RTA®

HIV-1 Real-Time PCR Kit

Handbook

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IVD

Quantitative detection of Human Immunodeficiency Virus RNA

For *in vitro* diagnostic use. For professional use only

REF 09013025 – 25 tests
09013100 – 100 tests

CE 0483

Kit Contents

	CapColor	Reagents	25 Tests	100 Tests
1	BROWN	HIV-1 Reaction Mix	500 µl	2x1000 µl
2	YELLOW	HIV-1 Enzyme Mix	42 µl	165 µl
3	BLUE	HIV-1 Internal Control	75 µl	250 µl
4	RED	HIV-1 Quantification Standart 1 (10^7 IU/ml)	100 µl	100 µl
5	RED	HIV-1 Quantification Standart 2 (10^6 IU/ml)	100 µl	100 µl
6	RED	HIV-1 Quantification Standart 3 (10^5 IU/ml)	100 µl	100 µl
7	RED	HIV-1 Quantification Standart 4 (10^4 IU/ml	100 µl	100 µl
8	WHITE	PCR Grade Water	100 µl	100 µl

Storage

All reagents of RTA HIV-1 Real-Time PCR Kit should be stored at -20°C. Storage at higher temperatures should be avoided (e.g. +4°C). Under these conditions, kit contents should be stable through the expiration date printed on the label. The reagents should not be freeze-thawed more than 2 times; otherwise the shelf of the kit will reduce. During the working steps all reagents should be kept on ice.

Intended Use

RTA HIV-1 Real-Time PCR Kit is an in vitro nucleic acid amplification assay for quantification of Human Immunodeficiency virus type 1 (HIV-1) RNA in human serum or plasma (EDTA) using RTA Viral Nucleic Acid Isolation Kit and Stratagene Mx3000p/Mx3005p instrument or Rotor-Gene 3000/6000 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or BIO-RAD CFX96-IVD Real-Time PCR Detection System for amplification. RTA HIV-1 Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HIV-1 infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings. RTA HIV-1 Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HIV-1 RNA or confirmation of the diagnosis of infection with HIV-1.

Product Use Limitations

- All reagents of the kit are for *in vitro* diagnostic use only.
- RTA HIV-1 Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HIV-1 RNA or confirmation of the diagnosis of infection with HIV-1.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Test with other sample types may result in inaccurate results.
- This kit has been validated for use with RTA Viral Nucleic Acid Isolation Kit. Using other isolation kits may adversely affect the performance characteristics of the kit.
- This kit has been validated for use with Stratagene Mx3000p/Mx3005p instrument or Rotor-Gene 3000/6000 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or BIO-RAD CFX96-IVD Real-Time PCR Detection System for amplification, detection and analysis. Using other instruments may adversely affect the performance characteristics of the kit.
- This kit has been optimized for use with specific PCR plastic consumables listed under Additional Materials Required section of the Handbook. Using other PCR plastic consumables may adversely affect the performance characteristics of the kit.
- Trustworthy results depend on proper sample collection, transport, storage and processing methods.
- It is intended for professional use by properly trained personnel.
- RTA HIV-1 Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HIV-1 infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings.
- The instructions in user manual should be followed strictly for optimum PCR results.
- The expired kits should not be used. Kit components from different lots should not be mixed.

Product Description

RTA HIV-1 Real-Time PCR assay is a one-step real time reverse transcription PCR assay in which RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by DNA polymerase-mediated cDNA amplification. During cDNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if cDNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number. RTA HIV-1 real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. The target region is situated in *gag gene* region of HIV-1 genome and is 121-bases long. HIV-1 RNA concentration is noted in International Units/ml (IU/ml). If it is needed to convert to copies/ml, our conversion factor for RTA HIV-1 Real-Time PCR Kit is 0.5 copies/IU. In other words, 1 IU/ml = 0.5 copies/ml.

