RAS Lifesciences Pvt Ltd
Striving for better health

# Amplisure™ HIV-1 RNA Quantitative Kit

## (Real Time PCR Kit)

**QT-HIV-25 : 25 rxns**
**QT-HIV-50 : 50 rxns**
**QT-HIV-100 :100 rxns**

IVD

**Product Insert**

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## Introduction

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers. HIV infects vital cells in the human immune system such as helper T cells, macrophages, and dendritic cells. When CD4+ T cell numbers decline below a critical level, cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections. HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about $10^{10}$ virions every day, coupled with a high mutation rate.

HIV-1 testing is initially done by ELISA to detect antibodies to HIV-1. Only specimens those are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate Western blot result, which may be either be due to an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person.

Detection of HIV-1 nucleic acid (RNA) by PCR can provide early evidence of HIV-1 infection (approximately 10-14 days after infection), when results of routine diagnostic assays are inconclusive. Clinical studies have indicated that detection of HIV-1 RNA in whole blood specimens by qPCR is highly sensitive (>95%) and specific (>98%) for the presence of early HIV-1 infection.

## Intended Use

The Amplisure™ HIV-1 RNA Quantitative Kit is used to detect the genome of HIV genotypes (A, B, C, D, AE, F, G, AA - GH) and quantify HIV-1 genome in plasma , using Real-Time PCR after nucleic acid extraction. The viral load is measured using a range of four quantification standards provided in the kit. Combined with other methods of biological investigation (clinical picture and other laboratory markers), the results obtained with the Amplisure™ HIV-1 RNA Quantitative Kit are used to monitor HIV infection.

This kit cannot be used for screening blood from donors or blood products.

## Product Description

Amplisure™ HIV-1 RNA Quantitative Kit is an *in-vitro* diagnostic kit for quantitation of Human Immunodeficiency Virus (A, B, C, D, AE, F, G, AA - GH subtypes) in human plasma. The kit contains the necessary reagents for performing HIV quantitation by Real-time PCR.

Pathogen detection by Real Time polymerase chain reaction (PCR) is based on the amplification of specific region (gag) of the pathogen genome. The assay principle is based on Taqman probes which allow higher specificity and sensitivity.

In addition, the Amplisure™ HIV-1 RNA Quantitative Kit contains a second amplification system to identify possible PCR inhibition by using an internal control (IC), without affecting the analytical sensitivity of the assay. External quantitation standards calibrated with WHO control are supplied, which allow the determination of the amount of Human Immunodeficiency Viral RNA.

## Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) Pre-PCR area/room - for setting up PCR reaction
c) PCR area/room – for performing PCR using the thermocyclers/real time PCR

As part of Good Laboratory Practices (GLP), it is recommended to have dedicated areas to avoid cross contamination.

## General Precautions

### *Precautions while extracting Nucleic acid*

Always wear proper attire (powder free gloves, facemask and Head cap) before starting the nucleic acid extraction procedure. During preparation of samples, open one sample tube at a time and close it before opening another tube, follow it at every step of isolation. Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of

Nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with Nucleic acid.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area. Sample Preparation Area must have dedicated laboratory coats, pipettes, pipette tips and cyclo-mixer and not to be moved to other areas. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Sample preparation should be performed in a Biosafety cabinet using aerosol free tips.

***Precautions while setting up a PCR reaction***

PCR assay is sensitive and any accidental introduction of product from previous amplification reactions can lead to incorrect results. Hence measures to reduce the risk of contamination in the laboratory should include physically separating the activities involved in performing PCR and complying with good laboratory practices. PCR hood should be used to avoid contamination.

It is recommended to have proper cleaning procedures to minimize the risk of cross contamination and carry over contamination (e.g. DNA OUT™, RNase OUT™, 0.1% Sodium Hypochlorite, Fumigation etc.).

It is recommended that areas should be defined in Pre-PCR room for preparation of mastermix and addition of templates. Laboratory coats and equipment used in the Pre-PCR Area must remain in this area and should not be moved to the Sample Preparation Area.

***Precautions for post PCR or equipment area/room***

The Real time PCR instrument/s should be kept in a separate segregated area away from Sample preparation area and Pre-PCR area.

***Precautions after completion of Real time PCR assay***

The reaction tubes or strips should be properly discarded without opening the caps, after the completion of run to avoid carry over contamination.

