WHO Prequalification of Diagnostics Programme
PUBLIC REPORT
Product: NucliSENS EasyQ® HIV-1 v2.0 (Automated)
Number: PQDx 0127-016-00

Abstract

The NucliSENS EasyQ® HIV-1 v2.0 (Automated) with product codes[1] 280140, 280130, 280131, 280132, 280133, 280134, 285056, 200309 and 285033, manufactured by bioMérieux SA, Chemin de l'Orme, 69280 Marcy L'Etoile, France, CE-marked regulatory version, was accepted for the WHO list of prequalified diagnostics and was listed on 23 December 2011.

The NucliSENS EasyQ® HIV-1 v2.0 (Automated) is a nucleic acid amplification assay for the quantitative determination of HIV-1 RNA in human EDTA plasma and EDTA whole blood spotted on cards (DBS). It is intended to be used for NASBA-based amplification and real-time detection of isolated HIV-1 RNA. The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of anti-retroviral therapy be measuring changes in plasma/DBS (from EDTA whole blood) HIV-1 RNA levels during the course of anti-retroviral treatment.

The NucliSENS EasyQ® HIV-1 v2.0 (Automated) consists of nucleic acid amplification combined with a simultaneous detection step. The process requires isolated nucleic acids a s starting material. The nucleic acids are isolated with NucliSENS® miniMAG® or NucliSENS® easyMAG®. The assay is compatible with NucliSENtral® to support the exchange of electronic data between NucliSENS EasyQ® Director software and NucliSENS® easyMAG® User software.

The NucliSENS EasyQ® HIV-1 v2.0 (Automated) assay must not be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of an HIV-1 infection.

In order to perform the assay, the following components are required:

Instrumentation:

- NucliSENS easyMAG configuration 280140
- NucliSENS Easy Q configuration 200309
- Mini Strip Centrifuge 285056

Reagents for nucleic acid isolation:

- NucliSENS easyMAG extraction Buffer 1 280130

[1] See page 2 for a list of components required to perform the assay.

- NucliSENS easyMAG extraction Buffer 2 280131
- NucliSENS easyMAG extraction Buffer 3 280132
- NucliSENS easyMAG extraction Lysis Buffer 280134
- NucliSENS easyMAG magnetic silica 280133

Reagents for amplification:

- NucliSENS Easy Q HIV-1 V2.0 285033

Consumables for nucleic acid isolation:

- Biohit Tips 280146
- EasyMAG disposables 280135
- Strip Plates Greiner 278303

Consumables for amplification:

- EasyQ 8-Tube Caps 285051
- EasyQ 8-Tube Strips 285048

Storage:

- The NucliSENS Easy Q HIV-1 V2.0 test kit, 285033 should be stored at 2-8 °C.
- The NucliSENS easyMAG extraction Buffer 1, 280130 should be stored at 2-30°C
- NucliSENS easyMAG extraction Buffer 2, 280131 should be stored at 2-30°C
- NucliSENS easyMAG extraction Buffer 3, 280132 should be stored at 2-8°C
- NucliSENS easyMAG extraction Lysis Buffer, 280134 should be stored at 2-30°C
- NucliSENS easyMAG magnetic silica, 280133 should be stored at 2-8°C

Shelf-life:
The NucliSENS Easy Q HIV-1 V2.0 test kit, 285033 has a shelf-life upon manufacture of 18 months.
Reagents for nucleic acid isolation have a shelf-life upon manufacture of:
NucliSENS easyMAG extraction Buffer 1, 280130: 24 months
NucliSENS easyMAG extraction Buffer 2, 280131: 18 months
NucliSENS easyMAG extraction Buffer 3, 280132: 15 months
NucliSENS easy MAG extraction Lysis Buffer, 280134: 24 months
NucliSENS easy MAG magnetic silica, 280133: 18 months

Summary of prequalification status for NucliSENS Easy Q® HIV-1 v2.0

	Initial acceptance	
	Date	**Outcome**
Status on PQ list	23 December 2011	listed
Dossier assessment	05 December 2011	MR
Inspection status	23 November 2011	MR
Laboratory evaluation	FT	MR

MR: Meets Requirements, NA: Not Applicable, FT: Fast-tracked

The NucliSENS EasyQ® HIV-1 v2.0 (Automated) was accepted for the WHO list of prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

bioMérieux SA submitted an application for prequalification of the NucliSENS EasyQ® HIV-1 v2.0 (Automated). Based on the established prioritization criteria, NucliSENS EasyQ® HIV-1 v2.0 (Automated) was given priority for prequalification.

Product dossier assessment

bioMérieux SA submitted a product dossier for the NucliSENS EasyQ® HIV-1 v2.0 (Automated) as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for NucliSENS EasyQ® HIV-1 v2.0 (Automated) for prequalification.

Commitments for prequalification:
The manufacturer committed to amend and submit additional documentation on the following issues:

1. a revised version of the instructions for use.

Manufacturing site inspection

An inspection was performed at the site of manufacture (Centre Christophe Mérieux, 5 rue des Berges, 38024 Grenoble Cedex 01, France) on 30,31 August 2011 as described in 'Information for manufacturers on WHO prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1)'.

The 'fast track' inspection found that the manufacturer had a well-established quality management system and manufacturing practices in place that would ensure the manufacture of a product of consistent quality.

The manufacturer has committed to respond to the observations and nonconformities identified during the inspection.

Commitments for prequalification:

1. Risk assessment will be reconsidered to include risk analysis and mitigation for end users in resource limited and environmentally challenging regions to which the product is distributed.
2. Minor nonconformities identified in the production of the product will be addressed.
3. bioMérieux SA will move towards improving customer feedback mechanisms from representative organizations of users in resource limited regions where communication may be problematic.

Laboratory evaluation

Given the regulatory version of the product submitted for prequalification and the quality of the data submitted as part of the product dossier to support the claims for its intended use, the NucliSENS EasyQ® HIV-1 v2.0 (Automated) assay has been found eligible to undergo the WHO fast track procedure. Subsequently, the product will not be required to undergo a laboratory evaluation for its use with human plasma.

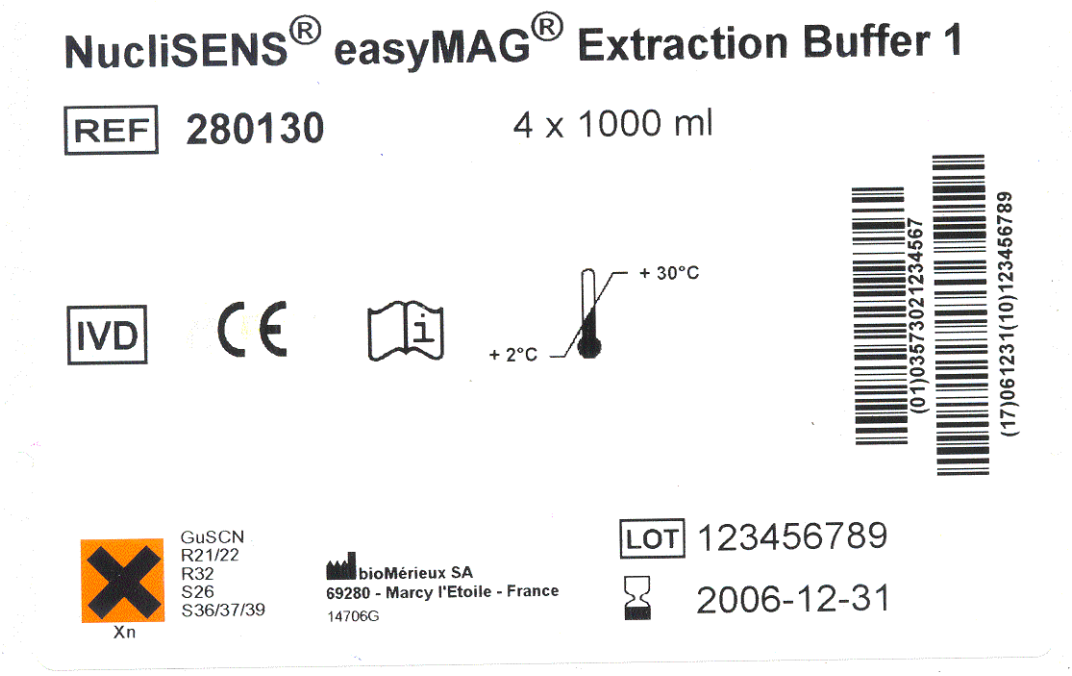
Nevertheless, taking into consideration the needs of WHO Member States and in an effort to support current attempts to increase access to HIV Viral Load testing, a laboratory evaluation of the NucliSENS EasyQ® HIV-1 v2.0 (Automated) will be carried out to assess its performance with the use of Dried Blood Spots (DBS). The results from the evaluation will not impact the WHO Prequalification status for use with human plasma and will serve as a source of information in order to advise WHO Member States in their efforts to scale up HIV Viral Load Testing.

Labelling

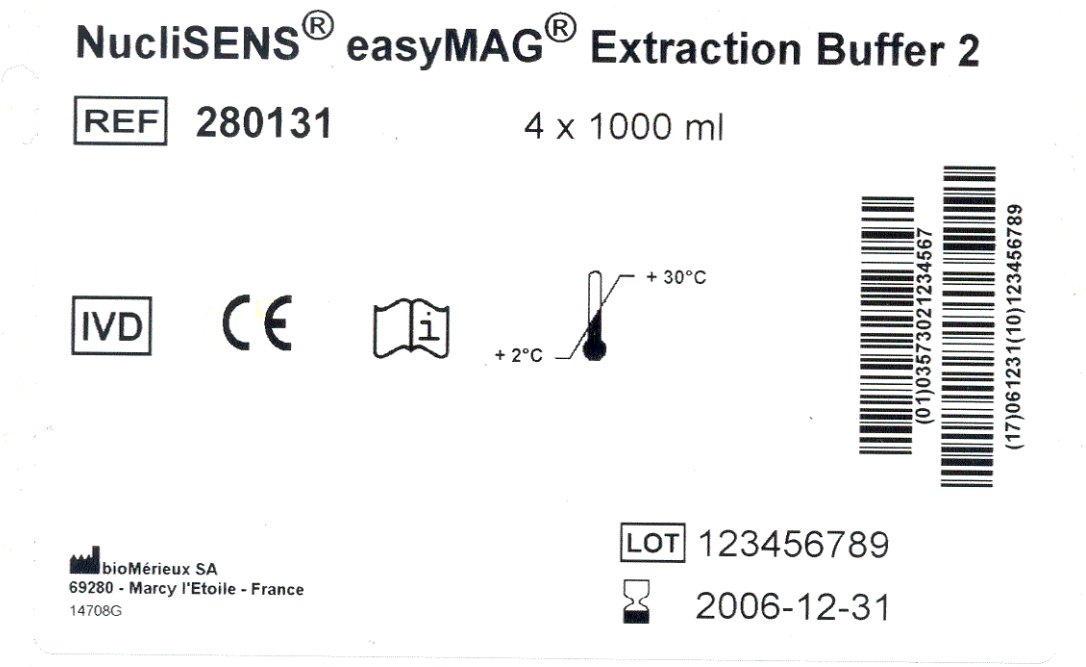
1. **Labels**
2. **Instructions for use**

1. Labels

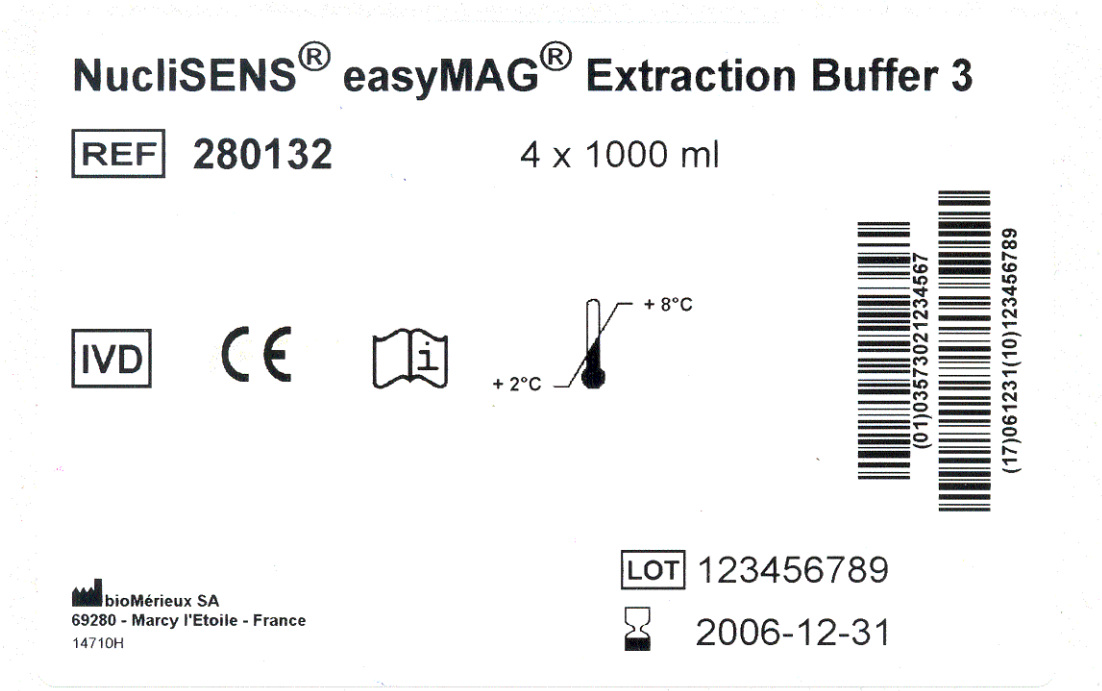
1.1 NucliSENS easyMAG extraction Buffer 1 280130



