September 2015

artus® HI Virus-1 RG RT-PCR Kit Handbook

Σ 24 (catalog no. 4513253)

Version 1

IVD

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q Instruments

REF 4513253

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Contents

Kit Contents

***artus* HI Virus-1 RG RT-PCR Kit**			**(24)**
Catalog no.			**4513253**
Number of reactions			**24**
Blue	HI Virus-1 RG* Master A		2 x 12 reactions
Violet	HI Virus-1 RG Master B		2 x 12 reactions
Red	HI Virus-1 RG QS† 1 (1x 10^4 IU/µl)	QS	200 µl
Red	HI Virus-1 RG QS 2 (1x 10^3 IU/µl)	QS	200 µl
Red	HI Virus-1 RG QS 3 (1x 10^2 IU/µl)	QS	200 µl
Red	HI Virus-1 RG QS 4 (1x 10^1 IU/µl)	QS	200 µl
Green	HI Virus-1 RG IC‡	IC	1000 µl
White	Water (PCR grade)		1000 µl
	Leaflet		1

* Rotor-Gene.

† Quantitation standard.

‡ Internal control.

Symbols

Symbol	Description
	Contains reagents sufficient for <N> tests
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Important note

Storage

The components of the *artus* HI Virus-1 RG RT-PCR Kit should be stored at –30°C to –15°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

Intended Use

The *artus* HI Virus-1 RG RT-PCR Kit is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma. This diagnostic test kit utilizes the reverse transcription polymerase chain reaction (RT-PCR) and is configured for use with Rotor-Gene Q Instruments. The test can quantitate HIV-1 RNA over the range of 120 – 1 x 10^8 HIV-1 IU/ml. Plasma samples containing Group M Subtypes A–H have been validated for use in the assay.

ⓘ The *artus* HI Virus-1 RG RT-PCR Kit may not be used with Rotor-Gene Q 2plex Instruments.

The *artus* HI Virus-1 RG RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in EDTA plasma HIV-1 RNA levels. The *artus* HI Virus-1 RG RT-PCR Kit is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection.

Limitations

All reagents may exclusively be used in in vitro diagnostics.

The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.

Strict compliance with the user manual is required for optimal PCR results.

Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases. Validity and performance of the assay design are evaluated at regular intervals.

Warnings and Precautions

For in vitro diagnostic use.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN® kit and kit component.

Discard sample and assay waste according to your local safety regulations.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* HI Virus-1 RG RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *artus* HI Virus-1 RG RT-PCR Kit constitutes a ready-to-use system for the detection of HIV-1 RNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The HI Virus-1 RG Master A and B contain reagents and enzymes for the reverse transcription and specific amplification of a 93 bp region of the HIV-1 genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q.

In addition, the *artus* HI Virus-1 RG RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Orange of the Rotor-Gene Q. The detection limit of the analytical HI Virus-1 RT-PCR (see "Performance characteristics", page 9) is not reduced. Quantitation standards (HI Virus-1 RG QS 1–4) are supplied, which allow the determination of the amount of viral RNA. For further information, see "Quantitation", page 22.

Principle

Pathogen detection by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real-time) allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run (1).

Pathogen information

The human immunodeficiency virus (HIV) is a retrovirus that causes acquired immunodeficiency syndrome (AIDS). There are two types of HIV responsible for human infections, HIV-1 and HIV-2, which differ in their virulence and prevalence. Most reported cases of AIDS around the world have been attributed to HIV-1. Infection with HIV occurs by the transfer of infected blood, vaginal fluid, breast milk and other body fluids. Within these body fluids HIV is present as both free virus particles and virus within infected immune cells. The three major routes of transmission are unprotected sexual intercourse, contaminated needles and transmission from an infected mother to her baby at birth or through breast milk.

HIV primarily infects cells in the human immune system such as helper T cells (specifically $CD4^+$). HIV infection leads to low levels of $CD4^+$ T cells. When $CD4^+$ T cell number decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

AIDS symptoms occur at an advanced stage of HIV infection when the compromised immune system cannot fight off opportunistic infections. At this stage, the infected person increasingly develops symptoms triggered by such infections. The most common infections include chronic cryptosporida diarrhea, cytomegalovirus-induced eye infection, pneumocystis pneumonia, toxoplasmosis, and tuberculosis as well as infections with members of the *Mycobacterium avium* complex. In addition, the development of different types of cancer, such as invasive cervical cancer, Kaposi sarcoma, or lymphoma, is frequently observed. At present, there is no cure for AIDS, and it is believed that most HIV infected people will eventually die of an AIDS-related illness. However, advancements in HIV/AIDS therapies, including those that fight the virus itself as well as those that prevent or treat opportunistic infections, have drastically improved life expectancy and quality of many HIV/AIDS patients.

Performance characteristics

Limit of detection

The limit of detection (LOD) as well as the LOD in consideration of the purification (sensitivity limits) were assessed for the *artus* HI Virus-1 RG RT-PCR Kit. The LOD in consideration of the purification is determined using HIV positive clinical specimens in combination with a particular extraction method. In contrast, the LOD is determined independent from the selected extraction method, using a standard of known concentration.

To determine the LOD of the *artus* HI Virus-1 RG RT-PCR Kit, a standard dilution series was set up from 0.0316 to 31.6 IU*/µl and analyzed on the Rotor-Gene 3000 in combination with the *artus* HI Virus-1 RG RT-PCR Kit. Testing was carried out on 3 different days on 8 replicates. The results were determined by a probit analysis. The LOD of the *artus* HI Virus-1 RG RT-PCR Kit in combination with the Rotor-Gene 3000 is 4.5 IU/µl ($p = 0.05$). This means that there is a 95% probability that 4.5 IU/µl will be detected.

The LOD in consideration of the purification (QIAamp® DSP Virus Kit, QIAGEN) of the *artus* HI Virus-1 RG RT-PCR Kit on Rotor-Gene Instruments was determined using a dilution series of the 2nd International WHO Standard of HIV-1 RNA for nucleic acid amplification technology (NAT) assays (NIBSC Code 97/650) from 10 to 3160 HIV IU/ml spiked in clinical plasma specimens. These were subjected to RNA extraction using the QIAamp DSP Virus Kit (QIAGEN, extraction volume: 0.5 ml, elution volume: 25 µl). Each of the dilutions was analyzed with the *artus* HI Virus-1 RG RT-PCR Kit on 3 different days on 8 replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 1. The LOD in consideration

* The standard is an in vitro transcribed RNA, the concentration of which has been calibrated using the 2nd International HIV standard (WHO).

of the purification of the *artus* HI Virus-1 RG RT-PCR Kit in combination with the Rotor-Gene 3000 is 71.6 IU/ml (p = 0.05). This means that there is a 95% probability that 71.6 IU/ml will be detected.