## Usage Limitations

1. All the kit components are for *in-vitro* diagnostics only.
2. The product is to be used by personnel specially trained in the *in-vitro* diagnostics procedures only.
3. Follow the product insert strictly for optimal results.
4. Do not use the kit beyond the expiry date mentioned on the box.
5. Follow the guidelines provided in product insert for sample collection, storage and transport.
6. For ideal performance, store the kit under recommended conditions only.

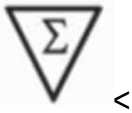
## Safety Precautions

1. All patient specimens should be considered as potentially infectious and handled in a BSL2 biosafety hood with BSL3 practices.
2. Wear personal protective equipment, including gloves, head cap, face mask and lab coats when handling kit reagents/sample extraction. Wash hands thoroughly using detergents before and after performing the test.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens as per regulatory guidelines.

## Storage Conditions and Product Stability

1. All the kit reagents should be stored at -20 °C. Replace all the kit components immediately at -20 °C after usage.
2. Repeated thawing and freezing (more than 6 x) of all kit reagents should be avoided, as it reduces assay sensitivity. If needed, make aliquots of the kit reagents according to the volume used in the protocol prior to freezing.
3. Allow reagents to be thawed completely on Ice/4 °C prior to use.
4. Kit reagents are stable through the end of the expiration date indicated on the box when stored at -20 °C.

## Symbols

| Description of Symbol | Denotation |
|---|---|
|  | *in-vitro* Diagnostic medical device |
|  | Consult Instruction manual (Product Insert) for use |
|  | Lot Number of the kit or Kit contents |
|  | Catalogue number of Kit |
|  <N> | Contains sufficient for <N> reactions (Pack Size) |
|  | Manufacturer |
|  | Temperature limitation (Storage Condition) |
|  | Use by MMM-YYYY (Expiry Date) |
|  | Biological risk (handle carefully) |
|  | Important Note |

## Kit Components

| Color Coding (Caps) | Contents | Description | 25 rxns (QT-HIV-25) | 50 rxns (QT-HIV-50) | 100 rxns (QT-HIV-100) 2 x 50 rxns |
|---|---|---|---|---|---|
| Yellow | RAS qRNA PCR Mix | DNA Amplification Reagent | 1 x 375 µL | 1 x 750 µL | 2 x 750 µL |
| Red | RAS RT Mix | cDNA synthesis Reagent | 1 x 175 µL | 1 x 350 µL | 2 x 350 µL |
| Brown | RAS HIV PPM | HIV Primer-Probe Mix | 1 x 50 µL | 1 x 100 µL | 2 x 100 µL |
| Lilac | RAS HIQS1 ($2 \times 10^4$ IU/ µL) | HIV Quantitation Standards | 1 x 30 µL | 1 x 60 µL | 2 x 60 µL |
| Lilac | RAS HIQS2 ($2 \times 10^3$ IU/ µL) | | 1 x 30 µL | 1 x 60 µL | 2 x 60 µL |
| Lilac | RAS HIQS3 ($2 \times 10^2$ IU/ µL) | | 1 x 30 µL | 1 x 60 µL | 2 x 60 µL |
| Lilac | RAS HIQS4 ($2 \times 10^1$ IU/ µL) | | 1 x 30 µL | 1 x 60 µL | 2 x 60 µL |
| Natural | RAS IC-A PCR Mix | Internal Control | 1 x 25 µL | 1 x 50 µL | 2 x 50 µL |
| Natural | RAS R-IC-A EX Mix | | 1 x 250 µL | 1 x 500 µL | 2 x 500 µL |
| White | MBGW | Molecular Biology Grade water | 1 x 500 µL | 1 x 500 µL | 2 x 500 µL |

## Materials required but not supplied

The materials which are required but not supplied are listed below:

1. Viral RNA Extraction kit
2. Biosafety Cabinet
3. PCR Hood
4. Calibrated variable micropipettes
5. Sterile pipette filter tips (aerosol free)
6. Vortex mixer
7. Dry Bath
8. Benchtop centrifuge with rotor for 1.5 mL reaction tubes
9. Real Time PCR machine
10. Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate
11. Cooling block (96 x 0.2 mL tubes)
12. 1.5 mL centrifuge tubes
13. 1.5 mL centrifuge tube stand
14. Cooling block (2 mL tubes)
15. Sterile Nitrile gloves
16. Facemask
17. Head cap
18. Lab coats

## Quality Systems

In accordance with ISO-certified Quality Management System (9001:2008 and 13485: 2003) of RAS Lifesciences, each lot of Amplisure™ HIV-1 RNA Quantitative Kit is tested against predetermined specifications to ensure consistent product quality.