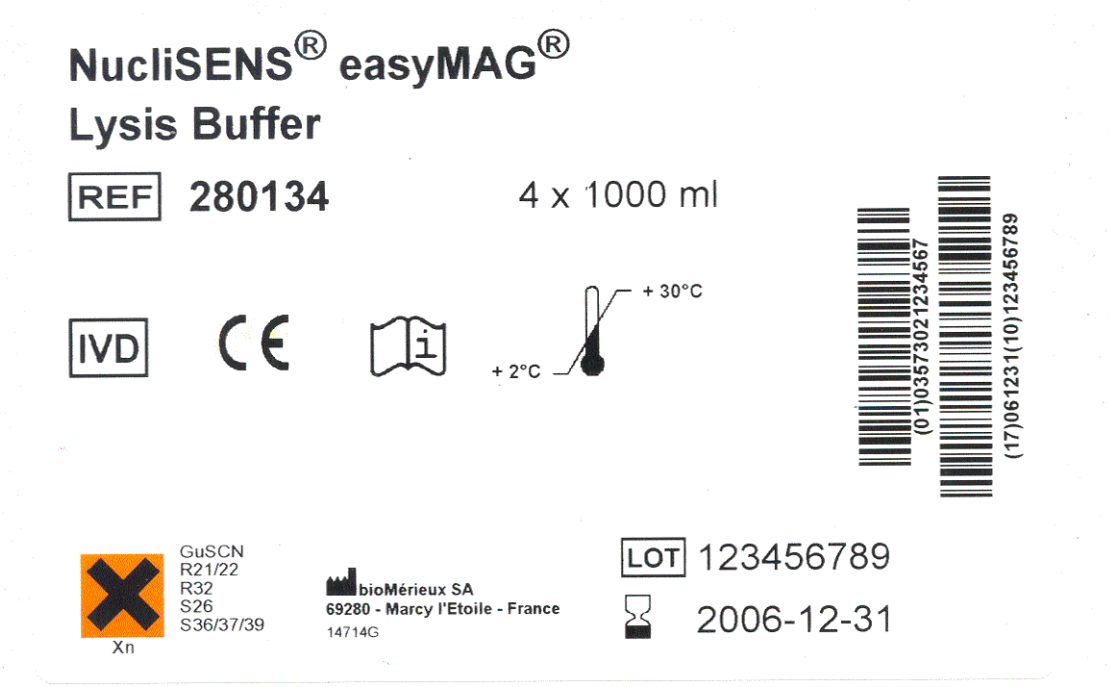
1.2 NucliSENS easyMAG extraction Buffer 2 280131



1.3 NucliSENS easyMAG extraction Buffer 3 280132

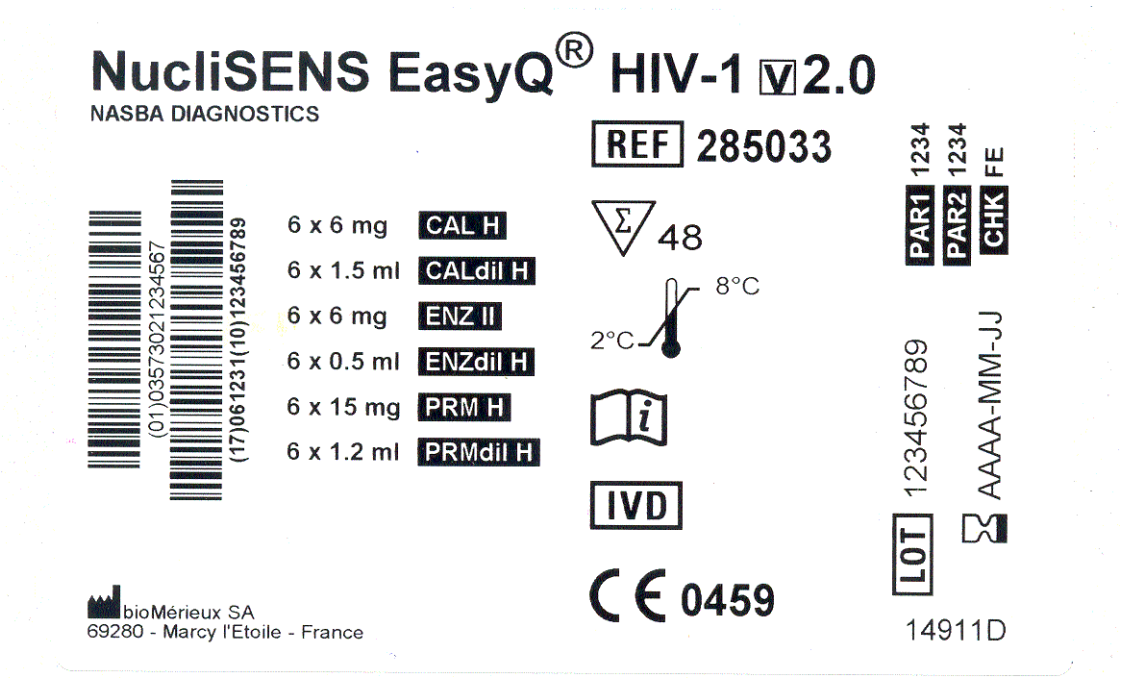


1.4 NucliSENS easyMAG extraction Lysis Buffer 280134



1.5 NucliSENS easyMAG magnetic silica 280133

1.6 Nuclisens Easy Q HIV-1 V2.0 285033



2. Instructions for use

2.1 NucliSENS easyMAG extraction Buffer 1 280130/ Buffer 2 280131 / Buffer 3 280132 / extraction Lysis Buffer 280134 / Magnetic silica 280133

NucliSENS® easyMag® accessory products
zusätzlich erforderliche Reagenzien und Materialien / Productos accesorios / produits complémentaires / prodotti accessori / produtos acessórios

BIOMÉRIEUX

REF 280130	4 x 1000 ml	NucliSENS® easyMAG® Extraction Buffer 1	⚠
REF 280131	4 x 1000 ml	NucliSENS® easyMAG® Extraction Buffer 2	
REF 280132	4 x 1000 ml	NucliSENS® easyMAG® Extraction Buffer 3	
REF 280133	48 x 0,6 ml	NucliSENS® easyMAG® Magnetic Silica	
REF 280134	4 x 1000 ml	NucliSENS® easyMAG® Lysis Buffer	⚠
REF 280135	48x	NucliSENS® easyMAG® Disposables	

English

For detailed instructions on the use of these products consult the NucliSENS® easyMAG® User Manual.

⚠ Warnings and precautions

Certain reagents contain guanidine thiocyanate.
- **R21/22**: Harmful in contact with skin and if swallowed.
- **R32**: Contact with acid liberates very toxic gas.
- **S26**: In case of contact with eyes rinse immediately with plenty of water and seek medical advice.
- **S36/37/39**: Wear suitable protective clothing, gloves and eye/face protection.

Warning: Buffers containing guanidine thiocyanate should not be mixed with cleaning solutions containing bleach. Liquid waste from extraction and isolation procedures containing guanidine thiocyanate must not be mixed with other laboratory waste. This will prevent potentially harmful chemical reactions from occurring.

Deutsh

Ausführliche Informationen entnehmen Sie bitte dem NucliSENS®easyMAG® User Manual.

⚠ Warnhinweise und Vorsichtsmaßnahmen

Bestimmte Reagenzien enthalten Guanidinthiocyanat.
- **R21/22**: Gesundheitsschädlich bei Kontakt mit der Haut und beim Verschlucken.
- **R32**: Entwickelt bei Kontakt mit Säure sehr giftige Gase.
- **S26**: Bei Berührung mit den Augen sofort gründlich mit Wasser abspülen und einen Arzt konsultieren.
- **S36/37/39**: Bei der Arbeit geeignete Schutzkleidung, Schutzhandschuhe und Augen-/Gesichtsschutz tragen.

Warnung: Puffer, die Guanidinthiocyanat enthalten, sollten nicht mit Reinigungslösungen gemischt werden, die Bleichmittel enthalten. Rückstände aus den Extraktions- und Isolierungsverfahren können Guanidinthiocyanat enthalten und dürfen daher nicht mit anderen Laborabfällen gemischt werden. Sollten diese gemischt werden, können potentiell gefährliche chemische Reaktionen auftreten.

Espagñol

Para instrucciones más detalladas sobre el uso de estos productos consultar el NucliSENS® easyMAG® User Manual.

⚠ Advertencias y precauciones

Ciertos reactivos contienen tiocianato de guanidina.
- **R21/22**: Nocivo por contacto cutáneo o ingestión.
- **R32**: El contacto con ácido libera un gas muy tóxico.
- **S26**: En caso de contacto con los ojos, aclare inmediatamente con abundante agua y acuda al médico.
- **S36/37/39**: Lleve ropa y guantes de protección adecuados y protección ocular/facial.

Advertencia: Los tampones con tiocianato de guanidina no deberían ser mezcladas con soluciones de limpieza que contengan lejía. Los desechos líquidos resultantes tras los procedimientos de separación y aislamiento que contienen tiocianato de guanidina no deben mezclarse con otros desechos de laboratorio. Esto evitará que se produzcan reacciones químicas potencialmente nocivas.

bioMérieux SA 14719 C 2009-07

2.2 NucliSENS EasyQ HIV-1 V2.0 285033

REF 285033 / 285043 NucliSENS EasyQ® HIV-1 v2.0
IVD CE EN

NucliSENS EasyQ® HIV-1 v2.0

1. INTENDED USE

In vitro diagnostic medical device.

- NucliSENS EasyQ® HIV-1 v2.0 is a nucleic acid amplification assay for the quantitative determination of HIV-1 RNA in human EDTA plasma and EDTA whole blood spotted on cards (DBS).
- NucliSENS EasyQ® HIV-1 v2.0 is intended to be used for NASBA®-based amplification and real-time detection of isolated HIV-1 RNA. The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of anti-retroviral therapy by measuring changes in plasma/DBS (from EDTA whole blood) HIV-1 RNA levels during the course of anti-retroviral treatment.
- The NucliSENS EasyQ® HIV-1 v2.0 assay must not be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of an HIV-1 infection.

2. EXPLANATION OF THE TEST

NucliSENS EasyQ® HIV-1 v2.0 consists of nucleic acid amplification combined with a simultaneous detection step. This process requires isolated nucleic acids as starting material. These processes are described in more detail below. The nucleic acids are isolated with NucliSENS® miniMAG® or NucliSENS® easyMAG®. The assay is compatible with NucliSENtral™ to support the exchange of electronic data between NucliSENS EasyQ® Director software and NucliSENS® easyMAG® User software.

2.1 Nucleic acid isolation

Human EDTA plasma or DBS is added to NucliSENS® Lysis Buffer containing guanidine thiocyanate and Triton X-100. Any cellular matter or viral particles present in the specimen will be disrupted in the presence of NucliSENS® Lysis Buffer releasing nucleic acids. NucliSENS® Lysis Buffer inactivates RNases and DNases present in the specimen. A synthetic calibrator is added in a known concentration at this stage, which functions as an internal standard for the isolation, amplification and detection procedure. For DBS, the filter paper is first removed before the synthetic calibrator is added. Magnetic silica is added to the lysate to initiate the isolation process. Nucleic acids present in NucliSENS® Lysis Buffer will bind to the magnetic silica dioxide particles under high salt conditions [(1)]. The magnetic silica is then washed several times and subsequently the nucleic acids are eluted from the solid phase thereby making the nucleic acids available for use in the amplification and detection procedure.

2.2 Nucleic acid amplification and detection

NucliSENS EasyQ® HIV-1 v2.0 utilizes a combined process of nucleic acid amplification and simultaneous detection with molecular beacons.

Amplification

NucliSENS EasyQ® HIV-1 v2.0 nucleic acid amplification uses primers that are specific for sequences found in wild type (WT) HIV-1 RNA and the synthetic NucliSENS EasyQ® HIV-1 v2.0 calibrator RNA. This calibrator RNA differs from the HIV-1 WT RNA by only a short nucleotide sequence designed to ensure similar amplification kinetics. Any WT HIV-1 RNA present in the eluted nucleic acids is co-amplified along with the internal calibrator. Other nucleic acid sequences will not be amplified.

NASBA® amplification is based on a repeated process of primer annealing, formation of double-stranded DNA containing a T7 promoter site and T7 RNA polymerase mediated transcription of multiple anti-sense copies of WT and calibrator RNA target sequences (amplicons) [2]. See figure 1.

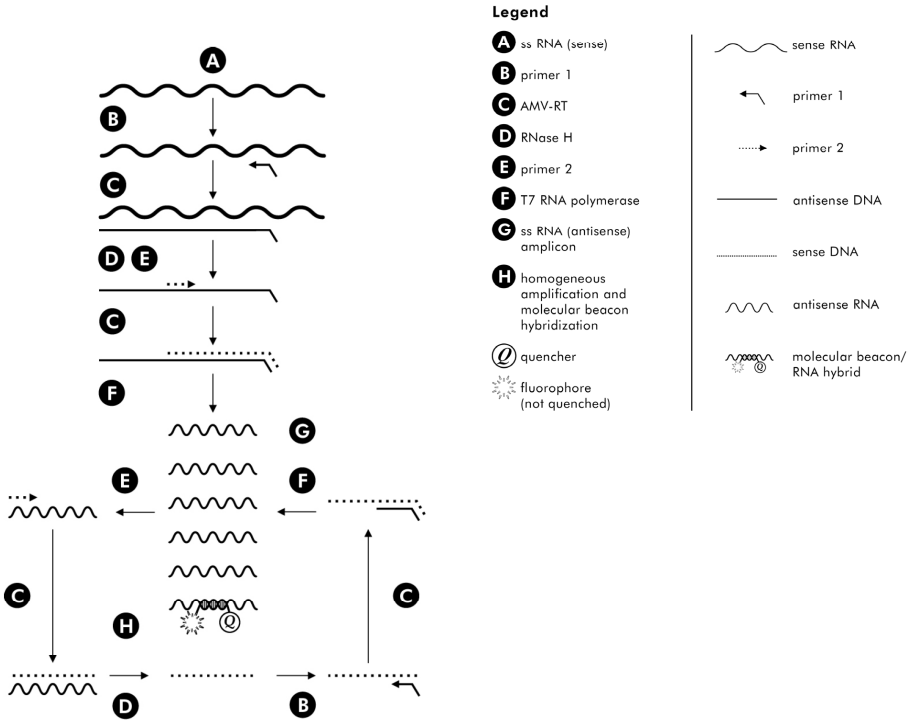


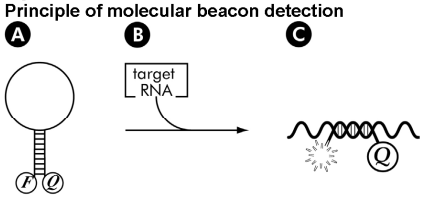
Figure 1 Principle of NASBA® amplification and molecular beacon detection

The DNA intermediate is initially generated through a process that involves the binding of a primer to the RNA template (primer 1). This primer, which contains a T7 RNA polymerase promoter site, is then extended by AMV-RT (Avian Myeloblastosis Virus Reverse Transcriptase) to form an RNA-DNA duplex. Degradation of the RNA strand of the duplex by RNase H permits the binding of a second primer to the remaining DNA strand (primer 2). The second primer is then extended by AMV-RT to form the double-stranded DNA intermediate, which contains the T7 RNA polymerase promoter needed for transcription. Once transcription is initiated, the resulting RNA transcripts, which are anti-sense to the original RNA present in the plasma specimen, can serve as a template to start a new amplification process.

Initially, amplification causes an exponential rise in the number of amplicons as more RNA copies, generated from each RNA target sequence, enter the amplification process. As the reaction progresses the primer pool will become depleted. After primer depletion, the accumulation of RNA transcripts proceeds solely due to the transcription of double-stranded DNA, leading to a linear increase in amplicons. It is this phase of the reaction that is used for the determination of transcription products.