Pathogen Information

HIV is classified in the family Retroviridae, subfamily Lentivirinae, and genus Lentivirus (1). The structure of HIV follows the typical pattern of the retrovirus family, comprising a singlestranded, positive-sense ribonucleic acid (RNA) genome of about 9.7 kilobases. There are two strands of HIV RNA and each strand has a copy of the virus's nine genes. The RNA is surrounded by a cone-shaped capsid which consists of approximately 2000 copies of the p24 viral protein. Surrounding the capsid is the viral envelope. The viral envelope is composed of a lipid bilayer membrane, formed from the cellular membraneof the host cell during budding of the newly formed virus particle. Host-cell proteins, such as the major histocompatibility complex (MHC) antigens and actin, remain embedded within the viral envelope, along with the viral envelope protein. Each envelope subunit consists of two non-covalently linked membrane proteins: glycoprotein (gp) 120, the outer envelope protein, and gp41, the transmembrane protein that anchors the glycoprotein complex to the surface of the virion. The envelope protein is the most variable component of HIV, although gp120 itself is structurally divided into highly variable (V) and more constant (C) regions. The variability of V regions may be a product of envelope functionality, as has been especially well described in V3, where amino acid changes alter coreceptor use. The variability of the HIV envelope also confers a uniquely complex antigenic diversity (1).
HIV-1 is divided into three quite distinct lineages: the groups M, N and O. Again, the worldwide distribution of these groups is not equal: group M (for Main) strains are substantially more common in the global epidemic than the group O (Outlier) strains, which are largely confined to Africa, with sporadic cases reported elsewhere. The group N (non-M, non-O) strains have only been isolated in Cameroon (2).

Warnings and Precautions

- All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.
- Before and after work all surfaces should be disinfected with a freshly prepared solution of 10% bleach or antiviral agents.
- Dispose of unused reagents, waste and specimens in accordance with country or local regulations.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wear protective disposable gloves, laboratory coats and eye-wear when handling clinical specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water,
- The procedures should preferably be performed in four separated areas (i.e. for RNA extraction, PCR setup, sample addition, amplification) to aid in preventing contamination.. All supplies for a particular procedure should be stored in the area where that procedure is performed and should not be moved between areas Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to an area and never worn outside of that area. The work should flow in one direction, beginning in the extraction area, moving to the PCR setup area in which PCR Master Mix is prepared, then moving to the third area in which samples, negative control and quantification standarts are added, finally moving to amplification area in which real-time PCR equipment is run.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control; to prevent sample contamination, use new, sterile aerosol barrier or positive displacement Rnase/DNase-free pipette tips and sterile pipettes.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
- Store the kit away from any source of contaminating DNA or RNA, especially amplified nucleic acid.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- A single type of HIV-1 RNA assay should be used for monitoring a patient. If RTA HIV-1 Real-Time PCR Kit substitutes another HIV-1 RNA assay, both tests should be used in parallel for at least two subsequent samples.
- Do not use a kit after its expiration date.

Performance Characteristics

Analytical Sensitivity Analytical sensitivity was analyzed by use of a dilution series of WHO standard, and the cutoff value of the kit was determined by probit analysis for STRATAGENE Mx3000p, BIO-RAD CFX96-IVD, INCEPTRA Cycler 9660 and Rotor-Gene 6000 Real-Time PCR Systems. A dilution series of a WHO International HIV-1 standard (NIBSC code: 97/650) was prepared to give the final concentrations of 640, 320, 160, 80, 40 and 20 IU/ml. Dilutions were extracted by RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029) according to RTA Viral Nucleic Acid Isolation Kit Handbook. Each dilution was tested in 24 replicates for each instrument. Lower limit was calculated by probit analysis done by PASW Statistics 18 program.
For BIO-RAD CFX96-IVD, RTA HIV-1 Real-Time PCR Kit can detect HIV-1 RNA at concentration of 78 IU/ml with a probability rate of 95 %. And, 95 % confidence range is 59.7 – 137.1 IU/ml.
For STRATAGENE, RTA HIV-1 Real-Time PCR Kit can detect HIV-1 RNA at concentration of 84 IU/ml with a probability rate of 95 %. And, 95 % confidence range is 67,5 – 122,6 IU/ml.
For INCEPTRA Cycler, RTA HIV-1 Real-Time PCR Kit can detect HIV-1 RNA at concentration of 63 IU/ml with a probability rate of 95 %. And, 95 % confidence range is 50.2 – 100 IU/ml.
For Rotor-Gene, RTA HIV-1 Real-Time PCR Kit can detect HIV-1 RNA at concentration of 94 IU/ml with a probability rate of 95 %. And, 95 % confidence range is 74.7 –140 IU/ml.