## Sample Type/Collection/Storage/Transport

### Sample Type

Plasma ($K_2$EDTA-Blood)
*Heparinized Blood must not be used as they inhibit the PCR reaction*

**Sample Collection, Storage and Transport**

3-5 mL of blood has to be drawn into a $K_2$EDTA vaccutainer. Cap and swirl the tubes for uniform mixing of blood and $K_2$EDTA. When $K_2$EDTA is used, whole blood can be collected in tubes with or without a gel separator. The $K_2$EDTA blood samples are centrifuged (20 minutes at 1000–1500 x *g)* to separate plasma from cellular material and in the case of a non gel separator tube, the plasma should be removed to a secondary sterile tube within 4 hours of phlebotomy.

Collection and storage of unstabilized whole blood is not recommended for PCR analysis, because RNA degradation occurs in blood stored *ex vivo*. The sensitivity of the assay can be reduced if whole blood samples are frozen or stored for a longer period of time.

Plasma separated in a gel separator tube may be transported to the laboratory *in situ.* Plasma should be shipped at 2 to 8 °C and stored at -20 °C as it is stable for up to five days at 2 to 8 °C and longer if frozen at -20 °C or -70 °C or lower. Do not store plasma samples in a “frost -free” freezer as the temperature is cycled several times per day on this type of freezer, causing degradation of nucleic acid targets.

Sample material should be transported in a leak proof, unbreakable transport container to avoid leakage of sample. The samples should be transported following the local and national instructions for the transport of pathogen material.

## Assay Procedure

## RNA Extraction

Amplisure™ HIV-1 RNA Quantitative Kit has been validated using the following Viral RNA extraction systems:

1- Roche High Pure Viral RNA kit (Cat. No. 11858882001)
2- QIAamp Viral RNA Mini Kit (Cat. No. 52904)

Follow the manufacturer’s instructions mentioned in the manual for Viral RNA extraction. Different pack sizes of the above mentioned kits can be used. However the customer can validate their own extraction process using other Viral RNA extraction systems.

Recommended sample volume for extraction and elution are as follows:

| Name of the RNA Isolation Kit | Recommended Sample volume *(to be taken for RNA Extraction)* | Recommended Final Elution volume |
|---|---|---|
| Roche High Pure Viral RNA kit (Cat. No.11858882001) | 200 µL | 50 µL |
| QIAamp Viral RNA Mini Kit (Cat. No. 52904) | 140 µL | 50 µL |

The analytical sensitivity of the assay in consideration of the purification was determined using the above defined volumes.

## Use of Internal Control (IC)

Internal controls are supplied (RAS R-IC-A Ex Mix and RAS IC-A PCR Mix) along with Amplisure™ HIV-1 RNA Quantitative Kit. This allows the user to control the Viral RNA isolation procedure as well as to check for possible PCR inhibition.

ⓘ Internal control should only be used once, either at the Extraction step or at the PCR step

### *Usage of Internal Control at the RNA extraction step*

If internal control (IC-A) is required to be added at the time of RNA extraction, add 10 µL of RAS *R-IC-A Ex mix* per isolation to the lysis buffer, along with other components of kit used for lysis (as per kit instructions) and vortex for 5 seconds prior to usage.

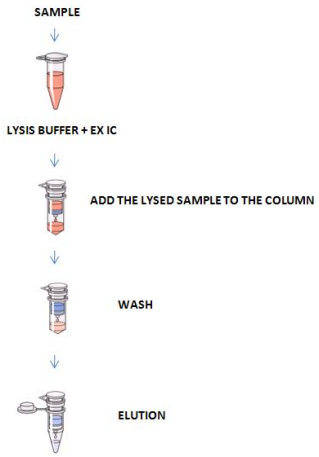



**Fig1. Viral RNA Extraction Overview**

***Usage of Internal Control at the Real time PCR step***

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the PCR master mix as described below. Addition of Internal control should be done only once, either at the time of sample extraction or during PCR setup.