Detection
The detection process of the NucliSENS EasyQ® HIV-1 v2.0 uses target-specific molecular beacons; see figure 2. A molecular beacon is a DNA oligonucleotide comprising a specific nucleotide sequence that recognizes a particular RNA target sequence coupled to a fluorophore and a quenching moiety. In the absence of complementary RNA the molecular beacon will form an internal hairpin structure causing the quencher to be brought into close proximity to the fluorophore. This results in quenching of the fluorescence of the molecular beacon. Binding to a complementary 'target' sequence opens the beacon allowing the molecular beacon to fluoresce, thereby 'reporting' the presence of the target sequence [3,4]. Molecular beacons have been used in various areas of nucleic acid research. These include the detection of single nucleotide polymorphisms, such as Factor V Leiden and allele discrimination in genotyping [5]. The application of molecular beacons for NASBA® was first described for a Potato Leaf-roll RNA virus target [6].

Figure 2
Principle of molecular beacon detection



Legend

A molecular beacon
B target RNA molecular beacon hybridization
C target RNA molecular beacon hybrid
F fluorophore (quenched)
Q quencher
fluorophore (not quenched)

Two different molecular beacons are used in the NucliSENS EasyQ® HIV-1 v2.0, one specific for the HIV-1 WT amplicon and one for the HIV-1 calibrator amplicon. The use of two fluorescent dyes (6-FAM for the WT, and 6-ROX for the calibrator) allows the synthesis of WT target and calibrator RNA to be followed simultaneously. Kinetic analysis of the fluorescent signals reveals the transcription rates of both the WT target and calibrator RNA and can be used to derive the quantity of HIV-1 RNA in the original plasma/DBS (from EDTA whole blood) specimen [7,13,14,15]. Quantitation of the viral load is then possible using a data reduction algorithm in the NucliSENS EasyQ® HIV-1 v2.0 assay software [8] (See section 8.1 for details).

3. NUCLISENS EASYQ® HIV-1 v2.0 REAGENTS

The NucliSENS EasyQ® HIV-1 v2.0 assay supports two formats.
Product numbers: 285033 (48T) and 285043 (480T)

3.1 Contents

285033 (48T): contains sufficient reagents for 48 tests.
285043 (480T): contains sufficient reagents for 480 tests.

1x		**CD-ROM** Instructions for Use for NucliSENS EasyQ® HIV-1 v2.0, NucliSENS EasyQ® HIV-1 v2.0 assay software for both the 48T and 480T format, NucliSENS® easyMAG® assay software and Adobe Acrobat Reader 4.0 or higher.
6 x 6 mg (48T) 60 x 6 mg (480T)	CAL H	**Calibrator** Lyophilized sphere containing synthetic calibrator RNA; each tube contained in a foil pack with silica gel desiccant. 1 tube contains 1 lyophilized sphere. Cap color: yellow
6 x 1.5 ml (48T) 60 x 1.5 ml (480T)	CALdil H	**Calibrator Diluent** Contains RNase/Dnase-free water and proclin. Cap color: yellow
6 x 6 mg (48T) 10 x 36 mg (480T)	ENZ II	**Enzymes** Lyophilized sphere containing AMV-RT, RNase H, T7 RNApolymerase, XCFF and BSA; each tube contained in a foil pack with silica gel desiccant. 48T: 1 tube contains 1 lyophilized sphere. 480T: 1 tube contains 6 lyophilized spheres. Cap color: red
6 x 0.5 ml (48T) 10 x 0.5 ml (480T)	ENZdil H	**Enzyme Diluent** Contains γ-radiated RNase/DNase-free water. Cap color: red
6 x 15 mg (48T) 10 x 90 mg (480T)	PRM H	**Primers** Lyophilized spheres containing synthetic primers, synthetic molecular beacon probes, nucleotides, dithiothreitoland $MgCl_2$; each tube contained in a foil pack with silica gel desiccant. 48T: 1 tube contains 2 lyophilized spheres. 480T: 1 tube contains 12 lyophilized spheres. Cap color: blue

6 x 1.2 ml (48T) 10 x 1.2 ml (480T)	PRMdil H	**Primer Diluent** TRIS/HCl, 30 % DMSO. KCl, sorbitol Cap color: blue

3.2 Reagent storage

NucliSENS EasyQ® HIV-1 v2.0 reagents should be stored at 2 to 8 °C. Remove only those quantities of reagents from storage that are needed for the number of tests to be performed. Protect from excessive heat or (sun)light. Do not mix reagents from different kit lots. Opened or prepared reagents cannot be stored for later use, but must be used immediately.

3.3 Reagent stability

Alterations in the physical appearance of test kit materials may indicate instability or deterioration. Expiry dates shown indicate the date beyond which components should no longer be used. Contact your local bioMérieux representative for assistance if you are in doubt about the suitability of reagents for use.

3.4 Safety aspects

- **Warning:** Handle all plasma/DBS (from EDTA whole blood) specimens as if capable of transmitting infectious agents. As no test method can offer complete assurance that infectious agents are absent, all materials of human origin should be handled as though they contain potentially infectious agents.
  Note: NucliSENS® Lysis Buffer will inactivate up to 5.3 ^{10}log of the HIV-1 virus.
- Laboratory reagents should always be handled with care. Chemical spillages (e.g. Lysis Buffer, Primer Diluent) should be dealt with promptly in an appropriate manner.

4. GENERAL PRECAUTIONS

Avoid contamination or plasma-to-plasma/DBS-to-DBS carry-over:

- Perform nucleic acid isolation and amplification in separate dedicated laboratory areas (preferably a self-contained area or dead air cabinet).
- The pre-amplification (see section 7.3) must be performed in a laboratory area that is free of amplicons.
- Prepare reagents in the laboratory area where they are to be used.
- Perform pre-amplification and amplification steps at ambient temperature. Temperatures between 18 and 25 °C are recommended.
- Use dedicated laboratory accessories; pipettes and other equipment that have been used in one laboratory area must not be used in other areas.
- Use a fresh pipette or pipette tip for each pipetting action.
- Pipettes with aerosol-resistant filter tips, or equivalent, must be used.
- Wear disposable gloves, powder free gloves are preferred, when working with clinical material possibly containing WT-RNA or amplified material. Change gloves after contact with potentially infectious material. Wash hands thoroughly after completion of the test procedure.
  Note: Glove powder entering the 8-tube strips may cause invalid results. Rinse gloves with water after putting them on.
- Collect used disposable material in a container or zip bag. Close and remove the container after each test run.
- After an amplification run has ended, discard the 8-tube strips in a container and close the container in order to prevent possible amplicon contamination in the area, e.g. from strip-vials that may open unexpectedly. Do not open the container after it has been closed!
- Do not open the 8-tube strips after amplification!
- Soak tube racks and strip holder used during nucleic acid isolation and amplification in a suitable detergent after each test run for at least one hour.
- Regularly clean the centrifuge rotor used for nucleic isolation and the inside of the centrifuge with a detergent and dry before re-use.
- Regularly clean the inside of the Mini-Strip Centrifuge lid with a detergent. Dry before re-use.
- Keep testing areas separate from areas where blood or blood products are stored.

- 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.
- Do not perform the test in the presence of reactive vapors (e.g. from sodium hypochlorite, acids, and alkalis of aldehydes) or dust as they may affect enzymatic activity.
- This kit contains substances of animal origin. All such materials should be handled with caution and treated as being potentially infectious.
- Handle all materials used in the test cautiously as though capable of transmitting infectious agents (including specimens, samples, reagents, pipettes etc.). Immediately consult a physician in the event that contaminated materials are ingested or come in contact with open lacerations, lesions or other breaks in the skin.
- Clean up any spillage containing potentially infectious agents immediately with a 1 % sodium hypochlorite solution (a freshly prepared 1: 5 dilution of liquid household bleach) or an equivalent disinfectant. Dispose of cleaning materials by an acceptable method in accordance with local regulations. Should the spillage occur inside the NucliSENS® easyMAG® or NucliSENS EasyQ® Analyzer, follow the disinfection instructions stated in the User manual.
- Dispose of all plasma specimens, DBS and materials used to perform the test as if they contain infectious agents in accordance with local regulations.
- Use only EDTA plasma and/or DBS (from EDTA whole blood) specimens for HIV viral load determination in the isolation and amplification run. Do not combine the specimens for HIV viral load testing with specimens for other quantitative or qualitative testing.
- The NucliSENS® easyMAG® has a maximum run size of 24 samples, the NucliSENS® miniMAG® has a maximum run size of 12 samples and the NucliSENS EasyQ® Analyzer has a maximum run size of 48 samples.
- The NucliSENS® easyMAG® system and the NucliSENS EasyQ® Analyzer must be kept free of computer viruses. bioMérieux advises the performance of a virus scan on all computers and portable electronic data devices prior to transferring data to or from the NucliSENS® System. Use an industry standard virus scanner and assure that it has been updated for the most recent virus detection files.
- In case the NucliSENS® easyMAG® and NucliSENS EasyQ® systems are linked via NucliSENtral™, refer to the NucliSENtral™ User Manual for more detailed information regarding computer virus protection in combination with the NucliSENtral™ network.
- The assay software on the CD-ROM supports both the 48T and the 480T formats. Select the correct protocol in the NucliSENS® easyMAG® (see section 6.5) and NucliSENS EasyQ® systems (see section 8.3), depending on the format of the kit:
    - HIV-1 2.0 for the 48T kit (Product number: 285033)
    - 480 HIV-1 2.0 for the 480T kit (Product number: 285043)

Note: In a standard NucliSENtral™ configuration **ONLY ONE** format can be supported.
If both formats need to be supported, please contact your local bioMérieux representative.

5. SPECIMEN HANDLING

The use of any sample matrix other than EDTA plasma or DBS from EDTA whole blood has not been validated.

5.1 Plasma specimens

5.1.1 Blood collection and plasma preparation

Blood should be collected in sterile tubes by normal venipuncture techniques using EDTA as anticoagulant and should be handled with the proper precautions [11,12]. After centrifugation (e.g. 10 minutes at 1500 g), the obtained plasma specimen should be used as sample input. No special specimen preparation or fasting of the patient is necessary. No adverse effects were observed using EDTA as the anticoagulant. Any deviations from the described procedures should be validated by users in their own laboratory setting.

5.1.2 Specimen storage

- Whole EDTA blood can be stored for 24 hours at 2 to 8 °C.
- EDTA plasma specimens can be stored at 2 to 8 °C for up to 7 days, 1 month at –20 °C or 1 year at – 70 °C without a significant loss of HIV-1 RNA.

- EDTA plasma specimens can be frozen and thawed up to three times without a significant loss of HIV-1 RNA.
- EDTA plasma specimens that are frozen and thawed more than three times or those containing particulate matter (e.g. gel-like substances) may give erroneous results.
- EDTA plasma specimens can be stored in NucliSENS® Lysis Buffer:
  - for a maximum of 14 days at 2 to 8 °C **or**
  - for a maximum of 24 hours at ambient temperature (2 to 30 °C) or
  - for a maximum of one year at –70 °C.

Note: Do not store specimens in NucliSENS® Lysis Buffer at –20 °C.

5.1.3 Specimen transport

Transportation of human whole blood or plasma specimens must comply with country, federal and local regulations for the transport of etiological agents. Transportation of EDTA plasma specimens and EDTA plasma in Lysis Buffer and the analysis thereof has to be accomplished within the timeframes mentioned in section 5.1.2 (Specimen storage).

5.2 Dry blood spot specimens

5.2.1 General Procedural notes

- Label the collection card with the appropriate sample identification and the date
- Do not reuse the desiccant sachet
- Cards should be physically separated (e.g. one card per an envelope or plastic zip-lock bag) to avoid cross contamination).

5.2.2 Materials required (but not supplied)

- Whatman 903® Specimen Collection Paper (e.g Proteinsaver™ 903® Card)
- Desiccant sachet (MiniPax® Sorbent sachet 2grams, MultiSorb Technologies)
- An envelope or plastic zip-lock bag
- NucliSENS® Lysis Buffer 2 ml (bioMérieux)
- Pipette tips of 2-100 µl without filter material (e.g. Eppendorf # 0030 073 223)
- Pipette tips of 5 ml with filter material (e.g Labsystems # 94052550)
- Centrifuge for NucliSENS® Lysis Buffer tubes
- Roller mixer with rocking and rolling action for gentle mixing (e.g. Stuart SRT6, Sigma-Aldrich)
- Pair of scissors
- 5 ml tubes with caps (e.g VWR #2110047/ #2110039)

5.2.3 DBS Collection

Collect whole blood in a tube with EDTA-anticoagulant and, on the same day, spot 50 µl blood on Whatman 903® Specimen Collection Paper (e.g. Proteinsaver™ 903® Card) using a calibrated device (pipette).
Note: Fill each printed circle with a SINGLE application of blood. Prevent spotting outside the circles.
Note: Avoid touching or smearing the blood spots
Two spots are needed for the nucleic acid extraction procedure described below.
If blood spots cannot be prepared immediately after blood drawing, the blood tubes should be stored in a refrigerator for up to 24 hours until spotting. Dry the filter paper for at least 3 hours (and at maximum overnight) at room temperature (15 to 30 °C).

5.2.4 DBS Storage

Store the filter paper containing the dried blood spots with desiccant sachets in an air-impermeable bag at room temperature (15-30°C) for a maximum period of 9 months . Prevent storage at high humidity conditions and/or at high temperatures since this will negatively affect sample stability.
Desiccant sachets should be used according to the manufacturer's specifications.
Dried and packed EDTA blood spots can alternatively be stored

- for a maximum of 3 weeks at 2 to 8°C
- for a maximum of 9 weeks at 37 ± 3°C (in case of high humidity: maximum 3 weeks)
- for a maximum of 3 months at –20°C

without a significant loss of HIV-1 RNA.