Linear Range To determine the upper limit, a dilution series of HIV-1 Reference material ranging from 1 x 104 IU/ml to 1 x 106 IU/ml were prepared. Viral RNA was extracted from standards by RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029) according to RTA Viral Nucleic Acid Isolation Kit Handbook. High concentration samples (1 x 107, 1 x 108 and 1 x 109 IU/ml) were prepared by using calibrated in vitro transcribed RNA bearing HIV-1 quantification standard. Within this range, the relationship between log of target RNA and Ct values is linear. Linear regression analyses comparing the Ct values-versus-log of target RNA were as follows:
For STRATAGENE, Ct value = -3.351(log of target RNA) + 45.49; with a correlation coefficient (R2) of 0.999. For INCEPTRA Cycler, Ct value = -3.416(log of target RNA) + 46.06; with a correlation coefficient (R2) of 0.999. For BIO-RAD CFX96, Ct value = -3.256(log of target RNA) + 44.78; with a correlation coefficient (R2) of 0.999. For Rotor-Gene, Ct value = -3.445(log of target RNA) + 47.04; with a correlation coefficient (R2) of 0.998. Upper limit is at least 1 x 109 IU/ml for STRATAGENE, INCEPTRA Cycler, Rotor-Gene and BIO-RAD CFX96-IVD.
Lower limit was calculated by probit analysis done by PASW Statistics 18 program according to the quantification results of HIV-1 Analytical Sensitivity Studies. 95 % lower confidence limit is 100 IU/ml for STRATAGENE, 94 IU/ml for INCEPTRA Cycler, 87 IU/ml for BIO-RAD CFX96-IVD and 111 IU/ml for Rotor-Gene. Dynamic ranges of RTA HIV-1 Real-Time PCR Kit:

For STRATAGENE	**100 - 1 x 10^9 IU/ml**
For INCEPTRA Cycler	**94 - 1 x 10^9 IU/ml**
For BIO-RAD CFX96	**87 - 1 x 10^9 IU/ml**
For Rotor-Gene	**111 - 1 x 10^9 IU/ml**

Precision For each experiment, 24 replicates of each concentration of 5x10^5 and 5x10^2 IU/ml of RTA HIV-1 Reference Genotype B calibrated with 3rd WHO International Standard (NIBSC code: 10/152) were used. The results on basis of log values are shown in the following tables:

Table 1: Descriptive statistics of HIV-1 precision data for 5x10^5 IU/ml sample.

	N	Minimum	Maximum	Mean	Std. Deviation	Variance	Coefficient of variation (%)
Intra_assay	24	5,55	5,76	5,6767	,04887	,002	0,86
Inter_assay	24	5,61	5,77	5,6846	,03741	,001	0,66
Inter_batch	24	5,52	5,82	5,7554	,05710	,003	0,99
Inceptra	24	4,69	6,03	5,7108	,23756	,056	4,16
Stratagene	24	5,68	5,76	5,7325	,02770	,001	0,48
RotorGene	24	5,69	5,80	5,7608	,02636	,001	0,46
VOLTRAN	24	5,45	5,76	5,6083	,07631	,006	1,36
TOTAL	168	4,69	6,03	5,7042	,11041	,012	1,94

Table 2: Descriptive statistics of HIV-1 precision data for 500 IU/ml sample.