## qPCR Protocol

### Preparation of Reaction Mastermix

Quantitation procedure with Amplisure$^{TM}$ HIV-1 RNA Quantitative Kit involves *1 step RT qPCR*. It is recommended that a minimum of three standards and a negative control (MBGW should be used as negative control) are required to be included in a single run for acquiring proper results.
Set up a real time single step RTPCR reaction as follows:

***1- qPCR reaction mix composition without Internal Control (When R-IC-A Ex Mix is added during RNA extraction)***

| Components | Volume per reaction ( µL) (for final vol. of 30 µL) |
|---|---|
| RAS qRNA PCR Mix | 15.0 |
| RAS RT Mix | 7.0 |
| RAS HIV PPM | 2.0 |
| RNA/HIQS/ MBGW | 5.0 |
| MBGW | 1.0 |

***2- qPCR reaction mix composition with Internal Control (When R-IC-A Ex Mix is not added during RNA extraction)***

| Components | Volume per reaction ( µL) (for final vol. of 30 µL) |
|---|---|
| RAS qRNA PCR Mix | 15.0 |
| RAS RT Mix | 7.0 |
| RAS HIV PPM | 2.0 |
| RNA/HIQS/ MBGW | 5.0 |
| RAS IC-A PCR Mix | 1.0 |

1. **Addition of IC-A PCR mix (if required) should be done at the time of preparation of master mix.**
2. **The results may be inconsistent, if the IC PCR mix is added individually.**

Place the PCR plate/tubes/strips in real time thermocycler.

## PCR Programming

Amplisure™ HIV-1 RNA Quantitative Kit is validated on the following instruments:

- Rotor-Gene™ 6000
- Rotor-Gene™ Q 5plex
- ABI 7500 DX Real-Time PCR System
- ABI 7300 Real-Time PCR System
- Eppendorf Realplex 4
- Bio-Rad™ CFX 96

## *Plate Setup*

1- Program the plate setup by labeling the slots as per tube/strip/plate labels. The sequence of labeling of slots should be the same way as the tube/strip/plate is kept in the machine.
2- Select the type of sample (Unknown/Standard/NTC) for each slot.
3- When Standard is selected as sample type, mention the quantitation value in IU/µL (2E4, 2E3 etc)
4- Select the channel for acquisition (FAM/Yakima Yellow)

| Name of channel | Source wavelength (nm) | Detection wavelength (nm) |
|---|---|---|
| FAM (Pathogen target) | 470 | 510 |
| Yakima Yellow (Internal Control) | 530 | 555 |

5- For background calibration in different instruments, follow the procedure described below:

| | |
|---|---|
| Rotor-Gene™ 6000 | - Perform 'Gain optimization' |
| Rotor-Gene™ Q 5plex | - Perform 'Gain optimization' |
| ABI 7500 DX Real-Time PCR System | - Select Passive Reference dye 'ROX' |
| ABI 7300 Real-Time PCR System | - Select Passive Reference dye 'ROX' |
| Eppendorf Realplex 4 | - Select 'ROX' for background calibration |
| Bio-Rad™ CFX 96 | - Select 'ROX' for background calibration |

**Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com**

## *Cycling conditions*

1- Configure the following program in the machine.

| Steps | No. of cycles | Temperature (°C) | Time |
|---|---|---|---|
| 1 (Reverse Transcription) | 1 | 42 | 15 min |
| 2 (Initial denaturation) | 1 | 95 | 10 min |
| 3 (PCR cycling 1) | 5 | 94 | 10 sec |
| | | 54 | 30 sec |
| | | 60* | 45 sec |
| 4 (PCR cycling 2) | 40 | 94 | 10 sec |
| | | 56 | 30 sec |
| | | 60* | 45 sec |
| * **Plate Read/Data Acquisition in FAM and Yakima Yellow channel** | | | |

2- Set the reaction volume as 30 µL.
3- Plate read/Data Acquisition for FAM and Yakima Yellow channel should be incorporated in the third and fourth stage; step 3 (60 ˚C/45 sec).
*4*- The ideal run time for the assay is 130 minutes. Note: *In case of Eppendorf Realplex 4, select RAMP rate as 35%.*

**Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com**

## Data Analysis

Analyze the data after completion of the run. Check the $R_n$/*Cycle* amplification plot and $\Delta R_n$/*Cycle* amplification plot to observe the amplification signal generated by different samples in the run. Compare both the plots for data analysis. Also look for noisy signals, if observed as it might not give you a proper result.

## Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to $R_n$/*Cycle* amplification plot. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals.

## Result

The values for unknown samples would appear in the result column in *IU/µL* in FAM Channel. Samples showing no amplification in FAM channel should show amplification in Yakima Yellow channel, and then only results should be considered. The negative control should not show any value in the result column.