5.2.5 Pre- extraction procedure

1. Cut out the complete blood spots (2 spots of 50 µl/extraction) from the filter paper by using a pair of scissors taking care not to touch the spotted blood. A new pair of scissors should be used for each new specimen and they must be decontaminated before re-use.
   Note: Take adequate provision to prevent accidents (cutting of operator, contamination of lab-environment) when handling the spots
   Note: Store remaining spots appropriately
2. Add the blood spots (2 spots of 50 µl/extraction) into a NucliSENS® Lysis Buffer 2 ml tube.
3. Incubate the tubes in a horizontal position on a roller mixer for 30 minutes at room temperature
   Note: Position the tubes on the roller mixer such that the blood spots are in constant contact with the Lysis Buffer.
   Note: Do not vortex the tubes. This can result in degradation of the filter material and the release of fibers that can interfere with the extraction process.
4. Centrifuge the tubes during 15 seconds at 1500 g to spin down the fluid.
5. For NucliSENS® easyMAG® extraction, attach a 100 µl pipette tip to a 5 ml pipette tip and use this combination to transfer the lysate for nucleic acid extraction directly into one of the vessels of a NucliSENS® easyMAG® disposable, leaving any possible remains of paper material in the NucliSENS® Lysis Buffer 2 ml tube. Select a volume of 1.8 ml on the 5 ml pipette to avoid formation of foam. Continue according to the standard instructions using the Off board extraction protocol (see Section 6.2.3)
   Note: Using the 100 µl pipette tip prevents paper material being transferred to the extraction vessel.
6. For NucliSENS® miniMAG® extraction, transfer the lysate for nucleic acid extraction into a 5 ml tube leaving any possible remains of the paper material in the NucliSENS® Lysis Buffer 2 ml tube. Continue according the standard instructions described for the NucliSENS® miniMAG® procedure (see Section 6.4.2).

6. NUCLEIC ACID ISOLATION PROCEDURES

Nucleic acid isolation can be performed according to the following two procedures:

- Manually, using the NucliSENS® miniMAG® magnetic extraction procedure.
- Automated, using the NucliSENS® easyMAG® magnetic extraction procedure.

6.1 General Procedural notes

- **Caution:** NucliSENS® Lysis Buffer, NucliSENS® miniMAG® Wash Buffer 1, NucliSENS® easyMAG® Lysis Buffer and NucliSENS® easyMAG® Extraction Buffer 1, contain guanidine thiocyanate. Guanidine thiocyanate is harmful when inhaled, in contact with skin and when swallowed. Contact with acid can liberate very toxic gasses. Read associated instructions for use for safety information before using these products.
- Plasma specimens that have been frozen and thawed more than three times or that contain particulate matter may yield erroneous results. Plasma specimens containing particulate matter may be used for testing after centrifugation for 2 minutes at high speed.
- Label tubes with appropriate specimen information.
- Make sure reagents, samples and specimens are at ambient temperature before starting.
- All isolation reagents must be mixed thoroughly before use.
- Opened or prepared reagents cannot be re-used, but must be used immediately.
- Return any unopened isolation reagents to storage immediately. See component labels for appropriate storage conditions.
- Store any remaining plasma (for possible use in additional testing) as described in section 5.1.2.
- Do not uncap tubes in the presence of other open tubes containing patient material/specimens.

- Make sure that all lyophilized material is at the bottom of the tube before opening a tube containing lyophilized material.
- Never open more than one tube at any time to minimize the risk of cross-contamination.
- The integrity of each individual result can be monitored by reference to the performance of the internal calibrator. It is, however, recommended that a positive control and a negative control are included in each run to verify product performance.
  Note: HIV-1 RNA positive and negative controls are commercially available and can be obtained from several suppliers, e.g. Seracare/BBI[9], Acrometrix[10].
- For instructions related to the NucliSENS® miniMAG® and/or NucliSENS® easyMAG®, read the accompanying User manual of NucliSENS® miniMAG® and/or NucliSENS® easyMAG®.
- If NucliSENtral™ is used to support electronic data transfer, read the accompanying NucliSENtral™ User Manual.

Note:

1. A gel-like mass may be produced after addition of EDTA plasma to NucliSENS® Lysis Buffer or after the thawing of a lysed EDTA plasma sample. When this occurs do not process this sample, discard the sample and start specimen lysis again using a fresh EDTA plasma specimen.
2. After addition of the pre-mix Calibrator-Silica, the plasma/lysis mixture may become (increasingly) gel-like and it might not be possible to aspirate the sample using a 5 ml pipette. If this occurs, do not process this sample, but discard the sample and start specimen lysis again using a fresh EDTA plasma specimen.

6.2 Additional materials required (but not supplied)

6.2.1 General

- NucliSENS® Lysis Buffer (2 ml/tube).
- Centrifuge (capable of 1 500 g) for NucliSENS® Lysis Buffer (2ml/tube).
- Water bath capable of heating NucliSENS® Lysis Buffer at 37 °C ± 1 °C.
- Calibrated micropipettes with variable settings for 5 to 5 000 µl delivery volumes
- Sterile, disposable, aerosol resistant tips.
- 1.5 ml micro tubes or 0.2 ml polyethylene microtube strips with separate lockable caps (e.g. Greiner).
- Timer.
- Vortex.
- Absorbent tissue.
- Sodium hypochlorite solution, 1 % (a freshly prepared 1 : 5 dilution of liquid household bleach).
- Waste container with cap.
- Disposable gloves, powder free gloves are preferred.

6.2.2 NucliSENS® miniMAG®

- NucliSENS® Magnetic Extraction Reagents.
- NucliSENS® miniMAG®.
- 1.5 ml micro tubes.
- Thermoshaker, with the following specifications: temperature range up to 85 °C; temperature accuracy of ± 2 °C at 60 °C; mixing frequency of 1 200 to 1 400 rpm (for example: Thermomixer Compact, Eppendorf).
- Magnetic stand suitable for 1.5 ml micro tubes, e.g. Promega.
- Vacuum system capable of aspirating 1.5 l/min, e.g. Vacusafe or calibrated dedicated micropipettes for the removal of supernatant.
  - Calibrated multipipettor for the addition of wash fluid, e.g. Eppendorf.
  - Fine tipped plastic transfer pipette (pastette)
- If applicable: disposable 10 ml plastic pipettes and disposable 10 ml tubes with screw-cap for removal of supernatant from NucliSENS® Lysis Buffer 2 ml/tube.

6.2.3 NucliSENS® easyMAG®

- NucliSENS® easyMAG® Lysis Buffer (1000 ml).

- NucliSENS® easyMAG® Extraction Buffers 1, 2 and 3.
- NucliSENS® easyMAG® Magnetic Silica.
- NucliSENS® easyMAG®.
- NucliSENS® easyMAG® Disposables.
- NucliSENS® easyMAG® Sample Vessel Carrier.
- RNase/DNase free, flat or flat C-shaped bottom uncoated micro-titer 8-well strips (for distributing the pre-mix of magnetic silica particles and calibrator).
- Electronic programmed multipipette with the following programs, e.g. Biohit:

Program 1:

Step	Volume	Action
1	550 µl	aspirate
2	550 µl	dispense

Program 2:

Step	Volume	Action
1	1050 µl	aspirate
2	25 µl	dispense
3	125 µl	dispense
4	125 µl	dispense
5	125 µl	dispense
6	125 µl	dispense
7	125 µl	dispense
8	125 µl	dispense
9	125 µl	dispense
10	125 µl	dispense
11	25 µl	dispense

Program 3:

Step	Volume	Action
1	100 µl	aspirate
	100 µl	dispense
	100 µl	aspirate
2	800 µl	aspirate
	900 µl	dispense
	1000 µl	aspirate
	1000 µl	dispense
	1000 µl	aspirate
	1000 µl	dispense
	1000 µl	aspirate
	1000 µl	dispense

6.3 Preparation of isolation reagents

6.3.1 Buffers containing guanidine thiocyanate

- In buffers containing guanidine thiocyanate, i.e. NucliSENS® Lysis Buffer, NucliSENS® miniMAG® Wash Buffer 1, NucliSENS® easyMAG® Lysis Buffer and Extraction Buffer 1, crystals may have formed in the solution if the buffers have been stored below ambient temperature, which is undesirable. Dissolve the crystals by pre-warming the buffers for about 30 minutes in a water bath before starting (recommended temperature: 37 °C). Do not use a dry stove for pre-warming buffers.
- During the incubation mix thoroughly every 10 minutes by inverting tube or bottle to make sure that any crystals in the buffer dissolve.
- Cool down to ambient temperature.

6.3.2 Guanidine thiocyanate-free buffers

- Ensure that the solution is at ambient temperature before use for guanidine thiocyanate-free buffers, i.e. NucliSENS® miniMAG® Wash Buffer 2 and Wash Buffer 3, Elution Buffer, NucliSENS® easyMAG® Extraction Buffer 2 and Extraction Buffer 3 and NucliSENS EasyQ® Calibrator Diluent.

- No special preparation is required.

6.3.3 Silica

- Ensure that the solution is at ambient temperature before use.
- No special preparation is required.

6.4 NucliSENS® miniMAG® extraction

Note: Use the pre-extraction procedure for DBS samples.

6.4.1 EDTA plasma sample lysis

- Spin NucliSENS® Lysis Buffer down to the bottom of the tubes by centrifugation for 10 seconds at 1 500 g.
- Spin plasma specimens down to the bottom of the tubes by centrifugation for 2 minutes at high speed (see section 5.1.1, 'Blood collection and plasma preparation').
- Transfer 0.1, 0.5 or 1 ml of EDTA plasma specimen into the NucliSENS® Lysis Buffer (2ml/tube) and close the tubes immediately.
- Vortex the NucliSENS® Lysis Buffer tubes containing plasma.
- Incubate the sample tubes for at least 10 minutes at ambient temperature.
- Continue with step 6.4.2 or store as described in section 5.1.2.

6.4.2 Preparation pre-mix Calibrator-Silica solution (hereafter called pre-mix)

- Reconstitute the Calibrator accusphere (yellow cap) in 550 µl NucliSENS EasyQ® Calibrator Diluent (yellow cap).
- Vortex the Calibrator solution
- Vortex the silica tube until a homogeneous suspension is formed.
- Add 550 µl magnetic silica to the calibrator solution.
- Vortex the pre-mix before each pipetting step as part of the isolation procedure.

Note: **Use within 20 minutes after preparation.**

6.4.3 Nucleic acid isolation

- Thaw frozen NucliSENS® Lysis Buffer tubes containing EDTA plasma specimen; e.g. by incubation for 30 minutes at 37 °C. Mix the sample tubes thoroughly and make sure that all crystals have dissolved.
- Centrifuge the sample tubes for 10 seconds at 1 500 g.
- For each NucliSENS® Lysis Buffer tube containing EDTA plasma or DBS:
  Add 100 µl pre-mix to the sample tubes and briefly vortex immediately after addition of the pre-mix.
  Note: Addition of the pre-mix to the samples must be done within 20 minutes after preparation of the pre-mix.
  Note: Vortex the pre-mix before each pipetting step.
- Leave the sample tubes for 10 ± 1 min at ambient temperature without further mixing.
- Centrifuge sample tubes for 2 min at 1 500 g to pellet the silica.
- Remove the supernatant from the silica pellet by manual aspiration or, preferably, by vacuum aspiration.
  Note: If a vacuum aspiration system is not available, use a fine tipped plastic transfer pipette (pastette) or use a 1000 µl micropipette (with aerosol-resistant disposable tip).
  Note: Use this pipette exclusively to remove supernatant. Do not use it for the addition of wash buffers.
  Caution: Avoid disturbing the silica pellet.
- Wash the silica pellet in the sample tubes according to the following procedure using the NucliSENS® miniMAG®:
  Note: Complete the wash procedure as quickly as possible.
  Caution: Do not pause while the samples are in Wash Buffer 3. Leaving the samples in Wash Buffer 3 may result in lower yields of nucleic acids.
- Label 1.5 ml micro tubes and place (with open lid) in the NucliSENS® miniMAG®.
  Note: Ensure that the tubes are labeled appropriately to be able to correctly identify each sample.

- Re-suspend the silica pellet by pipetting 400 µl Wash Buffer 1 into the sample tube.
- Verify that the magnetic field is in the OFF position and correct if necessary, i.e. the magnetic rack should be tilted back.
- Transfer a maximum of 450 µl of the mixture to a 1.5 ml micro tube placed in the NucliSENS® miniMAG®.
  Note: **Use a fresh sterile tip for each sample to avoid sample carry-over.**
  Note: Transfer as little foam as possible and avoid generating air bubbles.
- Repeat the above for each sample until all samples have been placed on the instrument. Place the magnetic rack in the up position (Magnetic field ON).
- Wash for 30 sec at speed STEP 1.
- Allow the silica particles to collect on the wall of the micro tube.
- Remove the supernatant by aspirating at the surface of the liquid.
- Tilt back the magnetic rack (Magnetic field OFF).
- Add the wash buffer to the micro tube by pipetting directly onto the silica aggregate in order to improve washing; for the amount, type and volume of wash buffer see table below.
- Place magnetic rack in up position (Magnetic field ON).
- Repeat until each sample has been washed a total of 5 times:

Wash 2	**400 µl** Wash Buffer 1, wash for 30 sec at STEP 1
Wash 3	**500 µl** Wash Buffer 2, wash for 30 sec at STEP 1
Wash 4	**500 µl** Wash Buffer 2, wash for 30 sec at STEP 1
Wash 5	**500 µl** Wash Buffer 3, wash for **15** sec at STEP 1

- Remove the supernatant as much as possible and without disturbing the silica pellet.
- Take the micro tubes from the instrument. Add 25 µl Elution Buffer onto the silica pellet in the micro tubes.
- Close the micro tubes and collect the silica at the bottom of the tube by gently tapping.
  Note: Do not collect the silica by centrifugation, as this may compact the silica, which could result in lower extraction yields due to difficulties in re-suspending the silica efficiently.
  Note: Ensure that the tubes are labeled appropriately to be able to correctly identify each sample.
- Place the micro tubes in the Thermoshaker and incubate for 5 min at 60 ± 2 °C at 1 400 rpm to elute the nucleic acid from the silica.
  Note: Validation of the NucliSENS® Magnetic Extraction Reagents was performed using the Thermomixer Compact (Eppendorf).
- Place the micro tubes in a magnetic stand (e.g. Promega) and pipette each nucleic acid extract without silica into a fresh 1,5 ml micro tube.
  Take care not to transfer any silica particles.
  Note: If any transfer of silica in the nucleic acid extract is suspected, place the tubes in the magnetic stand and again pipette the nucleic acid extracts into fresh 1,5 ml tubes.
- If nucleic acid extracts cannot be used for amplification immediately after preparation, the nucleic acid extracts (without silica) can be stored at 2 to 8 °C for up to 2 days or, preferably, at –20 °C for a maximum of three months. Storage of small quantities of nucleic acid extracts (i.e. 5 µl) is not recommended.

6.5 NucliSENS® easyMAG® extraction

Note: Use the pre-extraction procedure for DBS samples.
Note: For DBS, select the specimen type 'OTHER' in the NucliSENS® easyMAG®.
Note: Select the correct protocol that corresponds to the kit format.

6.5.1 EDTA plasma sample lysis

6.5.1.1 easyMAG® [OFF-BOARD LYSIS INCUBATION] option

- Spin NucliSENS® Lysis Buffer down to the bottom of the tubes by centrifugation for 10 seconds at 1 500 g.
- Spin plasma specimens down to the bottom of the tubes by centrifugation for 10 seconds at high speed (see section 5.1.1 'Blood collection and plasma preparation').