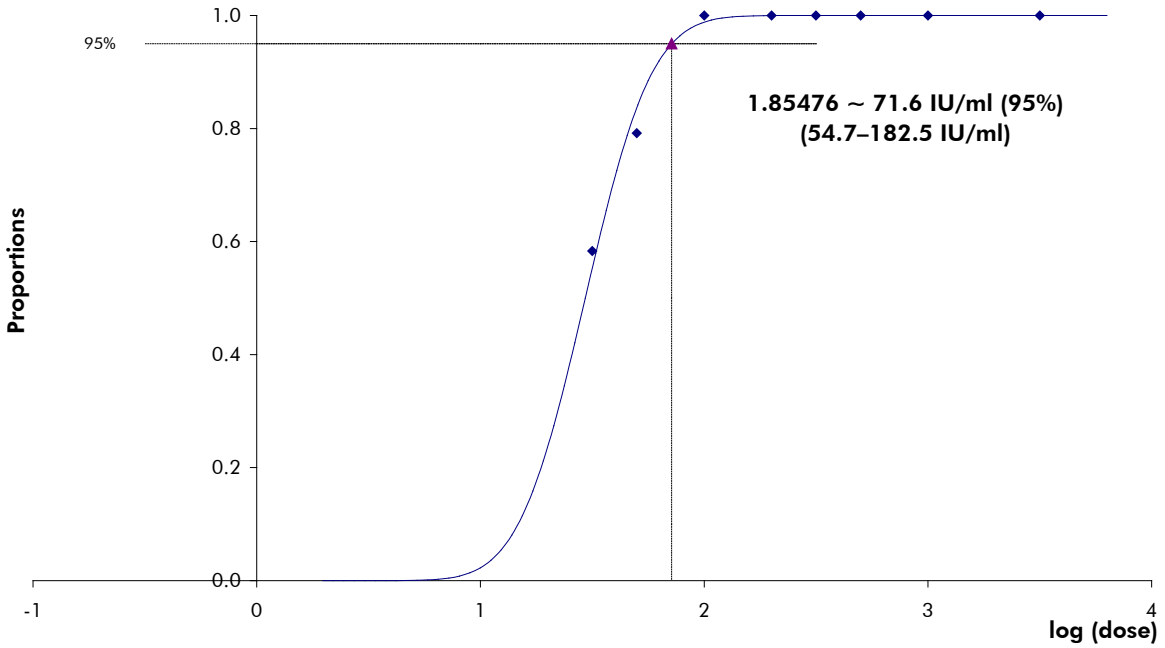


Figure 1. Probit analysis: HI Virus-1. LOD in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* HI Virus-1 RG RT-PCR Kit on the Rotor-Gene 3000.

The LOD in consideration of the purification of the *artus* HI Virus-1 RG RT-PCR Kit in combination with the Rotor-Gene Q/6000 is 66.9 IU/ml (p = 0.05). This means that there is a 95% probability that 66.9 IU/ml will be detected.

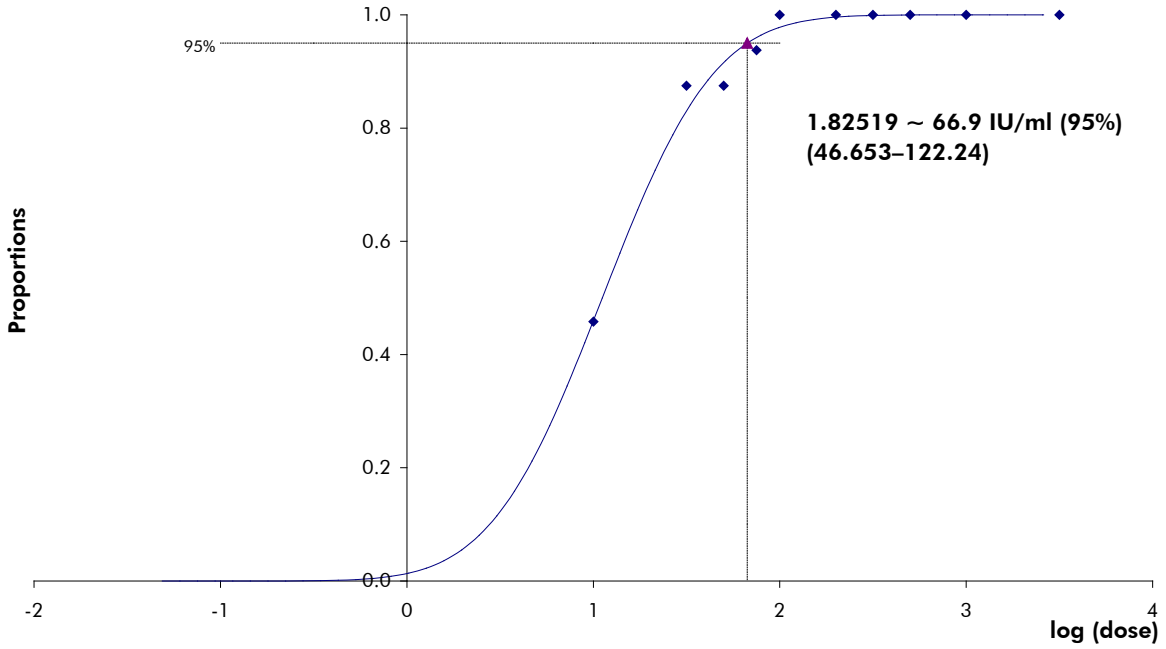


Figure 2. Probit analysis: HI Virus-1. LOD in consideration of the purification (QIAamp DSP Virus Kit, QIAGEN) of the *artus* HI Virus-1 RG RT-PCR Kit on the Rotor-Gene 6000.

Specificity

The specificity of the *artus* HI Virus-1 RG RT-PCR Kit is first and foremost 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 in gene banks published sequences by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured by a database alignment and by a PCR run on Rotor-Gene Instruments with the following genotypes (see Table 1).

Moreover, the specificity was validated with 100 different HIV negative plasma samples. These did not generate any signals with the HIV specific primers and probes, which are included in the HI Virus-1 RG Master.

A potential cross-reactivity of the *artus* HI Virus-1 RG RT-PCR Kit was tested using the control group listed in Table 2. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Table 1. Testing of the specificity of relevant genotypes

Virus	Genotype	Source	HIV (Cycling Green)	Internal control (Cycling Orange)
HI Virus-1	A	NIBSC*	+	+
HI Virus-1	B	NIBSC	+	+
HI Virus-1	C	NIBSC	+	+
HI Virus-1	D	NIBSC	+	+
HI Virus-1	E	NIBSC	+	+
HI Virus-1	F	NIBSC	+	+
HI Virus-1	G	NIBSC	+	+
HI Virus-1	H	NIBSC	+	+

* National Institute for Biological Standards and Control, Hertfordshire.

Table 2. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	HIV (Cycling Green)	Internal control (Cycling Orange)
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
Human herpesvirus 1 (herpes simplex virus 1)	–	+
Human herpesvirus 2 (herpes simplex virus 2)	–	+
Human herpesvirus 3 (varicella-zoster virus)	–	+
Human herpesvirus 5 (cytomegalovirus)	–	+

Table continued on next page

Table 2. Continued

Control group	HIV (Cycling Green)	Internal control (Cycling Orange)
Human T cell leukemia virus type 1 and type 2	–	+
Enterovirus	–	+
Parvovirus B19	–	+
Yellow fever	–	+
Aspergillus flavus	–	+
Aspergillus fumigatus	–	+
Candida albicans	–	+
Chlamydia trachomatis	–	+
Cryptosporidium parvum	–	+
Filobasidiella neoformans	–	+
Mycoplasma pneumoniae	–	+
Pneumocystis cariniie	–	+
Staphylococcus sp.	–	+
Streptococcus agalactiae	–	+
Staphylococcus aureus	–	+
Streptococcus pyogenes	–	+

Linear range

The linear range (analytical measurement) of the *artus* HI Virus-1 RG RT-PCR Kit was determined by analyzing a dilution series of an HIV in vitro transcript from 1 x 10^8 IU/µl to 1 IU/µl. The dilution series has been calibrated against the WHO International HIV RNA Standard.

Each dilution was tested in replicates (n = 8) using the *artus* HI Virus-1 RG RT-PCR Kit on Rotor-Gene Instruments.

The linear range of the *artus* HI Virus-1 RG RT-PCR Kit has been determined to cover concentrations from 5 IU/µl to at least 1 x 10^8 IU/µl.

The linear range in consideration of the purification of the *artus* HI Virus-1 RG RT-PCR Kit was determined by analyzing a dilution series of the OptiQuant HIV-1 RNA Quantification Panel from 1 x 10^8 IU/ml to 120 IU/ml. The purification was carried out in duplicates using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 25 µl). Each of the 9 samples were analyzed using the *artus* HI Virus-1 RG RT-PCR Kit. The linear range in consideration of the purification of the *artus* HI Virus-1 RG RT-PCR Kit has been determined to cover concentrations from 120 IU/ml to at least 1 x 10^8 IU/ml (see Figure 3).