## Interpretation

Interpret the values for unknown samples, only if the Slope of Standards is between -3.1 to – 3.6 and PCR efficiency is between 90%-110% (0.9 - 1.1) and there should be no amplification in negative control.

| Observation | Interpretation | Conclusion |
|---|---|---|
| Amplification signal detected in HIV channel (FAM) and in Internal control channel (Yakima Yellow) | Quantify HIV RNA | Proceed for further Analysis |
| Amplification signal detected in HIV channel (FAM) but no signal in Internal Control channel (Yakima Yellow) | HIV RNA detected For accurate quantitation repeat the quantitation with diluted (1:100) and undiluted sample | |
| Amplification signal not detected in HIV channel (FAM) but detected in Internal control channel (Yakima Yellow) | HIV RNA below detection limit | |
| No Amplification signal detected in HIV channel (FAM) as well as Internal control channel (Yakima Yellow) in unknown samples | Reaction can't be interpreted as Inhibition of PCR or incorrect extraction | Repeat the extraction and PCR |

To convert the results from *IU/µL* to IU/mL use the following formula:

$$\text{Result (IU/mL)} = \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

*** For calculating the result of diluted sample (1:100); multiply the observed IU/mL value by dilution factor, 100**

$$\text{Result of 1:100 diluted sample (IU/mL)} = \text{Dilution Factor (100)} \times \frac{\text{Result (IU/µL)} \times \text{Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

## Troubleshoot

| Observation | Possible cause | Solution |
|---|---|---|
| No amplification signal for Standards in FAM channel | 1. One of the components may not have been added.<br>2. Incorrect channel selection<br>3. Incorrect programming of the real time machine.<br>4. Instrument is not working properly | 1. Repeat the assay by following the correct protocol and addition of reagents<br>2&3. Recheck the PCR program<br>4. Contact manufacturer of thermocycler for technical support. |
| Weak amplification signal for standards (Signal below threshold) in FAM Channel | 1. Improper PCR programming.<br>2. Inaccurate dispensing of reagents<br>3. Possible deterioration of kit components due to improper storage | 1. Repeat the assay by following the correct protocol<br>2. Minimize Pipetting errors/Check for calibration status of pipettes |
| Amplification observed in FAM channel in negative control | 1. Aerosols generated<br>2. Environmental Pipettes, /Work area.<br>3. Possible contamination of Kit reagents . | 1. Use fresh aliquots of Standards/Kit Reagents (if available)<br>2. Clean the PCR rack/Pipettes thoroughly as per GLP<br>3. Clean and Fumigate the work area overnight prior to use |

**For any other technical query; please contact amplisure@raslifesciences.com**

# Assay Characteristics

## Analytical Sensitivity

The analytical sensitivity of PCR assay as well as the analytical sensitivity in consideration of the purification (RNA Extraction) was assessed for the Amplisure™ HIV-1 RNA Quantitative Kit.

The analytical sensitivity is determined using WHO standards/HIV positive clinical samples in combination with a particular extraction method.

### Analytical Sensitivity of qPCR assay

The analytical sensitivity is determined independent from the selected extraction method, using an international standard of known concentration ie, 3rd HIV-1 International Standard (NIBSC code: 10/152)

To determine the analytical sensitivity of the Amplisure™ HIV-1 RNA Quantitative Kit, a standard dilution series was set up from 0.03 IU/µL to 1480 IU/µL and analyzed with Amplisure™ HIV-1 RNA Quantitative Kit.

Testing was carried out on 5 different days on 3 replicates. The results were analyzed by statistical analysis.

***The analytical sensitivity of the Amplisure™ HIV-1 RNA Quantitative Kit is 0.36 IU/µL.***

### Analytical Sensitivity in consideration of Extraction Method

The analytical sensitivity in consideration of the purification method (using Roche High Pure Viral RNA kit Cat. No. 11858882001 of the Amplisure™ HIV-1 RNA Quantitative Kit was also determined using a dilution series of the 3rd HIV-1 International Standard (NIBSC code: 10/152) from 10.57 to 10571.4 IU/mL spiked in healthy plasma specimens (Negative for HIV by Real time PCR).

These were subjected to RNA extraction using the Roche High Pure Viral RNA kit (extraction volume: 0.2 mL, elution volume: 50 µL)

Each of the dilutions was analyzed with the Amplisure™ HIV-1 RNA Quantitative Kit on 5 different days on 3 replicates. The results were determined by statistical analysis.