- Transfer 0.1, 0.5 or 1 ml of the EDTA plasma specimen into NucliSENS® Lysis Buffer (2ml/tube) and close the tubes immediately.
- Vortex the NucliSENS® Lysis Buffer tubes containing plasma.
- Incubate the sample tubes at least 10 minutes at ambient temperature.
- Continue with step 6.5.2 or store as described in section 5.1.2.

6.5.1.2 easyMAG® [ON-BOARD LYSIS INCUBATION] option

- Create a run according to the instructions described in the operating procedure of the NucliSENS® easyMAG® User Manual. Select the correct HIV Protocol, the Matrix (Plasma for EDTA plasma), the Volume (0.100 ml, 0.500 ml or 1.000 ml) and select 'Primary'. (Eluate is 25 µl).
- Identify the samples and transfer 0.1 ml, 0.5 ml or 1 ml of the EDTA plasma to the sample vessel strips.
- Follow the instructions described in the operating procedure of the NucliSENS® easyMAG® User Manual and select [DISPENSE LYSIS].
- Incubate sample vessel strips containing the lysed samples for 10 minutes at ambient temperature and continue with nucleic acid isolation.

6.5.2 Preparation pre-mix Calibrator-Silica solution (hereafter called pre-mix)

- Reconstitute the Calibrator accusphere (yellow cap) in 550 µl NucliSENS EasyQ® Calibrator Diluent (yellow cap) using an electronic programmed multi-channel pipette e.g. **Program 1** of the Biohit multipipette (see section 6.2.3), or a mono-channel pipette.
- Vortex the solution.
- Vortex the silica tube until a homogeneous suspension is formed.
- Add 550 µl magnetic silica to the calibrator solution, using an electronic programmed multi-channel pipette e.g. **Program 1** of the Biohit multipipette, or a mono-channel pipette.
- Vortex the pre-mix before commencing the nucleic acid isolation procedure, which is described in section 6.5.3 below.
  Note: Use within 20 minutes after preparation.
- Vortex the pre-mix and add 125 µl pre-mix to each well of a micro-titer 8-well strip using an electronic programmed multi-channel pipette e.g. **Program 2** of the Biohit multipipette (see section 6.2.3), or a mono-channel pipette.
- When working with DBS (from EDTA whole blood) proceed at 6.5.3.2

6.5.3 Nucleic acid isolation

6.5.3.1 easyMAG® [OFF-BOARD LYSIS INCUBATION] option

- Thaw NucliSENS® Lysis Buffer tubes containing plasma specimen; e.g. by incubation for 30 minutes at 37 °C. Mix the sample tubes thoroughly and make sure that all crystals have dissolved. Centrifuge the sample tubes for 10 seconds at 1 500 g.
- Create a run according to the instructions described in the operating procedure of the NucISENS® easyMAG® User Manual. Select the correct HIV Protocol, the Matrix (plasma for EDTA Plasma, other for DBS), the Volume (0.100 ml, 0.500 ml or 1.000ml) and select 'Lysed'.
- Carefully transfer lysed samples to the sample vessel strips using a 5 ml pipette.
  Note:
  - Check that the correct sample is transferred to the assigned sample vessel.
  - Leave ± 50 µl behind to avoid transferring foam in the vessel.
  - Do not introduce foam by pipetting air into the sample vessel strip.
  - Do not touch the upper lid of the vessel with the pipette tips, and take care not to spill any droplets into the nearby sample vessels.

6.5.3.2 *easyMAG® [ON-BOARD LYSIS -INCUBATION] & [OFF-BOARD LYSIS INCUBATION] option*

- Add 100 µl pre-mix (see 6.5.2) to each NucliSENS® easyMAG® sample vessel containing sample, using an electronic programmed multipipette, e.g. **Program 3** of the Biohit multipipette (see section 6.2.3). Place the Biohit multipipette upright and halfway the sample vessels for addition of the pre-mix.
  Note: The addition of the pre-mix to the samples must be done within 20 minutes after preparation of the pre-mix.
  Pipette tips must be tightly fitted to the Biohit multipipette to guarantee delivery of the correct volume. Differences in liquid level in the tips after aspiration may indicate a loose pipette tip.
- Install sample vessel strips in the NucliSENS® easyMAG®.
- Follow the instructions described in the operating procedure of the NucliSENS® easyMAG® User Manual and select [START THIS RUN].
- Unload the sample vessel strips from the NucliSENS® easyMAG® after completion of the extraction run.
- For immediate amplification of the nucleic acid extracts, place the required number of 8-tube strips in a tube holder. Transfer **15 µl** of each nucleic acid extract, without silica, from the sample vessel strips to the bottom of a strip tube within 30 minutes after completion of the extraction run, using a multi-channel pipette or mono-channel pipette and cover the 8-tube strips with the tube tray lid. Take care not to transfer any silica particles.
- Remaining nucleic acid extract can be stored in micro tubes, e.g. 1.5 ml micro tubes, at 2 to 8 °C for up to 2 days or, preferably, at –20 °C for a maximum of three months. Storage of small quantities of nucleic acid extracts (i.e. 5 µl) for longer than one day is not recommended.
- Note: remaining nucleic acid extract volume is not sufficient to perform another amplification.
- If 48 samples are to be processed in one amplification run, store the covered 8-tube strips containing the nucleic acid extracts obtained from the first easyMAG® extraction run (24 samples) at 2 to 8 °C while preparing the nucleic acid extracts of the second easyMAG® extraction run.
- If amplification is not to be carried out directly on the extracts, the entire nucleic acid extracts can be stored in micro tubes, e.g. 1.5 ml micro tubes at 2 to 8 °C for up to 2 days or, preferably, at –20 °C for a maximum of three months.

6.5.3.3 *easyMAG® [OFF-BOARD SILICA INCUBATION] option*

- This workflow can be selected in NucliSENS® easyMAG® user software. This workflow will skip the silica incubation on the instrument and can be combined with both the on-board lysis as the off-board lysis incubation option.
- Follow the instructions selecting this workflow described in the operating procedure of the NucliSENS® easyMAG® User Manual.
- Make sure to incubate the sample vessel strips containing the previously lysed sample with silica for 10 minutes at ambient temperature and continue with nucleic acid isolation.

6.5.4 *Data Transfer*

6.5.4.1 *Electronic data transfer via NucliSENtral™*

The intended use of NucliSENtral™ is to support the exchange of electronic data between NucliSENS EasyQ® Director software, NucliSENS® easyMAG® User software and external information systems capable of sending test requests and/or receiving test results.

Please note NucliSENtral™ should be operated by or under the supervision of a qualified person who has undergone training by bioMérieux or local representatives.

Principle of NucliSENtral™
The NucliSENtral™ network software has been developed to integrate NucliSENS® instruments like NucliSENS® easyMAG® automated extraction systems and NucliSENS EasyQ® Analyzers, and also an LIS into a communicating system to optimize extraction and test request handling. The benefits of a NucliSENtral™ network will appear in enhanced routine operations and a better traceability of materials and information. NucliSENtral™ is a data distribution tool that takes over routine work from

the user by handling all the test request distribution in the background. Information received via an LIS is distributed to the NucliSENtral™ software to be processed. The current process state of every NucliSENtral™ software is permanently reported to NucliSENtral™, so that all applications can be used most efficiently. The NucliSENtral™ network is flexible, reliable and can be extended at any time.

The NucliSENtral™ solution will allow NucliSens EasyQ HIV-1 v2.0 samples to be processed efficiently by providing fully automated data management and an improved workflow for both the lab technicians and the laboratory. The automated workflow presents the user with less work to perform and reduces the chance of errors usually related to manual data entry.

The NucliSENS® easyMAG® and the NucliSENS EasyQ® assay protocols for HIV-1 v2.0 are developed to be compatible with NucliSENtral™ v1.0 or higher. To set up NucliSENtral™ please refer to the NucliSENtral™ User Manual.

6.5.4.2 Manual transfer of sample data

To transfer sample data manually (sample ID, assay definition, matrix, sample volume) from the NucliSENS® easyMAG® user software to the NucliSENS EasyQ® Director software, proceed as follows:

Make sure the NucliSENS® easyMAG® computer and the NucliSENS EasyQ® computer are switched on and the NucliSENS® easyMAG® User software and NucliSENS EasyQ® Director software are running.

Login to the NucliSENS® easyMAG® User software.

Insert a portable USB storage device (USB stick) into the computer.

Press the *Daily Use* menu button.

Press the *View Results* submenu button.

Select the run you wish to export and press the *Export Run* button.

Browse to the USB storage device, select the *.sl file type and enter the name of the file. Then press the *Save* button.

Remove the USB storage device from the NucliSENS® easyMAG® computer and insert it into the NucliSENS EasyQ® computer.

Select the *Sample Login* button from the *Routine* list in NucliSENS EasyQ® Director software.

Select *File* from the menu and then select *Open*.

Browse to the USB storage device then select the <file name>.sl file, which is to be transferred.

Press the *Open* button.

The transferred sample data is now visible in the *Sample Login* screen.

Important:

- NucliSENS® easyMAG® extraction samples in which errors or remarks occurred during their extraction will be marked with '_X_' preceding the Sample ID name in the export file. Check the NucliSENS® easyMAG® results (*Incidents view)* for such marked samples to identify the cause of the error or remark before continuing on the NucliSENS EasyQ® system.
- Decide if the sample is suitable for further processing on the NucliSENS EasyQ® Analyzer, based upon the specific error message or remark. A sample that is not suitable must be deleted in the NucliSENS® Director software sample login or worklist screen.
- To avoid sample mix-up, verify that the physical plate layout matches the sample assignment in the worklist screen of the NucliSENS EasyQ® Director software.
  Note: Make sure that the nucleic acid extracts transferred from the NucliSENS® easyMAG® to the NucliSENS EasyQ® Analyzer always correspond with the worklist prepared in the NucliSENS EasyQ® Director software.

7. NUCLEIC ACID AMPLIFICATION AND DETECTION PROCEDURES

7.1 General procedural notes

- Prepare reagents before starting nucleic acid amplification.
- The amplification area should be in an amplicon free area.
- Make sure reagents and samples are at ambient temperature before starting nucleic acid amplification.
- Make sure that the lyophilized material is at the bottom of the tube before opening a tube that contains lyophilized material.
- Return any unopened amplification reagents to storage immediately; see component labels for appropriate storage conditions.
- It is recommended to use the tube tray lid during the incubation steps and transfer to prevent cross contamination.
- Use the capping aid to close the 8-tube strips.
- Do not open the lid of the NucliSENS EasyQ® Analyzer during a run.
- The NucliSENS EasyQ® HIV-1 v2.0 assay has been validated as described in the Instructions for Use. If deviations are made from the procedure, users should revalidate the assay.

7.2 Additional materials/instruments required (but not supplied)

- 0.2 ml 8-tube strips with caps
- 0.2 ml tube tray holder with lid
- Capping aid
- Pipetting aid
- NucliSENS EasyQ® Incubator
- Mini-Strip Centrifuge (± 6 000 rpm, 2 000 g)
- Vortex mixer with a flathead dimpled adapter (for example Vortex Genie 2)
- NucliSENS EasyQ® Analyzer and NucliSENS EasyQ® Director software (version 2.6 or higher)
- Calibrated mono-channel micropipettes with variable settings for 5 to 1000 µl delivery volumes
- Calibrated multi-channel micropipette with variable settings for 5 to 20 µl delivery volumes
- Sterile-packaged, disposable, aerosol resistant tips
- Bench centrifuge for 1.5 ml test tubes (capable of centrifugation at 10 000 g)
- Waste container with cap (e.g. 50 ml tubes or zip bags)
- Printer for printing Instruction for Use, workflows and reports

7.3 Pre-amplification steps

1. Transfer the tubes with nucleic acid extracts to the amplification laboratory area.
2. Start up the NucliSENS EasyQ® Analyzer and NucliSENS EasyQ® Director software as described in the Operating Procedures in the NucliSENS EasyQ® User Manual and wait at least 15 minutes for the Analyzer to warm up.
3. Create or select a run as described in the Operating Procedures in the NucliSENS EasyQ® User Manual. Select the correct assay protocol that corresponds to the kit format.
4. Place the required 8-tube strips in the tube tray holder as indicated in the Director software.
5. Nucleic acid extract preparation:
    - If amplification is to be carried out immediately after isolation, transfer 15 µl portions of nucleic acid extract to the bottoms of the tubes on the positions indicated by the NucliSENS EasyQ® Director software. Cover the tubes with the tube tray lid.

    For stored nucleic acid extracts preparation should be as follows:
    - Thaw the nucleic acid extracts at ambient temperature, vortex the test tubes and spin before use at 10 000 g for 15 seconds.
    - Transfer 15 µl portions of nucleic acid extract to the bottoms of the tubes at the positions indicated by the Director software. Cover the tubes with the tube tray lid.

    Note:
    A run for the NucliSENS EasyQ® Analyzer is defined as a maximum of 48 samples, whereas the maximum run size for the NucliSENS® easyMAG® is 24 samples. In case two NucliSENS® easyMAG® runs are combined in one EasyQ® Analyzer run, verify if sample IDs in the worklist of the NucliSENS EasyQ® Analyzer correspond to the sample IDs in the 8-tube strip positions.

The placement rules of the samples follow the simple rule of alternate column positioning. Place the first 8-tube strip in column 1, column 2 is not used and the second 8-tube strip is positioned in column 3 and so on. The tray has the same grid numbering as the plate view. The graphic below (Figure 3) illustrates the relationship between the plate view and the tube tray.

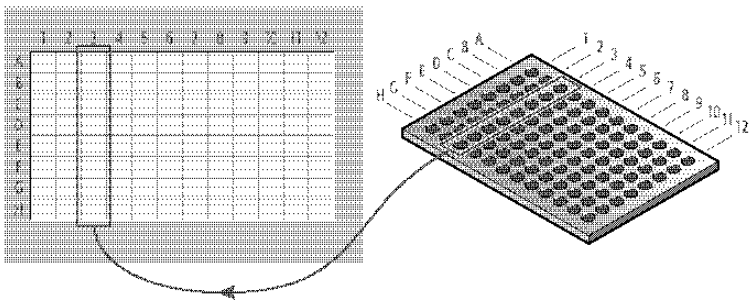


Figure 3 Relationship between plate view and sample placement in the tube tray.

6. Preparation of Primer solution (for 8 samples):
Add 180 µl Primer Diluent (blue cap) to the 2 lyophilized Primers (blue cap) and **vortex immediately** until completely dissolved. **Do not spin.**

If a larger volume of reconstituted Primer solution is required, primer accuspheres can be pooled and dissolved according to the following scheme:

48T		480T	
Primer accusphere (n)	Primer Diluent (µl)	Primer accusphere (n)	Primer Diluent (µl)
4	360	12	1080
6	540		
8	720		
10	900		
12	1080		

For the 480T add 1080µl primer diluent to the lyophilized primer accuspheres.