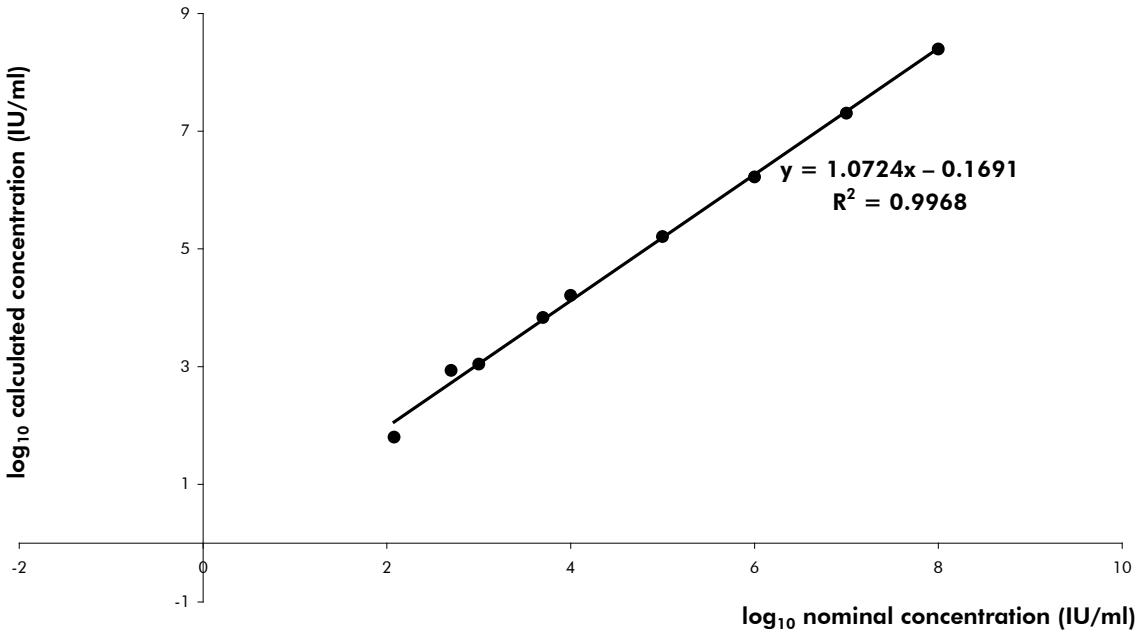


Figure 3. Linear Range of the *artus* HI Virus-1 RG RT-PCR Kit. Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Precision

The precision data of the *artus* HI Virus-1 RG RT-PCR Kit have been collected by means of Rotor-Gene Instruments and allow the determination of the total variance of the assay. The total variance consists of the intra-assay variability (variability of multiple results of samples of the same concentration within one experiment), the inter-assay variability (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the inter-batch variability (variability of multiple results of the assay using various batches). The data obtained were used to

determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the internal control PCR.

Precision data of the *artus* HI Virus-1 RG RT-PCR have been collected using the quantitation standard of the lowest concentration (QS4; 10 IU/µl). Testing was performed with 8 replicates. The precision data were calculated on basis of the C_T values of the amplification curves (C_T: threshold cycle, see Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.66% (C_T), and 2.15% (C_T) for the detection of the internal control. These values are based on the totality of all single values of the determined variabilities.

Table 3. Precision data on basis of the C_T values

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: HI Virus-1 RG QS 4	35.62	0.45	1.26
Intra-assay variability: Internal control	31.24	0.18	0.58
Inter-assay variability: HI Virus-1 RG QS 4	35.75	0.56	1.55
Inter-assay variability: Internal control	31.65	0.36	1.13
Inter-batch variability: HI Virus-1 RG QS 4	35.40	0.61	1.73
Inter-batch variability: Internal control	31.20	0.55	1.76
Total variance: HI Virus-1 RG QS 4	35.58	0.59	1.66
Total variance: Internal control	31.40	0.67	2.15

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HI Virus-1 RG RT-PCR Kit. 100 HIV negative samples of plasma were spiked with 4.5 IU/µl elution volume of HIV control RNA (threefold concentration of the LOD). After extraction using the QIAamp DSP Virus Kit, these samples were analyzed with the *artus* HI Virus-1 RG RT-PCR Kit. For all

HIV samples the failure rate was 0%. In addition, the robustness of the internal control was assessed by purification and analysis of 100 HIV negative plasma samples. The total failure rate was 0%. Inhibitions were not observed. Thus, the robustness of the *artus* HI Virus-1 RG RT-PCR Kit is ≥99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HI Virus-1 RG RT-PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

Diagnostic evaluation

The *artus* HI Virus-1 RG RT-PCR Kit was evaluated in a study. Comparing the *artus* HI Virus-1 RG RT-PCR Kit to the COBAS® TaqMan® HIV-1 Test, 241 plasma specimens were analyzed retrospectively. All plasma specimens had previously been analyzed positive or negative using the COBAS TaqMan HIV-1 Test for routine diagnostics.

HIV RNA for testing the *artus* HI Virus-1 RG RT-PCR Kit was isolated using the QIAamp DSP Virus Kit, and analysis was carried out on the Rotor-Gene 6000 Instrument. For comparative testing with the COBAS TaqMan HIV-1 Test, HIV RNA was isolated according to the instructions of the manufacturer provided in the package insert. The results obtained by using the *artus* HI Virus-1 RG RT-PCR Kit were compared to those of the COBAS TaqMan HIV-1 Test (see Table 4 and Figure 4).

105 of 126 samples that tested positive with the COBAS TaqMan HIV-1 Test also tested positive with the *artus* HI Virus-1 RG RT-PCR Kit. 113 of 115 samples that tested negative with the COBAS TaqMan HIV-1 Test also tested negative with the *artus* HI Virus-1 RG RT-PCR Kit.

If the results of the COBAS TaqMan HIV-1 Test are taken as reference, the diagnostic sensitivity is 98.1%, and the diagnostic specificity is 84.3%.

Table 4. Results of the 241 analyzed retrospective EDTA plasma samples

		COBAS TaqMan HIV-1 Test		
		+	–	Total
***artus* HI Virus-1 RG RT-PCR Kit**	+	105	21	126
	–	2	113	115