***The analytical sensitivity in consideration of the purification of the Amplisure™ HIV-1 RNA Quantitative Kit is 95 IU/mL with Roche High Pure Viral RNA kit.***

## Linear Range

The linear range (analytical measurement) of the Amplisure™ HIV-1 RNA Quantitative Kit was determined by analyzing a dilution series of HIV quantitation standards from $2x10^7$ IU/µL to 2 IU/µL. The dilution series has been calibrated against the 3rd HIV-1 International Standard (NIBSC code: 10/152).

Each dilution was tested in replicates (n= 3) using the Amplisure™ HIV-1 RNA Quantitative Kit.

The linear range of the Amplisure™ HIV-1 RNA Quantitative Kit has been determined to cover concentrations from ***0.4 IU/µL to 2 x $10^7$ IU/µL***.

## Conversion Factor (IU/µL to Copies/µL)

The 3rd HIV-1 International Standard (NIBSC code: 10/152) has been assigned an International Unit (IU) by the WHO as the number of genome equivalents.

The conversion factor value for Amplisure™ HIV-1 RNA Quantitative Kit has been assigned after calibration of the HIQS (HIV quantitation standards) with the 3rd HIV-1 International Standard (NIBSC code: 10/152). Conversion Factor (IU/µL to Copies/µL) is entirely dependent on the current PCR assay kit. A Correlation and Regression analysis was performed to compare the two standard curves of HIV RNA values obtained by Amplisure™ HIV-1 RNA Quantitative Kit (copies/µL) and HIV NIBSC standards (IU/µL).

The correlation coefficient between the expected and estimated values was very good

**r = 0.98**

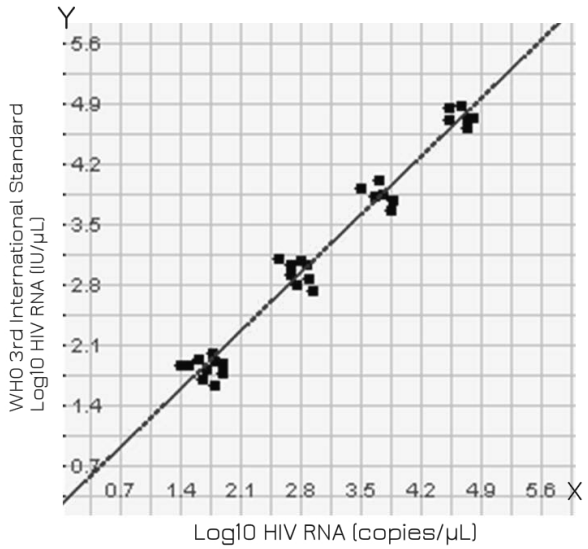



**Fig2. Correlation and Regression plot of HIV NIBSC standards (IU/µL) and HIV RNA (copies/µL)**

The conversion factor calculated for Amplisure™ HIV-1 RNA Quantitative Kit from IU/µL to Copies/ µL is 0.4, i.e. **1 IU/µL = 0.4 Copies/ µL**

To convert into copies/mL use the following formula:

$$\text{Result (copies/mL)} = \frac{\text{Calculated Value (copies/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

## Specificity

The specificity of the Amplisure™ HIV-1 RNA Quantitative Kit is ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all published sequences (Genbank) by BLAST analysis.

### Detection of Different HIV subtypes (A, B, C, D, AE, F, G, AA-GH) using International Standard

The detectability of all relevant genotypes (A, B, C, D, AE, F, G, AA-GH) was ensured by a Database alignment and by a qPCR run with the following genotypes, using

International standard (2nd WHO International Reference Panel preparation for HIV-1 subtypes for NAT; NIBSC code 12/224) and using ≥ 100 positive HIV samples covering all genotypes.

| International HIV Genotype Controls | Source of International controls | Result obtained with Amplisure™ HIV-1 RNA Quantitative Kit |
|---|---|---|
| Geno A | NIBSC | Detected |
| Geno B | NIBSC | Detected |
| Geno C | NIBSC | Detected |
| Geno D | NIBSC | Detected |
| Geno AE | NIBSC | Detected |
| Geno F | NIBSC | Detected |
| Geno G | NIBSC | Detected |
| Geno AA-GH | NIBSC | Detected |

## Cross Reactivity Data

A potential cross-reactivity of the Amplisure™ HIV-1 RNA Quantitative Kit was tested using the control group listed below. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