Note:
- For 12 primer accuspheres, pipette two times 540 µl primer diluent to the lyophilized primer accuspheres and immediately vortex until clear.
- Use within 30 minutes after preparation.

7. Preparation of Enzyme solution (for 8 samples):

Add 45 µl Enzyme Diluent (red cap) to the lyophilized Enzyme accuspheres (red cap). If a larger volume of reconstituted enzyme solution is required, Enzyme accuspheres can be pooled and dissolved according to the following scheme:

48T		480T	
Enzyme accusphere (n)	Enzyme Diluent (µl)	Enzyme accusphere (n)	Enzyme Diluent (µl)
2	90	6	270
3	135		
4	180		
5	225		
6	270		

For the 480T add 270µl enzyme diluent to the lyophilized enzyme accuspheres.

Allow to stand 15 minutes; ensure complete reconstitution of the lyophilized sphere by gently tapping the closed tube after addition of Enzyme Diluent. Spin before use at 10 000 g for 15 seconds.
Note: Use within 1 hour after preparation.

7.4 Nucleic acid amplification

1. Visually check that all nucleic acid extracts have been added and are at the bottom of the 8-tube strip.

2. For each tube (containing 15 µl nucleic acid extract):
   - Add 20 µl of Primer solution to the inside of the tube slightly above the nucleic acid extract. Use a mono-channel pipette and a fresh pipette tip for each pipetting step.
   - Visually check that Primer solution has been added to all tubes.

3. Place the tube tray in the NucliSENS EasyQ® Incubator, place the lid over the tube tray and incubate the 8-tube strips for 2 minutes at 65 °C and 2 minutes at 41 °C (Program 1 or program RNA).

4. During the incubation steps, place the tube caps upside down in a tube tray and add 5 µl of Enzyme solution to the inner side of all tube caps (cap opening oriented on the numbered side of the tray).
   - After the second incubation of step 3 (2 minutes at 41 °C), close the 8-tube strips by placing the caps using the capping aid.
   - Mark each individual strip. Make sure the caps are placed in the correct order (cap opening oriented on the numbered side of the tray), visually check if the 8-tube strips are closed properly and make sure the blue Enzyme solution is added to all caps.
     Note: If more than one strip is to be analyzed, the steps below should be performed as quickly as possible for each separate 8-tube strip.
   - Close all tubes before continuing with the next step.
   - Centrifuge the 8-tube strip for 2 seconds in the Mini-Strip Centrifuge.
   - Mix the 8-tube strip using a vortex with a flathead dimpled adapter.
     Turn on the vortex at continuous speed and choose a vortex speed such that the solution is moved up about halfway the height.
     Hold the 8-tube strip in the middle and place straight on the adapter during 1 second and remove. Repeat this two times.
   - Centrifuge the 8-tube strip for 2 seconds in the Mini-Strip Centrifuge.
     In case more than two strips are processed simultaneously, return the 8-tube strips to the NucliSENS EasyQ® Incubator in order to keep the temperature at 41 °C. Make sure to place the strips back in the original orientation.

5. Transfer the tube tray with the 8-tube strip(s) to the pre-warmed NucliSENS EasyQ® Analyzer (41 °C) and start the run immediately.
   Note: Be sure to start the run within 2 minutes after addition of the enzymes.

6. After the run has ended, carefully remove the tube tray with the 8-tube strips, and discard the 8-tube strips in a container and immediately close the container.
   Note: Make sure the 8-tube strips **remain closed** after amplification in order to prevent amplicon contamination of the area.

8. RESULTS

8.1 Calculations

This assay is standardized against a viral standard from the Virology Quality Assurance (VQA) Laboratory of the Clinical Trials Group [16]. By using NucliSENS EasyQ® HIV-1 v2.0 assay software and batch-specific parameters included on the CD-ROM, the NucliSENS EasyQ® Director software is capable of automatically calculating the number of VQA copies WT HIV-1 RNA ('cps/ml) in the plasma or DBS specimen added to the Lysis Buffer from the relevant kinetic information from the observed fluorescence curves. These fluorescence curves are the result of a number of biochemical processes that take place during the amplification process. These processes include the NASBA® driven increase in RNA levels and the binding of the molecular beacons to these RNA amplicons. As explained in section 2.2, the NASBA® process is characterized by an early exponential growth followed by a linear increase in the RNA levels. The transition between these two phases is marked by depletion of the

primer pool. As the WT RNA and calibrator RNA make use of the same primer pool, it follows that the ratio of the input concentrations of the two RNA species will be reflected in the ratio of the concentrations of double stranded DNA species. This ratio then determines the ratio of the RNA formation rates in the transcriptional phase, after primer pool depletion. The quantitation procedure is based on the assessment of the transcription rate ratio. Specific batch parameters are required to convert this ratio into an input ratio. To permit the assessment of the transcription rates in an amplification reaction, the biochemical processes of RNA formation and molecular beacon binding were studied using mathematical modeling. This permitted the generation of an equation that describes the fluorescence signal development with time as a function of several amplification process parameters. The relevant information on amplification kinetics of both WT and calibrator RNA, most notably the transcription rate ratio, is derived from the fluorescence curves by fitting this model through the data. The result of this fit is checked against a number of validation criteria, e.g. presence of a detectable calibrator signal, (statistical) quality of the curve fit and the timing of the onset of the fluorescence increase. If a result has been qualified as valid, a WT RNA concentration is calculated. A quantitative result is only reported when a WT signal has been detected and the estimated number of copies falls within the reportable quantity range. If a result has been qualified as valid and a WT signal is detected but falls outside the reportable quantity range, a specific result is reported indicating a positive detection, above or below the reportable quantity range. If a result has been qualified as invalid, the cause is indicated by the software.

8.2 Reviewing results

The following results can be observed:

8.2.1 Valid results

Sample volume	Reported result	Interpretation
1.0 ml	TND	Target not detected. Amplification signal increase of HIV-1 RNA below the limit for the assay.
	< 10 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA below the lowest reportable quantity limit of 10 cps. Corrected for the volume used, calculated cps/ml are below 10 cps/ml
	10 to 10,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA are within the reportable quantity range of 10 - 10,000,000 cps. Corrected for the volume used, calculated cps/ml are within the range of 10 - 10,000,000 cps/ml
	> 10,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA above the highest reportable quantity limit of 10,000,000 cps. Corrected for the volume used, calculated cps/ml are above 10,000,000 cps/ml. If quantitative results are desired, a smaller sample input volume (0.5 or 0.1 ml) should be used and the test repeated.
0.5 ml	TND	Target not detected. Amplification signal increase of HIV-1 RNA below the limit for the assay.
	< 20 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA below the lowest reportable quantity limit of 10 cps. Corrected for the volume used, calculated cps/ml are below 20 cps/ml If quantitative results are desired, a larger sample input volume (1.0 ml) should be used and the test repeated.

	20 to 20,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA are within the reportable quantity range of 10 - 10,000,000 cps. Corrected for the volume used, calculated cps/ml are within the range of 20 - 20,000,000 cps/ml
	> 20,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA above the highest reportable quantity limit of 10,000,000 cps. Corrected for the volume used, calculated cps/ml are above 20,000,000 cps/ml. If quantitative results are desired, a smaller sample input volume (0.1 ml) should be used and the test repeated.
0.1 ml	TND	Target not detected. Amplification signal increase of HIV-1 RNA below the limit for the assay.
	< 100 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA below the lowest reportable quantity limit of 10 cps. Corrected for the volume used, calculated cps/ml are below 100 cps/ml.If quantitative results are desired, a larger sample input volume (0.5 or 1.0 ml) should be used and the test repeated.
	100 to 100,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA are within the reportable quantity range of 10 - 10,000,000 cps. Corrected for the volume used, calculated cps/ml are within the range of 100 - 100,000,000 cps/ml
	> 100,000,000 cps/ml	Target detected. Amplification signal increase of HIV-1 RNA above the limit for the assay. Calculated copies HIV-1 RNA above the highest reportable quantity limit of 10,000,000 cps. Corrected for the volume used, calculated cps/ml are above 100,000,000 cps/ml. If quantitative results are desired, the original specimen should be diluted with HIV-1-negative human EDTA-plasma or lysis buffer, the test repeated and the reported result multiplied by the dilution factor.

The assay is standardized against a viral standard from the Virology Quality Assurance (VQA) Laboratory of the AIDS Clinical Trial Group. The assay results will be reported in VQA copies/ml. 1 IU WHO second international standard (code 97/650) corresponds to 0.48 copies.

For succession of viral load data obtained with NucliSENS EasyQ® HIV-1 v1.2, NucliSENS EasyQ® HIV-1 v1.2 results need to be multiplied with 1.74 to obtain results in copies (determined with subtype B standards).

8.2.2 Invalid results

If a sample is classified as invalid an error message will appear (see section 9.3 'Troubleshooting'). Refer to the NucliSENS EasyQ® User Manual for view, print and export of the results. The results assessment can be implemented in the NucliSENS EasyQ® Director software by approving results or ordering a retest. If a sample is classified as invalid a retest must be ordered.

8.3 Troubleshooting

8.3.1 Invalid results

The NucliSENS EasyQ® Director software can generate the following error messages:

Error code	Remark	Description	Solution
Below -1	Multiple remarks	Internal calculation error.	Contact your local representative
-1	Runtime Calculation Error	Internal calculation error due to unanticipated curve shape.	Retest starting with sample
1	No Beacon detected	Absolute signal level is too low.	Retest starting with sample.
2	Size of the data sets of the first and second candidate are not equal	The size of the calibrator data set differs from the size of the WT data set.	Contact your local bioMérieux representative.
3	Discontinuity in curve detected	A discontinuity in the curve is detected.	Retest starting with sample
4	No Amplification	Both calibrator and WT max/min signal ratios are below threshold values.	Retest starting with sample.
7	Aberrant amplification kinetics	The fitted curve does not accurately describe the observed data.	Retest starting with sample.
8	Lag time too short. Insufficient data for background fit	The onset of the fluorescence increase is too rapid.	Retest starting with sample. Be sure that the time between adding the enzymes and starting the run is less than 2 minutes.
9	Poor amplification. None of the curves sufficiently reached its plateau level	Neither calibrator nor WT signal reached a plateau value	Retest starting with sample
10	No calibrator detected. No calibrator was added or sample input level is too high	No calibrator signal is detected, possibly because no calibrator was added in nucleic acid isolation or WT input is too high.	Retest starting with (diluted) sample.
11	Too low measurement values encountered	Too low (or negative) signal level detected	Retest starting with sample
12	Lag time too long. Curves started rising later than expected	The onset of the fluorescence increase is too late	Retest starting with sample

8.3.2 NucliSENS EasyQ® Analyzer error

When an instrument error occurs during the execution of a run, the run will be disqualified. Results will not be calculated. Amplification and detection must be repeated for all samples in the run. The NucliSENS EasyQ® Director software will indicate the probable cause of the problem and will propose the relevant corrective action.

8.3.3 NucliSENS EasyQ® Incubator error

Refer to the User Manual of the NucliSENS EasyQ® Incubator.

9. LIMITATIONS OF THE PROCEDURE

- NucliSENS EasyQ® HIV-1 v2.0 is to be used by trained laboratory personnel only.
- The NucliSENS EasyQ® HIV-1 v2.0 assay must not be used as a screening test for HIV-1 or as a diagnostic test to confirm the presence of an HIV-1 infection.

- Although primers and probes are directed against highly conserved regions of the HIV-1 genome, it cannot be excluded that the quantitation of a specific HIV-1 strain is influenced by random sequence variability not related to a specific subtype.
- The NucliSENS EasyQ® HIV-1 v2.0 assay has been validated as described in the Instructions for Use. The assay must be performed in strict accordance with the Instructions for Use. Users should revalidate the assay if deviations are made from the procedure.
- Cross-reactivity with HIV-2 RNA cannot be excluded in the NucliSENS EasyQ® HIV-1 v2.0 assay.
- HIV Group O members are only occasionally detected. Quantification of group O is not validated.
- The use of the NucliSENS EasyQ® HIV-1 v2.0 assay has not been validated on capillary whole blood.

10. PERFORMANCE CHARACTERISTICS

10.1 Analytical specificity

Potentially cross-reacting analytes

The specificity of NucliSENS EasyQ® HIV-1v2.0 Reagents is safeguarded by the selection of the primers and probes. Primers and probes have been checked by sequence analysis on homologies with the following viruses and microorganisms:

Adenovirus type 2
Human Herpes virus type 6A
Human Herpes virus type 6B
Human Herpes virus type 7
Human Herpes virus type 8
Epstein Barr virus 1 (HHV-4)
Epstein Barr virus 2 (HHV-4)
Human Cytomegalo virus (HHV-5)
Herpes Simplex virus type 1
Herpes Simplex virus type 2

Hepatitis A virus
Hepatitis B virus
Hepatitis C virus
Human T-lymphotropic virus type 1
Human T-lymphotropic virus type 2
Influenza A (H1N1)
Influenza A (H1N2)
Influenza A (H3N2)
Propionibacterium acne
Staphylococcus aureus

To further exclude aspecificity of the NucliSENS EasyQ® HIV-1 v2.0 reagents, the following viruses and microorganisms were tested: HTLV-I, HAV, HBV, HCV CMV, HSV1/2, Influenza A, *S. aureus*, *P.acnes* and *C. albicans*. No cross-reactivity was observed, thus indicating that the reagents are specific for HIV. For HIV-2 positive specimens cross-reactivity was observed.

Potentially interfering substances

Quantitation performance of the assay to potential interfering factors was assessed by processing specimens with elevated levels of alanine aminotransferase (ALT), Aspartate AminoTransferase (ASAT), rheumatoid factor (RF), Systemic lupus erythematosus (SLE), lipemic and hemolytic samples, triglycerides, bilirubin, protein/albumin as well as specimens from multiparous women, all spiked with HIV-1 RNA. The outcome of the assay was not influenced by any of these factors.

10.2 Analytical performance

Linear quantitative range

Testing diluted samples from 9 to 79,000,000 cps/ml (0.9 ^{10}log to 7.9 ^{10}log cps/ml) derived from HIV-1 RNA reference material with two lots of NucliSENS EasyQ® HIV-1 v2.0 reagents, demonstrated a direct proportional relationship between the dilution factor and the number of HIV-1 RNA cps reported by the assay. The performance of the assay using EDTA plasma was found to give linear response over a range of 25 to 7.9 10^6 cps/ml (1.4 ^{10}log to 6.9 ^{10}log cps/ml) for 1 ml input of EDTA plasma, over a range of 50 to 1.5 10^7 cps/ml (1.7 ^{10}log to 7.2 ^{10}log cps/ml) for 0.5 ml input of EDTA plasma and over a range of 292 to 7.1 10^7 cps/ml (2.5 ^{10}log to 7.9 ^{10}log cps/ml) for 0.1 ml input of EDTA plasma (see Figure 4) and for DBS over a range of 500 to 21,000,000 cps/ml (2.70^{10}log to 7.33 ^{10}log cps/ml) (see Figure 4).

