of lysis tubes to the extractor cartridges and start extractor run.
- 9.1.10. After extractor run is completed, remove eluate tubes and store at -20°C if amplification procedure is not started immediately.

9.2. NUCLEIC ACID AMPLIFICATION

- 9.2.1. Thaw Enzyme and Enzyme Diluent and Primer and Primer Diluent.
- 9.2.2. Add 55uL Enzyme Diluent to the lyophilized Enzymes. Finger flick to mix. Allow to stand 15 minutes. **Do Not Vortex.**
- 9.2.3. Add 120uL Primer Diluent to the lyophilized Primers. Vortex immediately until solution is clear. **Do Not Spin. Note: If more than 10 samples are being run, additional Enzyme and Primer must be made up. Store leftover Enzyme and Primer at -20°C. Previously frozen primer solution must be heated at 65°C for 5 minutes before use.**
- 9.2.4. Prepare worksheet for your run, which may consist of a maximum of 12 samples: negative control in tube 1, patients in tubes 2 - 11 and positive control in tube 12.
- 9.2.5. Remove one negative control aliquot and one positive control aliquot from -20°C. Label 0.5mL bullets for patient samples.
- 9.2.6. Pipette 5uL of each patient sample to appropriately labeled tube. (If eluate tubes have been frozen, vortex and quick spin each tube before pipeting.)
- 9.2.7. Add 10uL Primer solution to each tube containing 5uL extracted RNA.
- 9.2.8. Place tubes in the PTC-100 thermocycler. Scroll to Program NAS1 and press Proceed. (NAS1 - 65°C for 5 min., 41°C for 15 min.)
- 9.2.9. Set timer for 10 min.
- 9.2.10. Press Pause. Add 5uL Enzyme to each tube. Press Pause to restart timer and incubate at 41°C for about 5 min.
- 9.2.11. Press Cancel. Immediately scroll to NAS2 and press Proceed. (NAS 2 - 41°C for 90 min, 4°C ∞.)
- 9.2.12. Amplified tubes that are not to be immediately used for detection may be stored for up to 1 year at -20°C.

9.3. NUCLEIC ACID HYBRIDIZATION

- 9.3.1. Allow Detection reagents to reach room temperature.
- 9.3.2. Transfer amplified samples to the NASBA Detection Room. Vortex tubes and quick spin.
- 9.3.3. Prepare the hybridization reagents as follows: (quantities shown are for a full run of 12 specimens including controls.)
 - 9.3.3.1. Prepare and label 4, 1.5ml tubes: WT, Qa, Qb, Qc.
 - 9.3.3.2. Vortex Bead-oligo (pink) until an opaque solution is formed.
 - 9.3.3.3. Immediately after vortexing, add 140uL Bead-oligo to each tube (WT, Qa, Qb, Qc). Recap the tubes.
 - 9.3.3.4. Add 140uL of WT-ruthenium labeled probe (white) to the WT tube. Recap the tube.

- 9.3.3.5. Add 140uL of Qa-ruthenium labeled probe (red) to the Qa tube. Recap the tube.
- 9.3.3.6. Add 140uL of Qb-ruthenium labeled probe (yellow) to the Qb tube. Recap the tube.
- 9.3.3.7. Add 140uL of Qc-ruthenium labeled probe (blue) to the Qc tube. Recap the tube.

Note: Use hybridization tubes within 1 hour of preparation.

- 9.3.4. Prepare and label 4, 5mL (12 x 75 mm) polypropylene tubes per sample and controls and 1 to use as blank. Label tubes 1 WT, 1 Qa, 1 Qb, 1Qc, 2 WT, etc.
- 9.3.5. Vortex the WT hybridization solution. Add 20uL (use long tips) of WT hybridization solution to the bottom of each hybridization tube designated WT and to an empty AN (assay negative) tube.
- 9.3.6. Vortex the Qa hybridization solution and add 20uL to Qa tubes. Do the same for Qb and Qc hybridization solutions.
- 9.3.7. Dilute the amplified sample:
 - 9.3.7.1. Prepare and label one 1.5mL tube for each sample and control. Add the indicated volume of Detection Diluent (specified on the outside label of the detection kit) to each tube and cap each tube.
 - 9.3.7.2. Add 5uL of each amplified sample or control the appropriate diluent tube using a fresh tip for each sample. Cap tubes, vortex and quick spin.
- 9.3.8. Add 5uL diluted sample and control to each of 4 hybridization tubes containing WT, Qa, Qb, and Qc. Use long tips and add sample into the hybridization solution.
- 9.3.9. Add 5uL Detection Diluent to the AN tube.
- 9.3.10. Cover the hybridization tubes and AN with adhesive cover.
- 9.3.11. Vortex entire rack. Place in 41°C water bath for 30 ± 2 min. During hybridization, mix the tubes by shaking the rack every 10 minutes.
- 9.3.12. Add 300uL Assay Buffer to each tube to stop the reaction.