The specificity was also validated with 50 different healthy plasma specimens/other various sample types. These did not generate any amplification signals with the HIV specific primers and probes, which are included in the Amplisure™ HIV-1 RNA Quantitative Kit.

| Pathogen Tested | Cross Reactivity with the HIV Primers/Probes |
|---|---|
| Adenovirus | -ve |
| Herpes Simplex Virus-1 | -ve |
| Herpes Simplex Virus-2 | -ve |
| Epstein Barr virus | -ve |
| Hepatitis C virus | -ve |
| Cytomegalo virus | -ve |
| Hepatitis B virus | -ve |
| Enterovirus | -ve |
| BK virus | -ve |
| MTB complex | -ve |
| Plasmodium sps. | -ve |

## Precision

The precision data of the Amplisure™ HIV-1 RNA Quantitative Kit have been generated for HIV positive clinical specimens and HIV quantitation standards.

## Intra-assay variability

The precision data consists of the *Intra-assay variability* which is variability of multiple results of samples of the same concentration within one experiment.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HIV-1 RNA Quantitative Kit have been collected using all the quantitation standards (HIQS1-HIQS4). Also included were three different samples with different viral loads.

## Inter-assay variability

The precision data consists of the *Inter-assay variability* which is variability of multiple results of samples of the same concentration within different experiments.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HIV-1 RNA Quantitative Kit have been collected using all the quantitation standards (HIQS1-HIQS4). Also included were three different samples with different viral loads. Testing was performed with 3 replicates for 10 days.

The precision data was calculated on basis of the CT values obtained.

| Sample Type | Mean [Log Value (IU/µL)] | Variability Testing | Standard Deviation | Coefficient of Variation (%) |
|---|---|---|---|---|
| HIQS1 | Log 4.3 | Intra-assay variability | 0.013 | 0.002 |
| | | Inter-assay variability | 0.083 | 0.005 |
| HIQS2 | Log 3.3 | Intra-assay variability | 0.017 | 0.002 |
| | | Inter-assay variability | 0.075 | 0.003 |
| HIQS3 | Log 2.3 | Intra-assay variability | 0.019 | 0.003 |
| | | Inter-assay variability | 0.130 | 0.004 |
| HIQS4 | Log 1.3 | Intra-assay variability | 0.023 | 0.002 |
| | | Inter-assay variability | 0.050 | 0.003 |
| HIV Control 1 | Log 3.6 | Intra-assay variability | 0.250 | 0.011 |
| | | Inter-assay variability | 0.273 | 0.013 |
| HIV Control 2 | Log 2.3 | Intra-assay variability | 0.165 | 0.015 |
| | | Inter-assay variability | 0.253 | 0.017 |
| HIV Control 3 | Log 1.1 | Intra-assay variability | 0.183 | 0.014 |
| | | Inter-assay variability | 0.310 | 0.018 |

**Performance study on samples**

Biological performances of the Amplisure™ HIV-1 RNA Quantitative Kit on plasma samples have been evaluated in an Indian Microbiology Laboratory, using samples collected during the laboratory's routine activity.

The laboratory's routine technique is a commercial real-time quantitative PCR kit which was used to amplify a fragment of 95 bp of the HIV-1 genome. The results were expressed directly in IU/mL.

The samples of plasma were extracted with the Roche High Pure Viral RNA kit (Cat. No.11858882001) using the protocol recommanded by the manufacturer on 200 µL with an elution volume of 50 µL. The extracted samples were amplified on Rotor-Gene Q using both kit reagents following manufacturer's instructions.
A total of 98 plasma samples was tested.

Results of the concordance obtained on the plasma samples

| | | COMMERCIAL PCR KIT | | Total |
|---|---|---|---|---|
| | | + | - | |
| Amplisure™ HIV-1 RNA Quantitative Kit | + | 74 | 2* | 76 |
| | - | 4* | 18 | 22 |
| | **Total** | 78 | 20 | 98 |

**Global concordance: 92/98** = 93.9% [87.2; 97.7] (95%exact CI)

Of 98 samples tested, 6 gave discordant results.

Two samples negative and 4 samples positive with the commercial HIV Real time kit , were sent for evaluation, at a third party testing center which uses another FDA approved platform. The results obtained at the center was similar to the result obtained by Amplisure HIV-1 kit during the clinical evaluation.

Quantitative analysis of positive plasma samples in the two kits

The quantitative analysis of results for plasma between the two techniques was carried out on confirmed positive samples.

It showed good correlation between the quantifications of the two techniques for plasma. Indeed, the mean difference in quantification between the two PCR techniques was around +0.08 $log_{10}$ IU/mL.