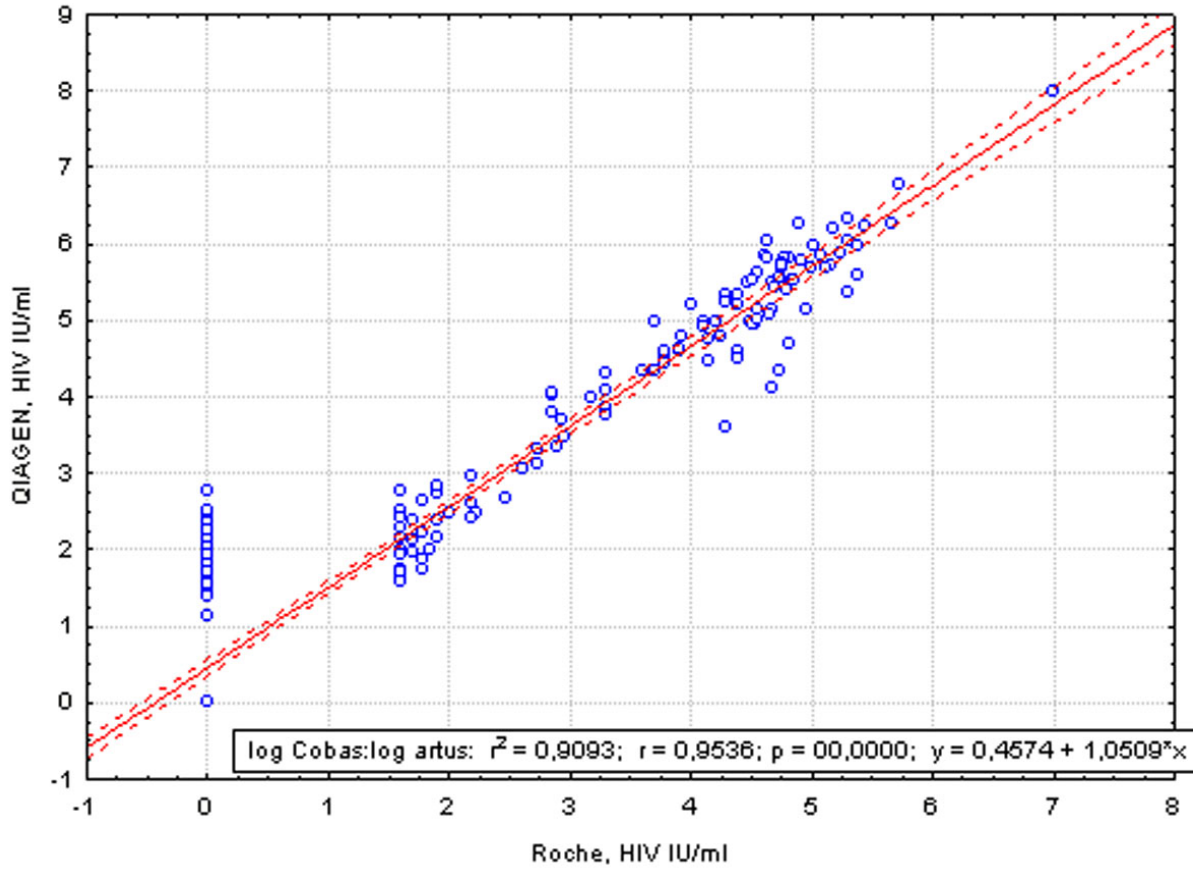


Figure 4. Comparison of the COBAS TaqMan HIV-1 Test (Roche, HIV; with sample purification using the COBAS AmpliPrep system) with the *artus* HI Virus-1 RG RT-PCR Kit (QIAGEN, HIV; with sample purification using the QIAamp DSP Virus Kit).
Correlation of quantitative results from both test systems (Table 4) was analyzed by linear regression. The results from both kits are shown in an XY (scatter) plot with log–log scale.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- RNA isolation kit (see "RNA isolation", page 20)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Rotor-Gene Q *†
- Rotor-Gene Q Software version 2.3 or higher*
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† The *artus* HI Virus-1 RG RT-PCR Kit may not be used with Rotor-Gene Q 2plex Instruments.

Important Notes

General precautions

The user should always pay attention to the following:

- Use sterile pipet tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Work quickly and keep components on ice or in the cooling block (72/96-well loading block).

Specimen collection, storage, and transport

ⓘ All samples have to be treated as potentially infectious material.

Only the following sample materials are permissible, for which the following rules and particular instructions regarding collection, transport, and storage have to be strictly observed.

ⓘ Current studies refer to EDTA or citrate plasma as the most suitable sample materials for HIV detection. Therefore, we recommend the use of these materials with the *artus* HI Virus-1 RG RT-PCR Kit.

The internal validation of the *artus* HI Virus-1 RG RT-PCR Kit has been performed using human EDTA plasma samples. Other sample materials are not validated. Please use only the recommended RNA isolation kit (see "RNA isolation", page 20) for sample preparation.

Using certain sample materials, particular instructions regarding collection, transport, and storage have to be strictly observed.

Specimen collection

Each blood withdrawal causes an injury of blood vessels (arteries, veins, capillaries). Only innocuous and sterile material should be used. For blood withdrawal appropriate disposables are available. For vein punctures, too fine capillary needles should not be employed. Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm, or the back of the hand. Blood has to be withdrawn with standard specimen collection

tubes (red cap, Sarstedt or equivalent tube of another manufacturer). A volume of 5–10 ml EDTA blood should be withdrawn. Tubes should be mixed over-head directly after sample collection (8 x, do not agitate).

ⓘ Samples from heparinized humans must not be used (see "Interfering substances", page 20).

Sample storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800–1600 x *g* within 6 hours. The isolated plasma has to be transferred into sterile polypropylene tubes. The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time. Virus encapsulated RNA is stable for days if stored at 4°C, for weeks if stored at –20°C, and even for months and years when stored at –70°C (2).

Sample transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material.*

The samples should be shipped within 6 hours. It is not recommended to store the samples where they have been collected. It is possible to ship the samples by mail, following the legal instructions for the transport of pathogen material. We recommend the sample transport with a courier. The blood samples should be shipped cooled (2–8°C) and the separated plasma deep frozen (–15 to –30°C).

Interfering substances

Elevated levels of bilirubin (≤15 mg/dl) and lipids (≤800 mg/dl) and hemolytic samples do not influence the system. Heparin (≥10 IU/ml) affects the PCR. Samples that have been collected in tubes containing heparin as an anticoagulant should not to be used. Also, samples of heparinized patients must not be used.

RNA isolation

The QIAamp DSP Virus Kit (QIAGEN, cat. no. 60704) is validated for viral RNA purification from human plasma for use with the *artus* HI Virus-1 RG RT-PCR Kit. Carry out the viral RNA purification according to the instructions in the *QIAamp DSP Virus Kit Handbook*.

* International Air Transport Association (IATA). Dangerous Goods Regulations.

ⓘ The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp DSP Virus Kit, we recommend to proceed according to the information about the reconstitution and storage of the carrier RNA given in the instruction manual ("Preparing reagents and buffers").

Before the beginning of each extraction, a mixture of lysis buffer and carrier RNA (and internal control, where applicable, see "Internal control", below) should be prepared freshly according to the pipetting scheme in Table 5.

Table 5. Pipetting scheme for use with the QIAamp DSP Virus Kit

Number of samples	**1**	**12**
Lysis Buffer (AL)*	550 µl	6600 µl
Carrier RNA (1 µg/µl)	6.2 µl	74.4 µl
Total volume	**556.2 µl**	**6674.4 µl**
Volume per extraction	**500 µl**	**500 µl each**

* Contains guanidine hydrochloride; see the *QIAamp DSP Virus Kit Handbook* for safety information.

ⓘ Use the freshly prepared mixture of lysis buffer and carrier RNA immediately for extraction. Storage of the mixture is not possible.

ⓘ The internal control of the *artus* HI Virus-1 RG RT-PCR Kit can be used directly in the isolation procedure (see "Internal control", below).

Internal control

An internal control (HI Virus-1 RG IC) is supplied. This allows the user both to control the RNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DSP Virus Kit, the RNA is eluted in 60 µl Elution Buffer (AVE). Hence, 6 µl of the internal control should be added initially.

ⓘ The internal control and carrier RNA (see "RNA isolation", page 20) should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer.

The internal control must not be added to the sample material directly. If added to the lysis buffer please note that the mixture of internal control and lysis buffer–carrier RNA has to be prepared freshly and used immediately (storage of

the mixture at room temperature or in the fridge for only a few hours may lead to internal control failure and a reduced extraction efficiency).

ⓘ Do not add the internal control and the carrier RNA to the sample material directly.

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the mixture of HI Virus-1 RG Master A and HI Virus-1 RG Master B, as described in step 2b of the protocol (page 25).

Setting the threshold for the PCR analysis

To ensure data generated by customers will be analogous to the performance characteristics defined in the associated handbook, the following parameters must be used to analyze all data generated using the *artus* HI Virus-1 RG RT-PCR Kit:

- Green Channel – threshold 0.05
- Orange Channel – threshold 0.03

This is further detailed on page 32.

Quantitation

The enclosed quantitation standards (HI Virus-1 RG QS 1–4) are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box as standards with the specified concentrations (see the instrument user manual).

ⓘ The quantitation standards are defined as IU/µl.* The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl)} \times \text{Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

As a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

* The standard has been calibrated using the International HIV standard (WHO).