9.4. NUCLEIC ACID DETECTION

During the 90 min amplification or 30 min hybridization the NucliSens Reader maintenance can be performed (Refer to maintenance sheet) and the Worklist prepared.

- 9.4.1. Prepare worklist as follows:
 - 9.4.1.1. Under the "Routine" menu, click on "New Run".
 - 9.4.1.2. Click "Yes" to flush worklist.
 - 9.4.1.3. Verify default file name click "OK."
 - 9.4.1.4. Click on "Select Assay" box; select "HIV-1 QT".
 - 9.4.1.5. Enter Acc #, patient last name or control.
 - 9.4.1.6. Type in volume of sample used (amount added to lysis buffer tube.)
 - 9.4.1.7. Click on "Add to list."
 - 9.4.1.8. Sample Data screen appears. Click on "Amp. Batch ID" field.
 - 9.4.1.9. Enter the Amplification batch ID (8 digit lot number). Note: Steps J-M require information that is found on the outside of the detection module and is lot specific.**
 - 9.4.1.10. Enter the Detection batch ID (8 digit lot number).

- 9.4.1.11. Enter the V parameter.
- 9.4.1.12. Enter the Calibrator log values for Qa, Qb, Qc.
- 9.4.1.13. Enter the Checksum value.
- 9.4.1.14. Click on "Change, Set Default"
- 9.4.1.15. Enter 2nd sample ID, volume, "add to list". Repeat for all other samples and controls.
- 9.4.1.16. Click on "Close".
- 9.4.1.17. Verify for correctness, then click "OK".
- 9.4.2. Vortex the Instrument Reference Solution until opaque. Add 250uL to fresh 5mL polypropylene tube. Place the tube in position #1 on NucliSens Reader carousel.
- 9.4.3. Place blank tube (AN) on carousel position #2. Load the hybridization tubes on the carousel according to the sample position on the worklist.
- 9.4.4. Under the "Routine" menu, click on "Run Worklist".
- 9.4.5. Click "Proceed".
- 9.4.6. When the run is complete click on "Routine" menu, "Assay Results."
- 9.4.7. Click on "Print All."

10. CALCULATIONS

The NucliSens Reader automatically calculates the number of Wild-Type (WT) HIV-1 RNA copies/mL in the plasma sample. Refer to the NucliSens Reader Operator Manual for the viewing, printing, storing or retrieving of results.

The three Calibrator signals (Qa, Qb, Qc) and total ECL signal (WT+Qa+Qb+QC) are corrected for background noise and checked against a number of validity criteria (e.g., total signal, calibrator deviation from the calibration curve). A sample is qualified as valid or invalid depending on the outcome of these validity checks. When the result has been qualified as valid, the calibrators are used to plot a calibration curve upon which the WT concentration is calculated. A quantitative result is only given if the samples estimated number of copies falls within the quantitative range of 25 to approximately 5 million copies per mL.

The following valid results may be observed:

Result Quantification	Example	Explanation
Below lower detection limit	<LDL	The number of WT copies is below the lower detection limit of the assay.
Within quantitation range	1000 copies/mL	Results are within the quantitative range.
Above upper limit of quantification	>>UDL copies/mL	The number of WT copies is more than 2 logs higher than the number of Qa input copies. Repeat the test using a reduced sample volume, so that the calculated number of copies will fall into the measurable range of the instrument.

11. ASSAY WARNINGS

- 11.1. Qc out of tolerance, Qc discarded:** This message appears when the observed signal for calibrator Qc is out of range. For calculation of the WT concentration only Qa and Qb are used, but the assay is still valid. An increased frequency of this message may indicate suboptimal performance of the assay (e.g. temperature of heating blocks or water baths).
- 11.2. Redetect:** If the test result is classified as invalid and the error messages **sensitivity shifted redetect, WT signal too high, or Qa signal too high** appear, a volume of Detection Diluent other than the one indicated on the box of the Detection module should be used to achieve optimum results. This means that the dilution factor is to be adjusted (see equation below) in a way that the Qa signal will yield approximately 1,000,000 counts at estimated WT inputs of less than 10^4 copies. The detection should subsequently be repeated to make the optimum use of the dynamic rang of the NucliSens Reader.