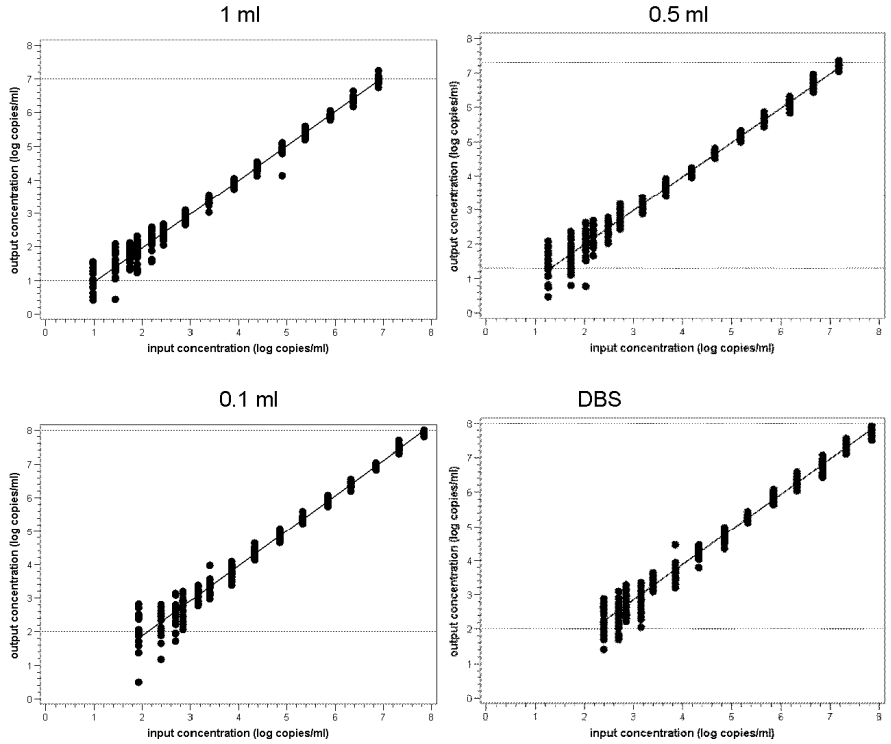


Figure 4 **Input versus output plot**
The solid line presents the regression line. Upper left graph shows data obtained with 1 ml EDTA plasma, the upper right graph shows data obtained with 0.5 ml EDTA plasma input and the lower left graph shows data obtained with 0.1 ml EDTA plasma input and the lower right shows data obtained with DBS. Horizontal dashed lines reflect the cut-off (i.e. lowest result reported in the assay) of the assay.

Precision

The dilution panel was also used to determine the overall precision. For EDTA plasma it was found that for concentrations of 794 (2.90 ^{10}log) cps/input and higher the precision was between 0.09 to 0.12 ^{10}log cps/ml. The precision between 79 and 794 (1.90 to 2.90 ^{10}log cps/input was found to be between 0.19 to 0.28 ^{10}log cps/ml and below 79 cps/input (1.90 ^{10}log cps/input) the precision was found to be between 0.29 and 0.54 ^{10}log cps/ml. For DBS it was found that at concentrations of 7100 (3.85 ^{10}log) cps/ml and higher the precision was between 0.15 to 0.16 ^{10}log cps/ml. The precision between710 and 7100 (2.85 to 3.85 ^{10}log cps/ml) was found to be between 0.26 to 0.28 ^{10}log cps/ml and below 2.85 ^{10}log cps/ml the precision was found to be between 0.38 and 0.47 ^{10}log cps/ml.

Limits of detection

The limit of detection was determined by multiple testing of HIV-1 negative specimens spiked with a range of HIV-1 RNA from 5 to 7,900,000 cps per input. Probit analysis was used to determine the relationship between the percentage positive results and the ^{10}log nominal input (see Figure 5). For 1 ml EDTA plasma input, the 95 % detection rate was determined to be 15 cps/ml (95% confidence interval 11-34 cps/ml). For 0.5 ml EDTA plasma input the 95% detection rate was 50 cps/ml 95% confidence interval 33-121 cps/ml), and for 0.1 ml EDTA plasma input the 95 % detection rates was

292 cps/ml 95% confidence interval 193-634 cps/ml). For DBS the 95 % detection rate was determined to be 802 cps/ml (95% confidence interval 510-1602 cps/ml).
The limit of detection claim for the different input EDTA plasma volumes for the NucliSENS EasyQ® HIV-1 v2.0 assay is 25 cps/ml for 1 ml input, 50 cps/ml for 0.5 ml input, 292 cps/ml for 0.1 ml input and 802 cps/ml for DBS.

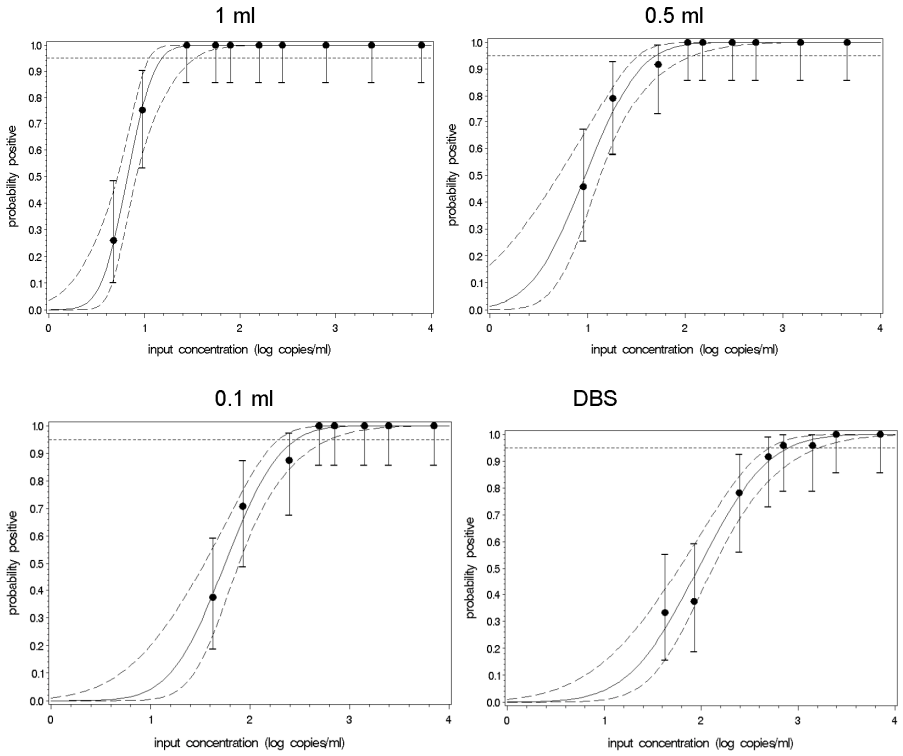


Figure 5 Results of the probit analysis.
Upper left graph with 1 ml EDTA plasma, upper right graph with 0.5 ml EDTA plasma, lower left graph with 0.1 ml EDTA plasma input and the lower right shows data obtained with DBS. Solid lines reflect the estimated probit model, dashed lines the two-sided 95 % confidence intervals for the predicted hit rates. Dots show the observed hit rates with two-sided 95 % confidence intervals based on a binomial distribution.

10.3 Subtype reactivity

NucliSENS EasyQ® HIV-1 v2.0 demonstrated the capability to detect and quantify RNA from representative specimens of the major HIV-1 group M subtypes A to J. For EDTA plasma specimens from BBI panel PRD 201, and the WHO NIBSC panel, were all detected. Figure 6A and 6B present the quantitative results obtained with the BBI panel members and WHO NIBSC in the NucliSENS EasyQ® HIV-1 v2.0, NucliSENS EasyQ® HIV-1 v1.1, Amplicor HIV-1 Monitor v1.5, Cobas Amplicor HIV-1 Monitor v1.5 and Versant Quantiplex HIV-1 RNA 3.0.

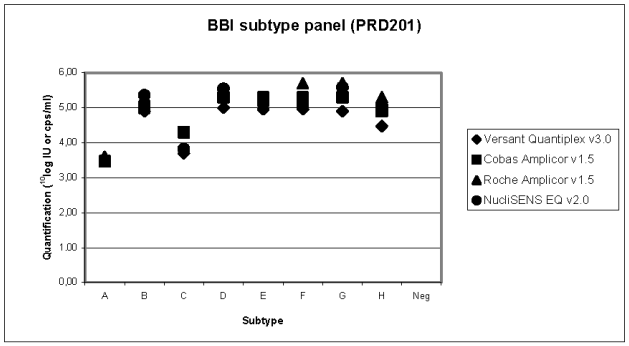


Figure 6A Quantitation results Cobas Amplicor HIV-1 Monitor v1.5, Amplicor HIV-1 Monitor v1.5, Quantiplex HIV-1 RNA 3.0 and NucliSENS EasyQ® HIV-1 v2.0 of BBI panels PRD 201.

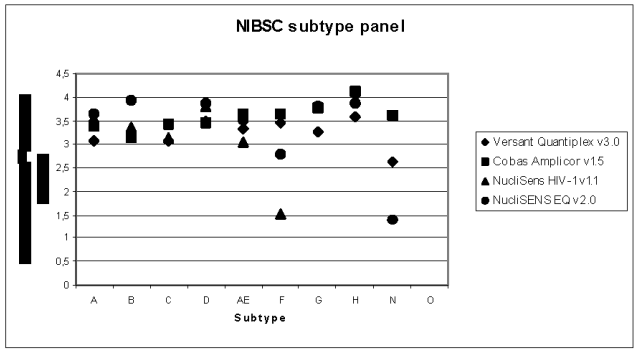


Figure 6B Quantitation results Cobas Amplicor HIV-1 Monitor v1.5, Quantiplex HIV-1 RNA 3.0, NucliSENS EasyQ® HIV-1 v1.1 and NucliSENS EasyQ® HIV-1 v2.0 of NIBSC subtype panel.

For DBS, NucliSENS EasyQ® HIV-1 v2.0 demonstrated the capability to detect and quantify RNA from the following subtypes: A, B, C, D, F, G, H and circulating recombinant form CRF02_AG.

In addition to the above-described panels, various circulating recombinant forms and additional subtypes were tested in serial dilutions for EDTA plasma of the following NIBSC samples: ARP1050 (CRF01_AE), ARP1066 and ARP1037 (CRF02_AG, consisting of subtype A and G), ARP1038 (CRF11-cpx, consisting of subtype A, G, J and CRF01_AE), ARP1034 (CRF14_BG, consisting of subtype B and G) and ARP176 (GH-AA, consisting of GH recombinant and A subtype), ARP1036 (subtype K) ARP1017.1 and ARP1017.2 (subtype J), ARP1043 (subtype H) and ARP190 (HIV-1 group N). All specimens were detected and the intended dilution factor used is well reflected by the results (data not shown).

10.4 Clinical specimens

EDTA plasma

Clinical reactivity was assessed by testing 228 EDTA plasma specimens in the NucliSENS EasyQ® HIV-1 v2.0 assay and NucliSENS EasyQ® HIV-1 v1.2 assay.

Figure 7 represents correlation plots between NucliSENS EasyQ® HIV-1 v1.2 and EasyQ® HIV-1 v2.0.

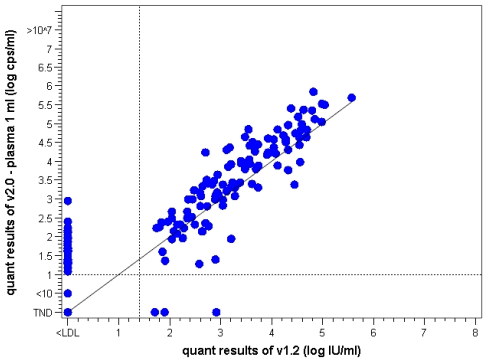


Figure 7 Correlation plot clinical specimens tested in NucliSENS EasyQ® HIV-1 V2.0 and EasyQ® HIV-1 v1.2.
Solid line presents the line y=x. Dashed lines reflect the cut-off of the assays.

It was found that the NucliSENS EasyQ® HIV-1 v2.0 assay has an improved ability to detect HIV in specimens from HIV-1 infected patients as compared to the NucliSENS EasyQ® HIV-1 v1.2 assay, showing that the NucliSENS EasyQ® HIV-1 v2.0 assay is more sensitive than version 1.2.

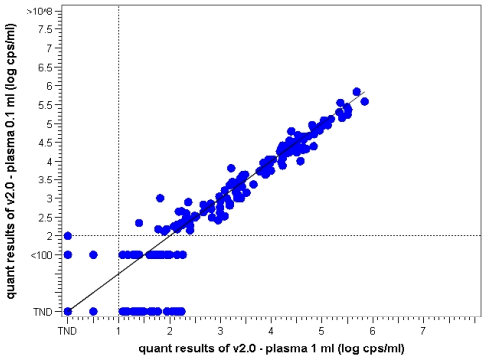


Figure 8 Correlation in quantitation result (cps/ml) between 1 ml and 0.1 ml sample specimen input.
Solid line presents the line y=x. Dotted line presents cut-off of the assay.

To assess the effect of input volume on the quantitation result, 244 individual clinical specimens were tested using 2 different input volumes (0.1 ml and 1.0 ml). On the119 samples that were detected and quantified both with 1 ml input and 0.1 ml input, correlation coefficients are found to be equal to 0.969 (Pearson) and 0.971 (Spearman). The mean difference (0.1 ml minus 1 ml) is estimated to –0.02 log cps/ml (std=0.26), with a 95% dispersion interval equal to [-0.54; +0.50]).

DBS

Clinical reactivity was assessed by testing 224 DBS specimens in NucliSENS EasyQ® HIV-1 v2.0 in comparison with 0.1 ml EDTA plasma samples which were also tested in the NucliSENS EasyQ® HIV-1 v2.0.

Figure 9 represents the correlation plot between NucliSENS EasyQ® HIV-1 v2.0 0.1 ml plasma input and DBS (2 spots of 50 µl).

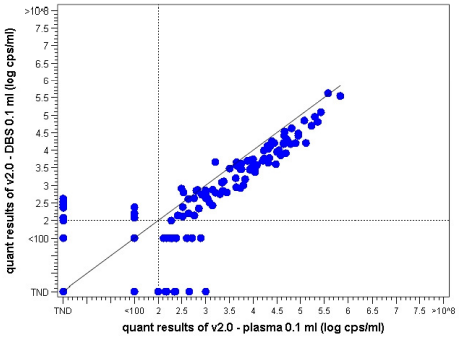


Figure 9 Correlation in quantitation result (cps/ml) between DBS and 0.1 ml sample specimen input. Solid line presents the line y=x. Dotted line presents cut-off of the assay.