The quantitation standards are calibrated to the HIV WHO standard during the manufacturing process. Results generated using Rotor-Gene Q will be output as IU/µl which must then be converted to IU/ml using the above equation.

Conversion factor

1 IU/ml corresponds to 0.50 copies/ml for detection of HIV-1 RNA on the Rotor-Gene Q in combination with manual sample preparation using the QIAamp DSP Virus Kit. The conversion factor is an approximation based on an average factor across the assay's dynamic range.

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure, read "Important Notes", pages 19–22.
- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- Make sure that at least one quantitation standard as well as one negative control (Water, PCR grade) are included per PCR run. To generate a standard curve, use all 4 quantitation standards supplied (HI Virus-1 RG QS 1–4) for each PCR run.

Things to do before starting

- Make sure that the cooling block (accessory of the Rotor-Gene Q Instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

1. **Place the desired number of PCR tubes into the adapters of the cooling block.**
2. **If you are using the internal control to monitor the RNA isolation procedure and to check for possible PCR inhibition, follow step 2a. If you are using the internal control exclusively to check for PCR inhibition, follow step 2b.**

2a. The internal control has already been added to the isolation (see "Internal control", page 21). In this case, prepare a master mix according to Table 6.

The reaction mix typically contains all of the components needed for PCR except the sample.

Table 6. Preparation of master mix (internal control used to monitor RNA isolation and check for PCR inhibition)

Number of samples	1	12
HI Virus-1 RG Master A	12 µl	144 µl
HI Virus-1 RG Master B	18 µl	216 µl
HI Virus-1 RG IC	0 µl	0 µl
Total volume	**30 µl**	**360 µl**

2b. The internal control must be added directly to the mixture of HI Virus-1 Master A and HI Virus-1 Master B. In this case, prepare a master mix according to Table 7.

The reaction mix typically contains all of the components needed for PCR except the sample.

Table 7. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
HI Virus-1 RG Master A	12 µl	144 µl
HI Virus-1 RG Master B	18 µl	216 µl
HI Virus-1 RG IC	2 µl	24 µl
Total volume	**32 µl***	**384 µl***

* The volume increase caused by adding the internal control is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

3. Pipet 30 µl of the master mix into each PCR tube. Then add 20 µl of the eluted sample RNA (see Table 8). Correspondingly, 20 µl of at least one of the quantitation standards (HI Virus-1 RG QS 1–4) must be used as a positive control and 20 µl of water (Water, PCR grade) as a negative control.

Table 8. Preparation of PCR assay

Number of samples	1	12
Master mix	30 µl	30 µl each
Sample	20 µl	20 µl each
Total volume	**50 µl**	**50 µl each**

4. **Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene Instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.**
5. **For the detection of HIV-1 RNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 5, 6, 7**
Reverse transcription of the RNA	**Figure 8**
Initial activation of the hot-start enzyme	**Figure 9**
Amplification of the cDNA	**Figure 10**
Adjusting the fluorescence channel sensitivity	**Figure 11**
Starting the run	**Figure 12**

All specifications refer to the Rotor-Gene Q Software version 2.3 or higher. Please find further information on programming Rotor-Gene Instruments in the instrument user manual. In the illustrations these settings are framed in bold black. Illustrations are included for Rotor-Gene Q Instruments.

6. **First, open the "New Run Wizard" dialog box (Figure 5). Check the "Locking Ring Attached" box and click "Next".**

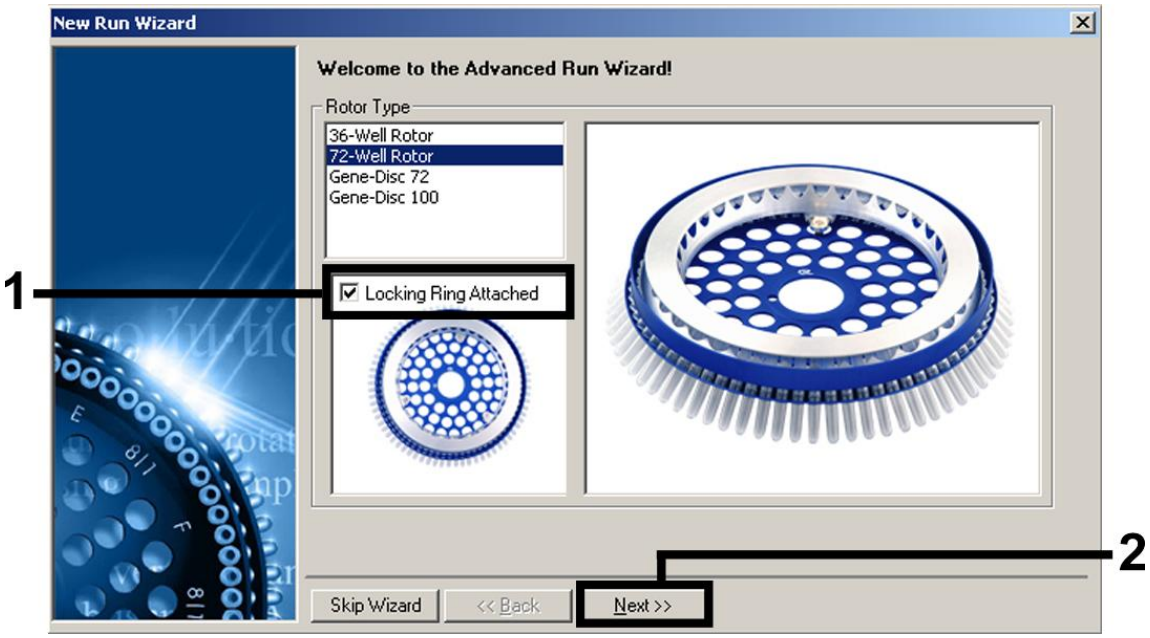


Figure 5. The "New Run Wizard" dialog box.

7. **Select *50* for the PCR reaction volume and click "Next" (Figure 6).**

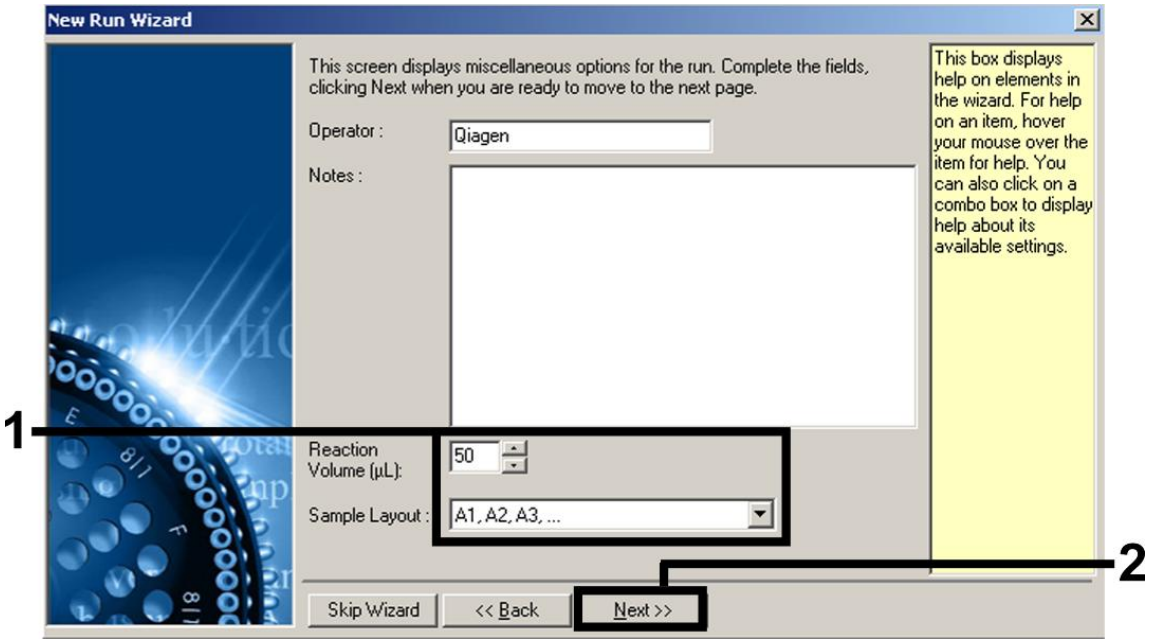


Figure 6. Setting the general assay parameters.