	N	Minimum	Maximum	Mean	Std. Deviation	Variance	Coefficient of variation (%)
Intra_assay	24	2,46	2,99	2,7513	,12088	,015	4,39
Inter_assay	24	2,31	2,88	2,7425	,12400	,015	4,52
Inter_batch	24	2,37	2,93	2,6525	,14161	,020	5,34
Inceptra	24	2,51	3,13	2,7521	,15013	,023	5,46
Stratagene	24	2,53	2,84	2,6700	,09278	,009	3,48
RotorGene	24	2,54	2,84	2,7358	,06157	,004	2,25
VOLTRAN	24	2,43	2,96	2,7467	,14666	0,22	5,34
TOTAL	168	2,31	3,13	2,7215	,12719	,016	4,67

Genotype Detectability The performance of RTA HIV-1 Real-Time PCR Kit was evaluated with 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (Main) (NIBSC code: 12/224) and 1st WHO International Reference Preparation for HIV-1 CRF's (NIBSC code: 13/214). The data gathered by RTA HIV-1 Real-Time PCR Kit is compatible with the results of the WHO ECBS reports. As a result of this study, RTA HIV-1 Real-Time PCR Kit can detect and quantify all subtypes of HIV-1 M group and Circulating Recombinant Forms.

Diagnostic Specificity HIV-1 negative clinical specimens were analyzed to determine the diagnostic specificity of RTA HIV-1 Real-Time PCR Kit. 54 HIV-1 RNA negative clinical serum specimens and 52 HIV-1 RNA negative clinical EDTA plasma specimens were used.
None of the HIV-1 negative clinical specimens gave positive test result for HIV-1 RNA. Diagnostic specificity of RTA HIV-1 Real-Time PCR Kit is 100 %. All of the Internal Controls of tests gave positive result.

Cross Reactivity To examine the specificity of an assay, cross-reactivity studies should be performed for potential cross-reactive markers. In this study, the specificity of the assay was evaluated by testing 7 reference organism and 9 clinical specimens which were positive. RTA HIV-1 Real-Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table below:

Organism	Source	Test Result
HCV for NAT (4th WHO International Standard)	NIBSC Code: 06/102	Negative
1st WHO International Standard for EBV for NAT	NIBSC Code: 09/260	Negative
1st WHO International Standard for HCMV for NAT	NIBSC Code: 09/162	Negative
3rd WHO International Standard for HBV for NAT	NIBSC Code: 10/264	Negative
Human Herpes Simplex virus type 2 for Nucleic Acid Amplification Techniques	NIBSC Code: 08/226	Negative
Human Herpes Simplex virus type 1 for Nucleic Acid Amplification Techniques	NIBSC Code: 08/224	Negative
Varicella Zoster Virus(Type B) Working Reagent for Nucleic Acid Amplification Testing (NAT)	NIBSC Code: 08/310	Negative
HTLV-1 (13 samples)	13 Clinical specimens	Negative

Cross-Contamination In this study, cross-contamination between samples was evaluated. To do this, five different runs were performed for each instrument. In every run, 4 high positive HIV-1 sample and 4 HIV-1 negative samples were used. Samples were extracted by RTA Viral Nucleic Acid Isolation Kit according to RTA Viral Nucleic Acid Isolation Kit Handbook. BIO-RAD CFX96-IVD, STRATAGENE Mx3000p, Rotor-Gene 6000 and INCEPTRA Cycler 9660 Real-Time PCR Systems were used for amplification, signal detection and analysis of, the results. Then, the kit was evaluated accordingly whether or not any cross-contamination was observed.
No cross-contamination was observed during the whole process, and none of the human samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.

Whole System Failure 50 HIV-1 RNA negative clinical serum specimens and 50 HIV-1 RNA negative clinical EDTA plasma specimens were spiked with WHO International HIV-1 standard (NIBSC code: 97/650) to give final concentrations of 234 IU/ml in the elution volume which is 3 times the 95% positive cutoff value determined by analytical sensitivity study. Whole system failure rate of RTA HIV-1 Real-Time PCR Kit is ≤1 %.

Clinical Comparative Study Total 199 clinical samples were tested. 107 of the specimens were positive, and the rest of them were negative. According to the results, the data gathered by RTA HIV-1 Real-Time PCR Kit is compatible with the results of other CE-marked devices. Log concentrations of all of 107 positive clinical samples are between ±1 log concentrations of the result of comparative device.