In the 84 samples that were detected and quantified both with plasma and DBS, correlation coefficients are found to be equal to 0.950 (Pearson) and 0.948 (Spearman). The mean difference (DBS minus plasma) is estimated to –0.36 log cps/ml (std=0.27), with a 95% dispersion interval equal to [-0.89; +0.17]).

10.5 Specificity

Diagnostic specificity of NucliSENS EasyQ® HIV-1 v2.0 was assessed by testing specimens from randomly selected healthy blood donors found to be non-reactive for antibodies to HIV-1 and HIV-2 with an CE marked assay.
261 1 ml input EDTA plasma specimens and 129 0.1 ml EDTA plasma specimens were tested. The observed specificity in this population was 100% [95% conf. interval, 98.6 – 100.0] for plasma 1ml input, and 100% [95% conf. interval, 97.2 – 100.0] for plasma 0.1ml input.
100 Dried Blood Spots were also tested and the observed specificity of NucliSENS EasyQ® HIV-1 v2.0 in this population was 100% [95% conf. interval, 96.4 – 100.0].

11. AVAILABILITY

	Catalog number
Reagents	
NucliSENS® Lysis Buffer (2 ml/tube)	200292
NucliSENS® Lysis Buffer (4 x 1000 ml/bottle)	280134
NucliSENS® Magnetic Extraction Reagents	200293
NucliSENS® easyMAG® Extraction Buffer 1 (4 x 1000 ml/bottle)	280130
NucliSENS® easyMAG® Extraction Buffer 2 (4 x 1000 ml/bottle)	280131
NucliSENS® easyMAG® Extraction Buffer 3 (4 x 1000 ml/bottle)	280132
NucliSENS® easyMAG® Magnetic Silica (48 x 0.6 ml/vial)	280133
NucliSENS EasyQ® HIV-1 v2.0 (48T)	285033
NucliSENS EasyQ® HIV-1 v2.0 (480T)	285043
Instruments/software	
NucliSENS® miniMAG®	200305
NucliSENS® easyMAG®	200110
NucliSENS EasyQ® Incubator II	285208
NucliSENS EasyQ® Analyzer 110V/220V	285060
NucliSENS EasyQ® Computer 110V/220V	285059
Monitor 110V/220V	93443
Printer 110V	99393
Printer 220V	99223
NucliSENS EasyQ® Director software v2.6	280107
NucliSENS® easyMAG® User software v2.0	280106
NucliSENtral™ v1.0	280108
Mini-Strip Centrifuge (220V)	285056
User Manuals	
NucliSENS EasyQ® Director User Manual v2.6	271444
NucliSENS® miniMAG® User Manual	70087331
NucliSENS® easyMAG® v2.0 User Manual	280163
NucliSENS EasyQ® Incubator II User Manual	285210
NucliSENtral™ v1.0 User Manual	280109
Additional materials	
Promega Magnetic rack (12 holes)	200299
Micro tubes; 1.5 ml (500 pcs)	200294
NucliSENS® easyMAG® Disposables	280135
NucliSENS® easyMAG® Sample Vessel Carrier	280145
Biohit electronic multipipette	280141
0.2 ml 8-tube strips (125 pcs)	285048
0.2 ml 8-tube caps (125 pcs)	285051

Tube tray holder	45685049
Capping aid	45685050
Pipetting aid	45685207

For technical assistance, please contact your local bioMérieux representative.

12. EXPLANATION OF SYMBOLS

International symbols

REF	Catalog number
IVD	*In vitro* diagnostic medical device
LOT	Batch code
V	Version
Σ	Contains sufficient for <n> tests
	Consult instructions for use
	Use by …
	Temperature limitation
	Manufacturer

Batch specific parameters

PAR1	Batch parameter 1
PAR2	Batch parameter 2
CHK	Check sum, required for manual installation of batch specific parameters.

13. REFERENCES

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NUCLISENS EASYQ® HIV-1 V2.0 ASSAY SOFTWARE

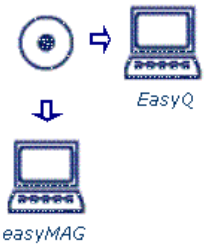
A. SOFTWARE INSTALLATION

INTRODUCTION

This chapter describes the installation procedure for HIV-1 v2.0 assay software via the supplied CD-ROM.

Use the procedure described in section A1 *NucliSENS EasyQ® HIV-1 v2.0 Assay Software Installation* for the installation of a HIV assay on the NucliSENS EasyQ® system.

Use the procedure described in section A2 *NucliSENS® easyMAG® Assay Software Installation* for the installation of an HIV specific extraction protocol on the easyMAG® system.



The assay software on the CD-ROM supports both the 48T and the 480T formats. Please install the correct protocol in the NucliSENS® easyMAG® and NucliSENS EasyQ® systems depending on the kit:
HIV-1 2.0 for the 48T kit (Product number 285033)
480 HIV-1 2.0 for the 480T kit (Product number 285043)

A.1 NucliSENS EasyQ® HIV-1 v2.0 Assay Software Installation

Step 1 Preparation

In this step, the NucliSENS EasyQ® system will be prepared for installation of NucliSENS EasyQ® HIV-1 v2.0 assay software.

Take the NucliSENS assay software CD-ROM out of the NucliSENS EasyQ® kit.

Make sure the NucliSENS EasyQ® computer is turned on and the NucliSENS EasyQ® Director software is ***not*** running.

Insert the CD-ROM into the NucliSENS EasyQ® computer. The NucliSENS EasyQ® HIV-1 v2.0 Assay software will start automatically.

The application will check the system for previously installed NucliSENS EasyQ® Director software. Depending on the system, this may take a while.

Most likely there will only be a single version of NucliSENS EasyQ® Director software installed on the system.

If the system only contains one NucliSENS EasyQ® Director installation, the application will automatically select this directory.

If multiple installations of NucliSENS EasyQ® Director software are present on the system, please select the desired target directory.

Step 2 Installation

In this step the assay(s) that has (have) to be installed must be selected.

The software will automatically determine whether the assay protocols and reagent lots as present on the CD-ROM need to be installed. REF lists the available assay protocols and LOT lists the available reagent lots.

Three situations are possible:

- Neither the assay software nor the lot information have previously been installed on the system. Both will have a check mark.
- The assay software has already been installed on the system but the lot has not. Only the lot will have a check mark.
- Both assay software and lot have previously been installed on the system. Neither will have a check mark.

If neither of the two boxes have a check mark, it is not necessary to transfer information from the CD-ROM. Please continue with Exiting this application (select *Exit*)

In other cases press the *Install* button on the screen. Only install the assay protocol or reagent lots that correspond to your HIV kit.

The application will then start transferring data from the CD-ROM to the system.

Press the *Exit* button to close the application.

A.2 NUCLISENS® EASYMAG® ASSAY SOFTWARE INSTALLATION

Step 1 Preparation

In this step, the NucliSENS® easyMAG® system will be prepared for installation of a HIV specific nucleic acid extraction protocol.

Make sure that the NucliSENS® easyMAG® computer is turned on and the NucliSENS® easyMAG® User software is running.

Login to the NucliSENS® easyMAG® User software. Make sure to use a 'Laboratory Manager' or 'Maintenance Engineer' account. Only laboratory managers and maintenance engineers are allowed to import assays.

Insert the CD-ROM into the NucliSENS® easyMAG® computer. Installation will start automatically.

If the computer asks to 'Install Program As Other User' press the *Cancel* button and proceed with *Step 2 Import*.

The NucliSENS® Assay software CD-ROM will warn that it cannot find a NucliSENS EasyQ® Director installation. Press the *OK* button to dismiss this message.

Step 2 Import

In this step a specific nucleic acid extraction protocol will be imported in the NucliSENS® easyMAG® User Software.

Press the *Maintenance* menu button.

Press the *Protocol Inventory* submenu button.
The *Protocol Inventory* work area is displayed.

Press the *Import Assay or Protocol* button. The *Import Assay or Protocol* dialog is shown.

Browse to the folder of the extraction protocol on the CD-ROM:

E:\easyMAG® Assays\<assay ID>\
Select the correct protocol file that corresponds to your kit and press Import. The *Import Assay or Protocol* dialog is closed.

B. FURTHER INFORMATION ABOUT NUCLISENS EASYQ® HIV-1 v2.0 ASSAY SOFTWARE

B.1 Dependencies and automatic selection

The application will recognize certain dependencies that exist between assays and lots. It will aid the user in making correct selections by acting upon selections made by the user, automatically selecting or deselecting assays or lots that are dependent on others.

Automatic selection or de-selection only affects assays and/or lots, or versions thereof, which are not yet installed with the targeted Director.

The dependencies recognized by the application are:

***When* the User…**	***Then***	**NucliSENS EasyQ® Assay software *will automatically…***
Selects an assay	…	select lots required by this assay.
Deselects an assay	…	deselect lots required by this assay.
Selects a lot	…	select the assay requiring this lot.
Deselects a lot	…	deselect the assay requiring this lot if the lot was the last remaining selected lot of that assay.

B.2 Repairing an assay or lot data file

In Step 2 of the installation process for the EasyQ® system, assays and/or lots that are to be installed can be selected. In most cases the EasyQ® system will need to be updated with new lots or an enhanced assay. An existing assay or lot can also be re-installed. For example, when one or more of a lot's constituting files have become corrupted, the Director software is unable to use that lot data file. In that event, the damaged lot data file can be repaired by selecting it in Step 2 of the installation procedure. In order to maintain full traceability of historic measurement data, prior to attempting to repair it the application will backup the lot that was in use prior to repair.

Assays can be repaired in the same way.

B.3 Troubleshooting

If an error message occurs it is referred to the error codes listed below. Also check if the instructions have been followed carefully.
If the problem persists contact your local representative. Make sure to have the following information available:

- Version number of the application (see title bar).
- Log file.

The most recent log file can be found in directory D:\NucliSENS EasyQ® Assay Software\Logs. (Here D: is the letter of the drive where the NucliSENS EasyQ® Assay software has been installed.).

C. ERROR MESSAGES IN NUCLISENS EASYQ® HIV-1 v2.0 ASSAY SOFTWARE

The following section lists the errors that the application may generate. Please use the description of possible causes and actions to try to resolve the error. Should the problem persist please contact your local representative.

C.1 Start up errors

This section lists errors that may occur when the application is started.

Error 1000

Message:	Insufficient free disk space. Please free up disk space first.
Possible cause:	The disk carrying the normal target Director does not have enough free space.
Action:	• Exit the application, then • Free disk space by deleting files. Make sure to free space on the disk where the target Director is installed! Please back up files first, before deleting them. • Then restart the application and try again.

Error 1010

Message:	Cannot create log file. Installation disabled.
Possible cause:	When the application starts it cannot create the folder for storing its log file for this and subsequent sessions, due to: File system problems. The application is running on a non-NucliSENS EasyQ® system, such as a laptop or office pc.
Action:	• When the application is running on a NucliSENS EasyQ® system, please contact your local representative. • Safety regulations dictate that the application should be run exclusively on a NucliSENS EasyQ® system.

Error 1100

Message:	NucliSENS EasyQ® Director software is running. To install assays/reagents, please close Director first. Do you wish to continue?
Possible cause:	NucliSENS EasyQ® Director software is currently running.
Action:	• To install assays the running Director software needs to be closed first. Then press 'Yes' and proceed with the installation. • Pressing 'Yes' without closing the running Director software first the application does not permit installation of assays and/or lots. The IFU can still be read. • If the running Director is not to be closed press 'No' to close the NucliSENS EasyQ® Assay software. As a consequence, the IFU cannot be read.

Error 1110

Message:	NucliSENS EasyQ® Assay software is running.
Possible cause:	Attempt to start NucliSENS EasyQ® Assay software while it is running.
Action:	The current running instance of the application will be displayed. It is not possible to run two instances of the application simultaneously.

Error 1200

Message:	Cannot find compatible NucliSENS EasyQ® Director software. Installation disabled.
Possible cause:	• NucliSENS EasyQ® Director software is not installed on the system. • NucliSENS EasyQ® Director software is installed, but this version of NucliSENS EasyQ® Assay software is not suitable for installing assays or lots with the installed Director(s). • NucliSENS EasyQ® Director is installed, but a required configuration file is missing. • NucliSENS EasyQ® Director is installed on a network disk rather than on a fixed disk.
Action:	Exit the application, then either • Install NucliSENS EasyQ® Director software or • If NucliSENS EasyQ® Director is installed, please contact your local bioMérieux representative.

Error 1300

Message:	Failed to read assay definition + <filename>.
Possible cause:	• An Assay definition file is missing or corrupt.
Action:	• Please contact your local representative.

C.2 Installation errors

This section lists the errors that may occur during the installation of assays and/or lots. Identifying assay or lot names to which the error applies, text enclosed in angle brackets is generated by the application at run-time.

Error 2000

Message:	Cannot install assay <assay name>. Installation aborted.
Possible cause:	A directory could not be created or a file could not be copied or deleted while installing the assay with the given name. This can be due to: • Files that are protected against being overwritten or deleted. • An unknown error in the file system. • Defective CD-ROM.
Action:	Please contact your local representative.

Error 2010

Message:	Cannot install reagent <lot name>. Installation aborted.
Possible cause:	A directory could not be created, a file could not be copied or deleted, or a required file could not be retrieved while installing the lot with the given name. This can be due to: • Files that are protected against being overwritten or deleted. • An unknown error in the file system. • Defective CD-ROM.
Action:	Please contact your local representative.

Error 2100

Message:	Cannot backup assay <assay name>. Installation aborted.
Possible cause:	A directory could not be created, a file could not be copied or deleted, or a required file could not be retrieved while creating a backup copy of the assay with the given name. This can be due to: • Files that are protected against being overwritten or deleted. • An unknown error in the file system. • Defective CD-ROM.
Action:	Please contact your local representative.

Error 2110

Message:	Cannot backup reagent <lot name>. Installation aborted.
Possible cause:	A directory could not be created, a file could not be copied or deleted, or a required file could not be retrieved while creating a backup copy of the lot with the given name. This can be due to: • Files that are protected against being overwritten or deleted. • An unknown error in the file system. • Defective CD-ROM.
Action:	Please contact your local representative.

C.3 IFU errors

This section lists errors that may occur when trying to read the Instructions for Use

Error 3000

Message:	Cannot find IFU. Reading IFU disabled.
Possible cause:	Defective CD-ROM.
Action:	Please contact your local representative.

Error 3010

Message:	Cannot open IFU.
Possible cause:	Defective CD-ROM.
Action:	Please contact your local representative.

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