8. **Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 7), and program the temperature profile as shown in Figures 7–10).**

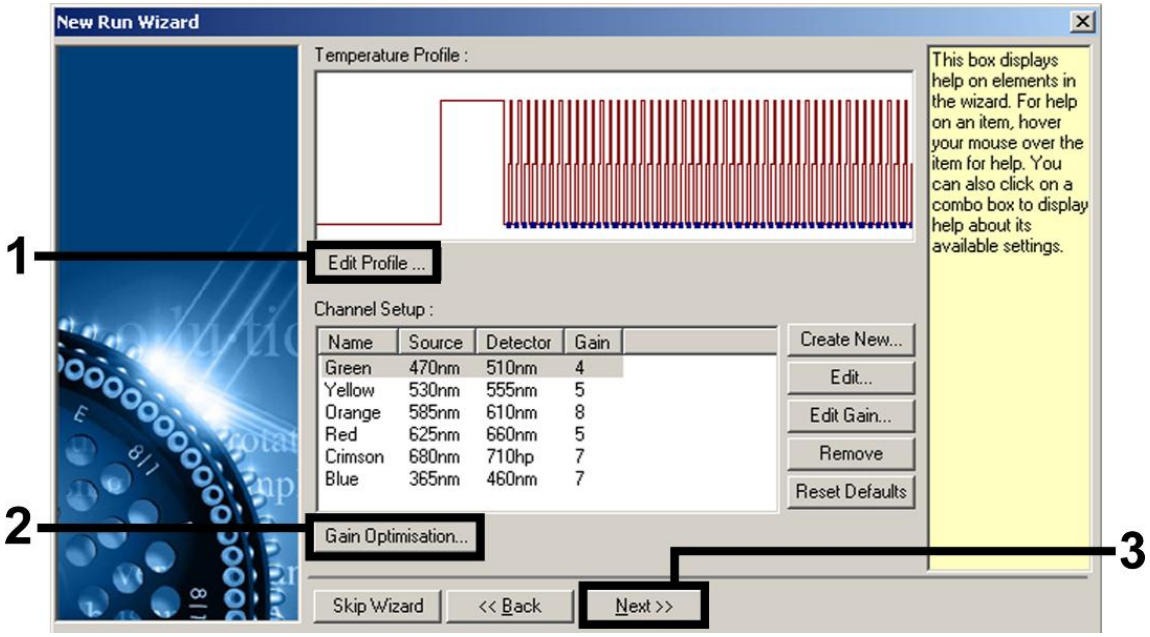


Figure 7. Editing the profile.

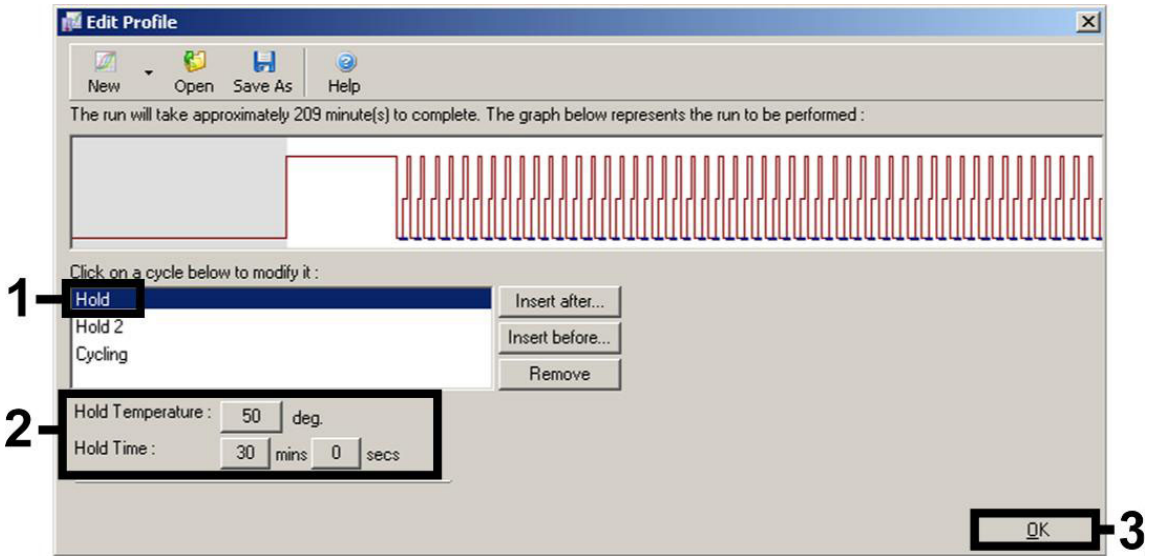


Figure 8. Reverse transcription of the RNA.

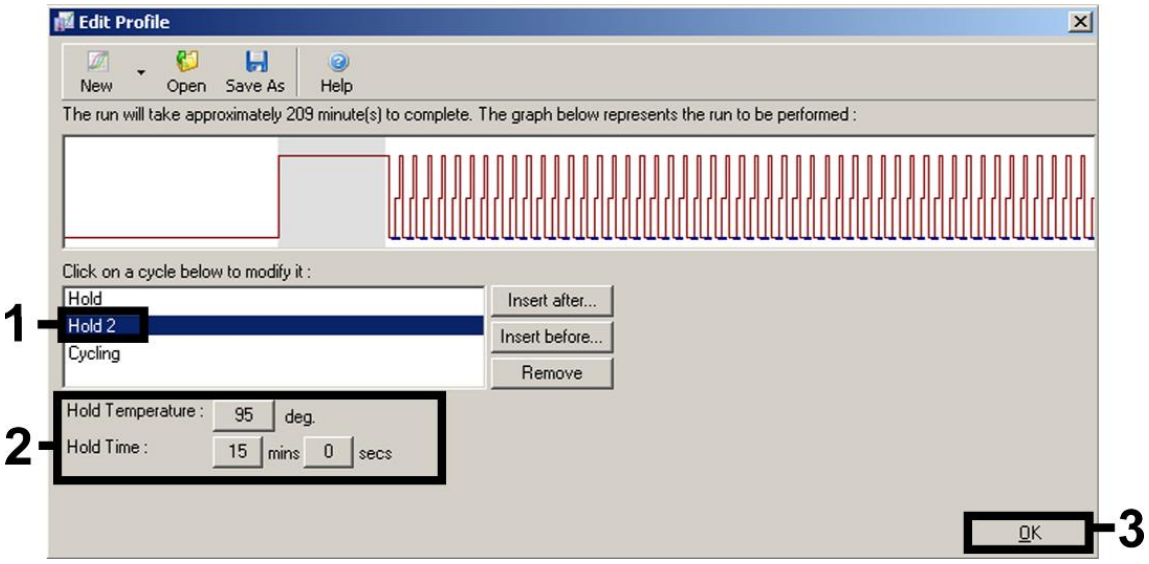


Figure 9. Initial activation of the hot-start enzyme.