Additional Materials Required
- RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey)
- Real-Time PCR system
- Disposable powder-free gloves
- Micropipettes (0.5 µl – 1000 µl)
- Sterile micropipette tips with filters
- Microcentrifuge tubes
- Vortex mixer
- Desktop microcentrifuge for 2.0 ml tubes and for PCR strip tubes
- PCR workstation
- Real-Time PCR reaction tubes/plates/capillaries
- For BIO-RAD CFX96-IVD:
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates with Bar Codes (BIO-RAD, Cat#: HSP-9955)
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001)
- Individual PCR Tubes, Low Tube Strips, 8-tubes strip, 0.2 ml Low Profile, White (BIO-RAD, Cat#: TLS0851)
- Flat Cap Strips, Optically Clear, 8-cap strip, 0.2 ml (BIO-RAD, Cat#: TCS0803)
- For STRATAGENE MX 3000p/MX 3005p:
- Optical tube strips (8x Strip) (Agilent Technologies, Cat#: 401428)
- Strip caps for PCR and qPCR applications (Agilent Technologies, Cat#: 401425)
- For Rotor-Gene 3000/6000:
- Strip Tubes and Caps, 0.1 ml (Qiagen, Cat#: 981103)
- For INCEPTRA Cyclers:
- 96x0,2 ml plate (Bioplastics, Cat#: B70501)
- EU flat cap plate (Bioplastics, Cat#: B57601)
- EU 0.1ml 8-tube strip attached Optical wide area cap (Bioplastics, Cat#: K72810B)

Sample Preparation
This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant.
Aseptic techniques must be employed during collection to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection.
All samples should be regarded as potentially infectious and standard precautions guidelines should be followed by all healthcare workers during sample collection and handling.
Samples must be collected into appropriate containers before despatch to the laboratory.
Be careful to check for cracks in the containers and to ensure that the lids of containers are properly tightened to prevent leakage of samples during handling and transportation. This can pose infection hazards to transport and laboratory staff.
Ensure that the outer surfaces of the containers are not contaminated by the patients' samples.
Store whole blood at room temperature for no longer than 4 hours. Centrifuge blood within 4 hours of collection. Transfer serum or plasma to a screw cap cryovial tube.
Transportation of whole blood, serum or plasma must conform to country or local regulations for the transport of etiologic agents.
Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for long-term storage.
Avoid multiple freeze/thaw cycles of specimens.

Protocol
Viral RNA Isolation RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey) should be used for viral RNA extraction from clinical samples. Please follow the manufacturer's instructions as stated in the kit manual.

Internal Control During RNA isolation, addition of the supplied internal control (IC) is necessary. IC allows the user to monitor RNA extraction step as well as to determine any PCR inhibition. For each sample, add 2.5 µl IC together with Solution RL of the Viral Nucleic Acid Isolation Kit for a 50 µl elution. Depending on your final elution volume, the volume of IC to be added can be calculated (0.05 µl IC/1 µl Elution Buffer). There was no amplification of internal control in the tests where high positive HIV-1 samples were amplified because there was a competition between internal control template and HIV-1 RNA template for using PCR primers and other components. The Ct value of internal control of a negative sample should be equal to 33 ± 5, otherwise, it denotes a problem during purification.

Quantification Standards For generating a standard curve to obtain accurate quantification data on the Real-Time system, four quantification standards should be used. For each standard the corresponding concentration should be defined properly to the Real-Time PCR system before each run and the standard curve will be generated accordingly at the end of the reaction. Work with HIV-1 Quantification Standards after preparation of clinical samples and negative control in a separate area. Caps of the tubes or capillaries of Clinical Samples SHOULD be closed in that area.

PCR Protocol
1. Thaw all components, except HIV-1 Enzyme Mix, at room temperature. Thaw HIV-1 Reaction Mix at 37°C for 5 min if there is a precipitate. Put HIV-1 Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

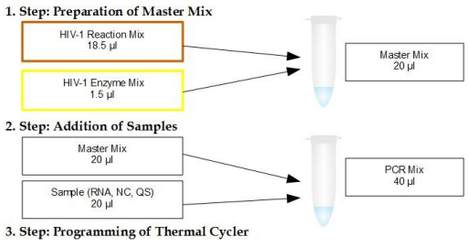
2. The final volume of Master Mix is calculated by multiplying single reaction volumes of Reaction Mix and Enzyme Mix by the total sample size. The number of negative controls, quantification standards and the clinical samples should be included when calculating total sample size. Against possible pipetting errors, addition of an extra sample to the total sample size is recommended. PCR Grade Water should be used as the negative control.