The new dilution factor = current dilution factor x (Qb signal/200,000)

- 11.2.1. Sensitivity shifted redetect with other dilution factor
- 11.2.1.1. WT < LDL and ECL signal for the calibrators too low
 - 11.2.1.2. Repeat the detection using a lower dilution factor.
- 11.2.2. WT signal too high
- 11.2.2.1. WT ECL signal too high and second excitation signal of WT not within the accepted range.
 - 11.2.2.2. Repeat the detection using a higher dilution factor, so that the WT signal will fall into the range of the instrument. If necessary, dilute the sample with negative human plasma and repeat the test starting from the nucleic acid release.
- 11.2.3. Qa signal too high
- 11.2.3.1. Qa ECL signal too high and second excitation signal of Qa not within the accepted ranged.
 - 11.2.3.2. Repeat detection using higher dilution factor so that the Qa signal will fall into the range of the instrument.
- 11.3. Retest:** If the test result is classified as invalid with the error messages mentioned below appear, the sample must be retested:
- 11.3.1. Sensitivity shifted due to less efficient amplification
- 11.3.1.1. WT < LDL and Qc < LDL
 - 11.3.1.2. Repeat the test starting from nucleic acid release.
- 11.3.2. Calculated copies for Qa and /or Qb out of tolerance
- 11.3.2.1. Qa and/or Qb deviate from the calibration
 - 11.3.2.2. Repeat the test starting from nucleic acid release
- 11.3.3. No amplification
- 11.3.3.1. Total ECL signal too low
 - 11.3.3.2. Repeat the test starting from nucleic acid release

12. REPORTING RESULTS

The NucliSens HIV-1 QT is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The test can quantitate HIV-1 RNA over the range of 176 to 3.47×10^6 copies/mL. The NucliSens

assay can accurately detect a 0.3 log₁₀ (2-fold) or greater change in the HIV-1 RNA for patients whose viral load is between 3,500 copies/mL and 3,500,000 copies/mL. The NucliSens HIV-1 QT can accurately detect a 0.9 log₁₀ (9-fold) change in HIV-1 RNA for patients whose viral load is between 180 and 850 copies/mL.

The limit of detection (LOD) is defined as the lowest HIV-1 RNA input level where at least 95% of the tests produce a result indicative of reactivity of the input sample for HIV-1. The limit of quantitation (LOQ) is defined as the lowest HIV-1 RNA input level where at least 95% of the tests produce quantifiable (within linear range) results with reasonable accuracy and precision. The LOD and LOQ for NucliSens HIV-1 QT were determined by multiple testing at three sites of specimens containing HIV-1 RNA concentrations ranging from 41 to 659 copies/mL. Five specimens representing a range of HIV-1 RNA concentrations were selected and tested 72 times each using three lots of reagents.

Logistic regression was used to determine the relationship between the proportion detected and the log nominal input. The LOD was calculated to be 176 HIV-1 RNA copies/mL. This HIV-1 RNA input level for the LOD was verified by the results of the testing panel members. For one panel member (nominal input = 164 HIV-1 RNA copies) the observed proportion of tests with positive results was 95.8%. The 95% detection rate was determined by logistic regression analysis to be 176 copies/mL. The coefficients of variation (CV) for panel members 15 and 16 with input concentrations of 329 and 164 copies/mL, were 53% and 94%, respectively (see table below). Based on these results, the LOQ has been determined to be equal to the LOD, i.e. 176 copies/mL.

Panel Member	HIV-1 RNA Input	HIV-1 log ₁₀ Input	Total Tested	Number Tested Positive	Observed Fraction Positive	%CV
14	659	2.8189	72	72	1.0000	72
15	329	2.5172	72	72	1.0000	53
16	164	2.2148	72	69	0.9583	94
17	82	1.9138	72	51	0.7083	98
18	41	1.6128	72	38	0.5278	100

13. INTERFERING SUBSTANCES

Elevated levels of lipids, bilirubin, and hemoglobin in specimens do not interfere with the quantitation of HIV-1 RNA by the NucliSens HIV-1 QT assay. The presence of antinuclear antibodies or rheumatoid factor showed no detrimental effect of the quantitation of HIV-1 RNA load, as were the specimens from multiparous women or from pregnant women. The presence of platelets in plasma does not appear to interfere. The following compounds have been found not to interfere with the quantitation of viral load by this assay: AZT, ddl, d4T, ddC, 3TC, indinavir, ritonavir, saquinavir, gancyclovir, acyclovir, zithromax-azithromycin, biacin-clarithromycin, clofazamine, ethionamide, pentamidine, bactrin-trimethoprim sulfamethoxazole, dapsone, and diflucan.

14. LDMS

Refer to the LDMS user manual. The most recent version for the NucliSens assay may be viewed on the DMC website at http://www.fstrf.org/ldms/manual/3.9/virology_3.9.pdf. In order to access the website, you must first register with the DMS at <http://www.fstrf.org>.

15. REFERENCES

Refer to the Package Insert.

Procedure: ACTG Lab Man BioMerieux NucliSens

Prepared by: ACTG Laboratory Technologist Committee

Preparation Date: 01 June 2004

Date Implemented into the Laboratory: _____

Updated on:

Reviewed by:

Date:

Supersedes Archived Manual: DAIDS Virology Manual for HIV Laboratories, Version
January 1997