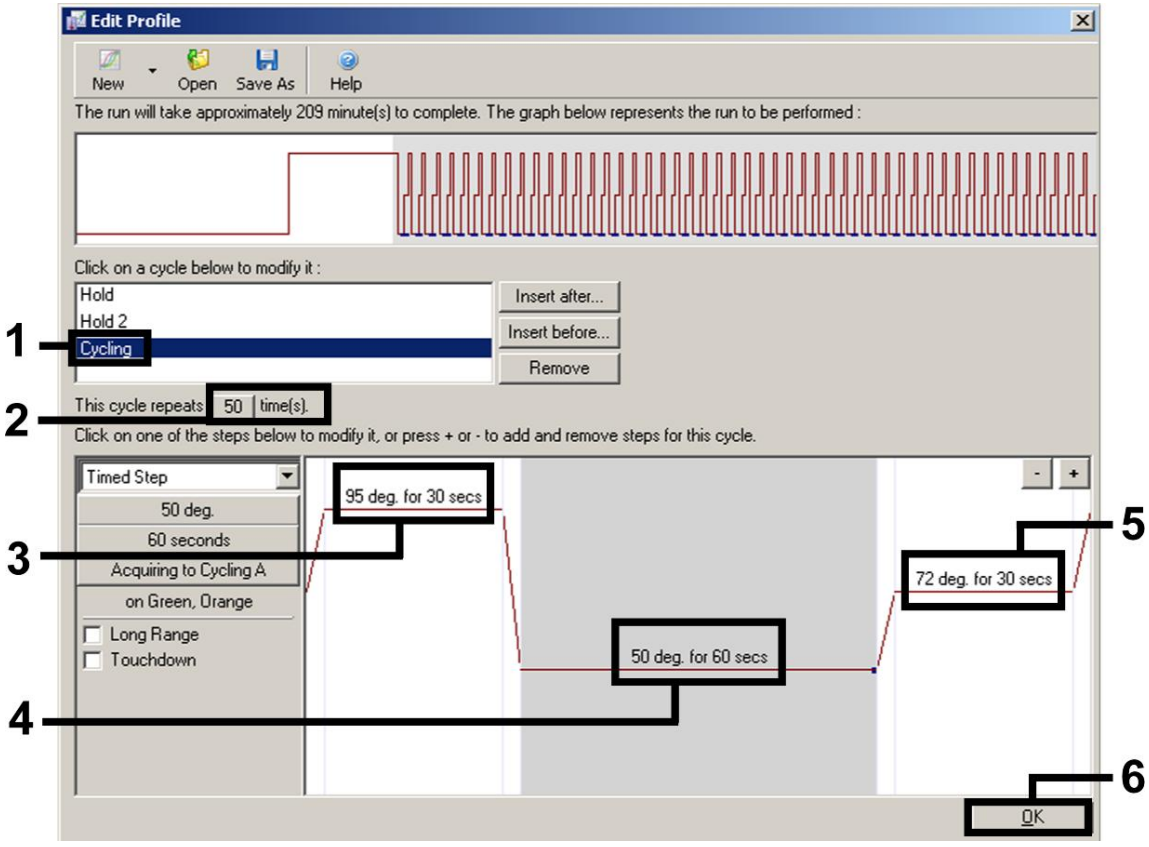


Figure 10. Amplification of the cDNA.

9. **The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (see Figure 7) to open the "Auto-Gain Optimisation Setup" dialog box. Set the calibration temperature to *50* to match the annealing temperature of the amplification program (Figure 11).**

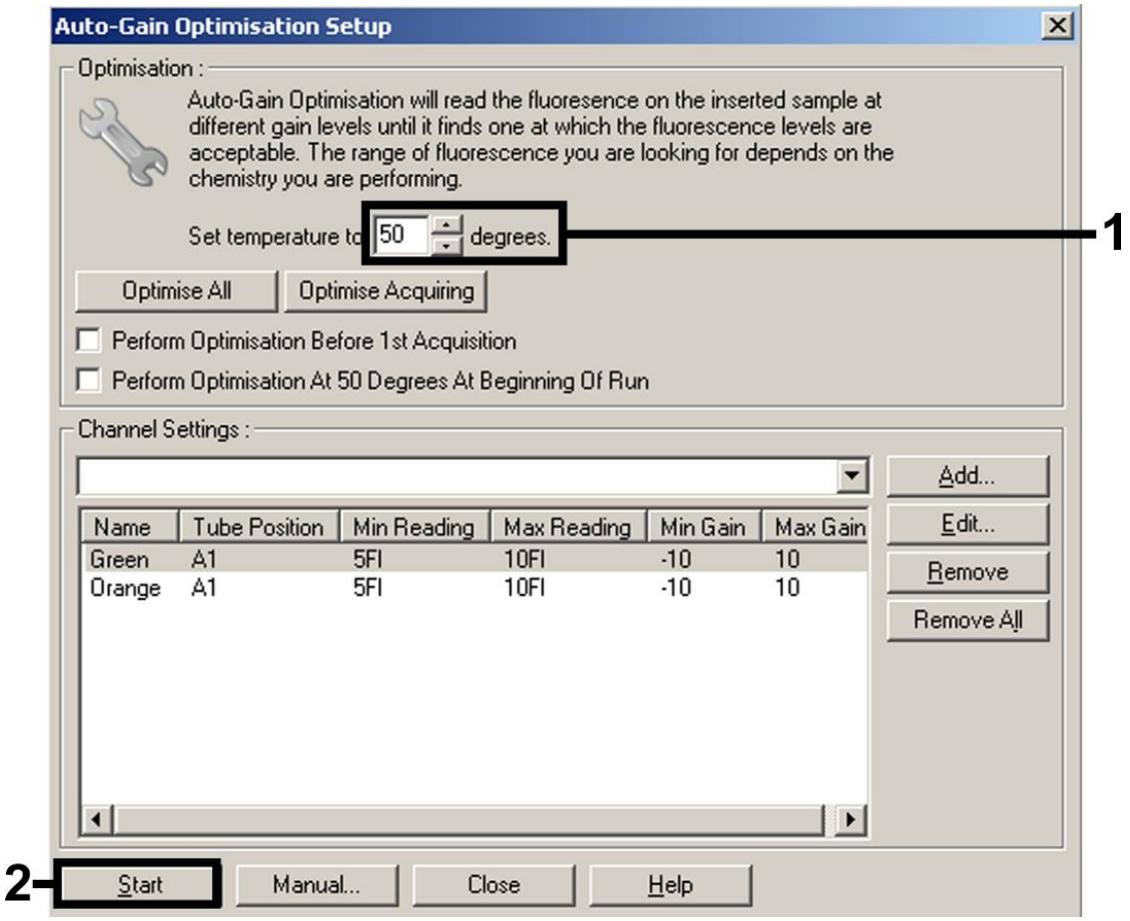


Figure 11. Adjusting the fluorescence channel sensitivity.

10. **The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 12). Click "Start Run".**