3. To prepare master mix, add 18.5 µl of HIV-1 Reaction Mix (brown tube) and 1.5 µl of HIV-1 Enzyme Mix (yellow cap) for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 20 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 20 µl RNA of each sample, negative control and quantification standards into the tubes. Spin down briefly.

4. Perform the following protocol for all PCR cyclers: 45°C for 30 min,95°C for 10 min, 1 cycle; 95°C for 30 sec, 63°C for 60 sec, 72°C for 30 sec, 45 cycles.

5. Fluorescence is measured at 72°; FAM and HEX channels should be chosen (See the schema in the next page).

6. Refer to the Operator's Manual of the related instruments to program and analyze the results.



Program Name	Cycles	Program for All Cycler
cDNA Synthesis	1	45ºC, 30 min
Hot Start	1	95ºC, 10 min
Amplification*	45	95ºC, 30 sec
		63ºC, 60 sec
		72ºC, 30 sec

*** Fluorescence is measured at 72°C; FAM and HEX/VIC/JOE channels should be chosen**

Data Analysis
To be able to evaluate the experiment, PCR efficiency of the Standard Curve must be between 90% 110% and R2 value must be more than 0.98. Otherwise, the experiment should be repeated.
During analysis on STRATAGENE software, adjust threshold fluorescence value manually by entering 1000 for FAM and 500 for HEX. During analysis on BIO-RAD CFX96-IVD software, adjust threshold fluorescence value manually by entering 500 for FAM and 500 for HEX.
During analysis on INCEPTRA Cycler software, the threshold should be set empirically. At the beginning, the threshold can be set at 50 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Rotor-Gene software, the threshold should be set empirically. At the beginning, the threshold can be set at 0,04 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
Concentration of each positive sample will be calculated by the software according to the standard curve as International Unit per milliliter (IU/ml) . If it is needed to convert the quantitative results from IU/ml to copies/ml, the conversion factor for RTA HIV-1 Real-Time PCR Kit is 0.5 copies/IU. In other words, 1 IU/ml = 0.5 copies/ml.
Due to different starting sample volumes and elution volumes during viral RNA isolation, the following formula SHOULD be used to calculate the concentration of the original clinical sample:

$$\text{Concentration of the Original Sample (IU/ml)} = \frac{\text{Concentration from Software (IU/ml)} \times \text{Elution Volume (µl)}}{\text{Original Sample Volume (µl)}}$$

The interpretation on the calculated results can be done as follows:

Signal detected in FAM channel	Signal detected in HEX channel	Calculated concentration of the original clinical sample	Conclusion
+	+	<87 IU/ml for BIO-RAD <100 IU/ml for STRATAGENE <94 IU/ml for INCEPTRA <111 IU/ml for Rotor-Gene	The result is valid. Quantitation is not possible since the quantitative result is below the analytical sensitivity value of the assay. Reproducibility of the positive result is not guaranteed.
+	+/-	≥87 IU/ml for BIO-RAD ≥100 IU/ml for STRATAGENE ≥94 IU/ml for INCEPTRA ≥111 IU/ml for Rotor-Gene and ≤1 x 109 IU/ml	The result is valid. HIV RNA is detected at the concentration calculated by the software since the quantitative result is within the linear range of the assay.
+	+/-	>1 x 10^9 IU/ml	The result is valid. HIV RNA is detected at a concentration >1 x 109 IU/ml. Quantitation is not possible since the quantitative result is above the linear range of the assay.
-	+	N/A	The result is valid. Target (HIV RNA) is not detected.
-	-	N/A	The result is invalid. No diagnostic interpretation can bedone.

References:

1. Chiu IM, Yaniv A, Dahlberg JE, Gazit A, Skuntz SF, Tronick SR, et al. Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. Nature 1985;317(6035):366-8.

2. Conte D, Simon F, Mauclere P, Roques P, Loussert-Ajaka I, Muller- Trutwin MC, Saragosti S, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med 1998;4(9):1032-7.

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