**Pearson coefficient correlation: R=**0.795

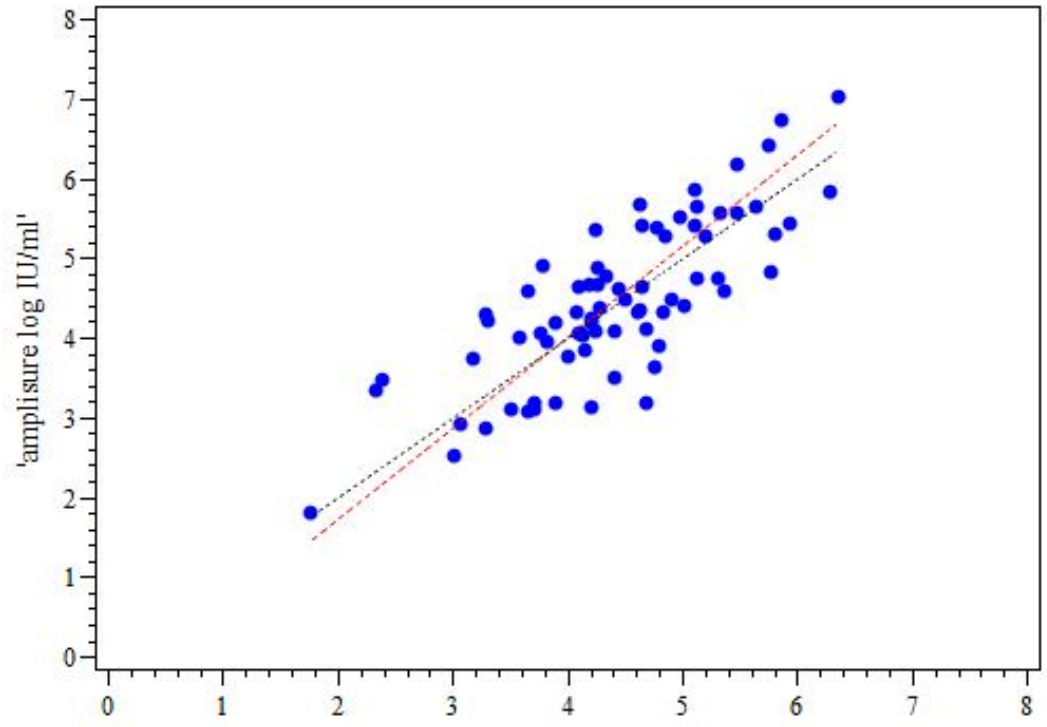



**Fig.3 : The quantification performance of the Amplisure HIV kit on blood plasma was therefore demonstrated.**

## Abbreviations

| ***Abbreviation*** | ***Expansion*** |
|---|---|
| HIV | Human Immunodeficiency Virus |
| RNA | Ribonucleic Acid |
| IC | Internal Control |
| RNases | Ribonucleases |
| PCR | Polymerase Chain Reaction |
| BSL2 | Bio Safety Level 2 |
| BSL3 | Bio Safety Level 3 |
| mL | Milli Liters |
| µL | Micro Liters |
| $K_2$EDTA | Di Potassium Ethylene Diamine Tetra Acetate |
| g | Relative Centrifugal Force |
| qPCR Protocol | Quantitative PCR protocol |
| MBGW | Molecular Biology Grade Water |
| RT PCR | Reverse Transcriptase PCR |
| NTC | No Template Control |
| FAM | Carboxyfluorescein |
| ROX | Carboxy-X-rhodamine |
| NIBSC | National Institute for Biological Standards and Control |
| IU | International Units |
| WHO | World Health Organization |
| BLAST | Basic Local Alignment Search Tool |
| Rxn | Reaction |
| NAT | Nucleic Acid Amplification Techniques |

## References

1. Douek DC, Roederer M, Koup RA (2009). "Emerging Concepts in the Immunopathogenesis of AIDS". *Annu.Rev.Med.* 60:471–84.Doi 10.1146/annurev.med.60.041807.123549.PMC 2716400. PMID 18947296.
2. DeSimone JA, Pomerantz RJ: New methods for the detection of HIV. Clin Lab Med 2002;22:573-592

3. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

## Ordering Information

**QT-HIV-25 : 25 rxns**
**QT-HIV-50 : 50 rxns**
**QT-HIV-100 : 100 rxns**