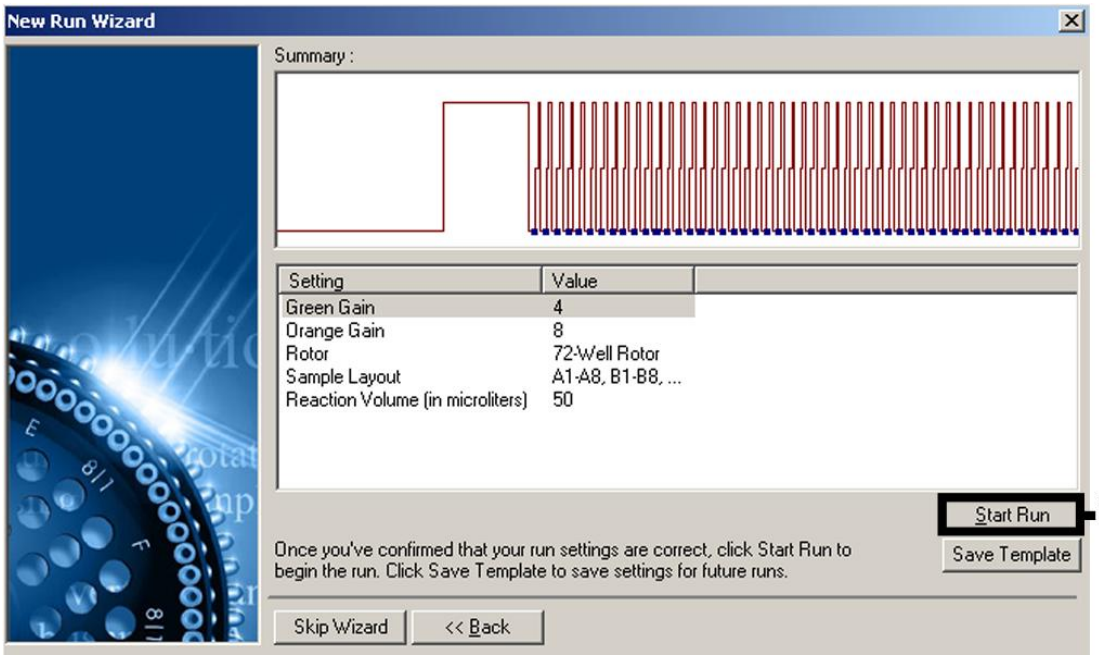
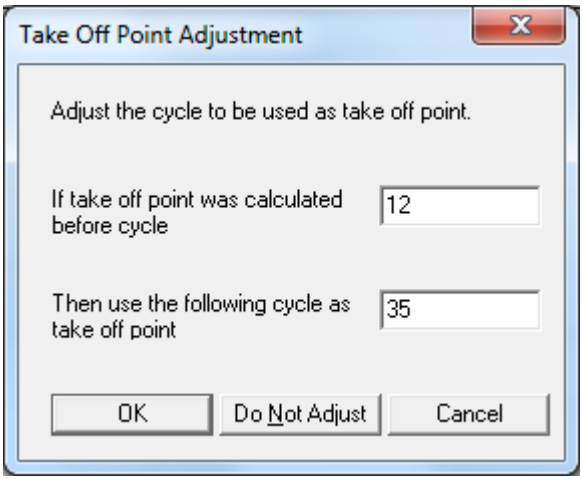


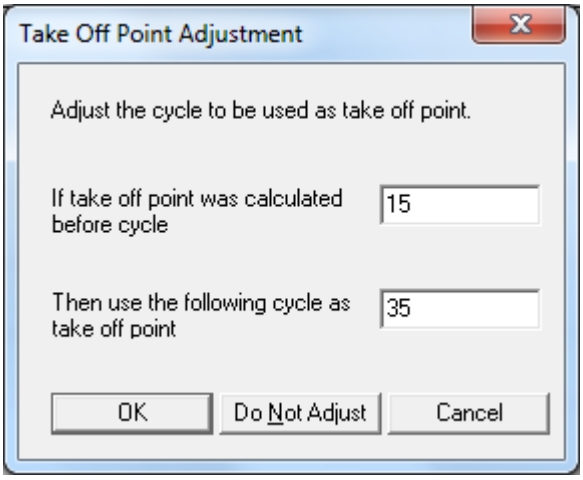
Figure 12. Starting the run.

11. **After the run is finished, analyze the data using the RGQ Software.**
12. **Open the run file (if closed), and select "Analysis" and "Cycling A.Green" for the analysis of HIV titers.**
13. **Select "Dynamic Tube".**
14. **Ensure "Slope Correct" is not selected.**
15. **Select "Take Off Adj." and enter "12" in the top and "35" in the bottom cell.**



16. **Set the graph to linear scale and set the threshold to 0.05.**
17. **Data can be exported by right clicking in the "Results" window and using the "Export to Excel" function.**

18. **For the analysis of the IC values, select “Analysis” and “Cycling A.Orange”.**
19. **Select “Dynamic Tube”.**
20. **Select “Slope Correct”.**
21. **Select "Take Off Adj." and enter "15" in the top and "35" in the bottom cell.**



22. **Set the graph to linear scale and set the threshold to 0.03.**
23. **Data can be exported by right clicking in the “Results” window and using the “Export to Excel” function.**

Channel	Target	Threshold	Dynamic tube	Slope correct	Take-Off Adjustment*
Green	HIV	0.05	On	Off	12/35
Orange	IC†	0.03	On	On	15/35

* Take-Off Adjustment requires RGQ Software version 2.3 or higher.

† IC: Internal Control

24. **Convert titer values from IU/µl to IU/ml using the equation on page 21.**
25. **The following results (25a, 25b, and 25c) are possible.**

    Examples of positive and negative PCR reactions are given in Figure 13 and Figure 14.

    Table 9 shows guidelines for interpretation of quantitative results.

25a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains HIV-1 RNA.

In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of HIV-1 RNA (positive signal in the Cycling Green channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Orange channel (competition).

25b. In fluorescence channel Cycling Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling Orange channel.
In the sample no HIV-1 RNA is detectable. It can be considered negative.

In the case of a negative HI Virus-1 RT-PCR, the detected signal of the internal control rules out the possibility of RT-PCR inhibition.

25c. No signal is detected in the Cycling Green or in the Cycling Orange channels.
No result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 35.

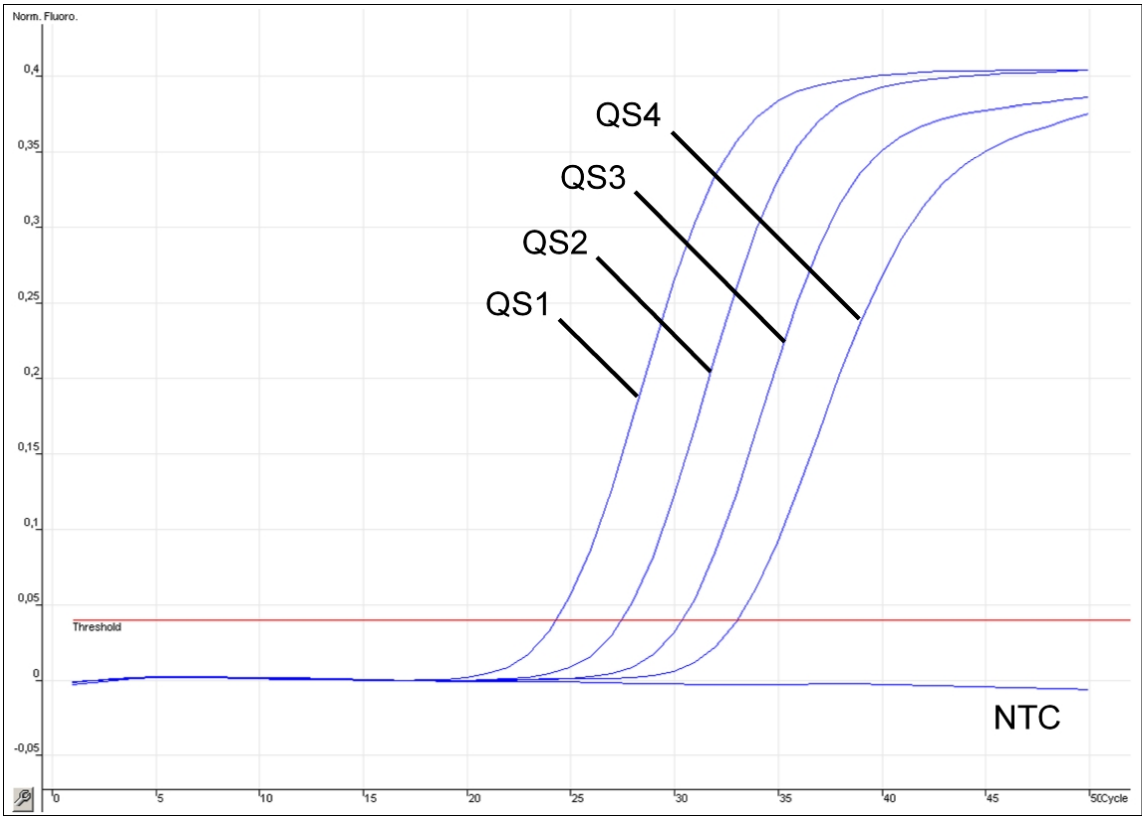


Figure 13. Detection of the quantitation standards (HI Virus-1 RG QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).

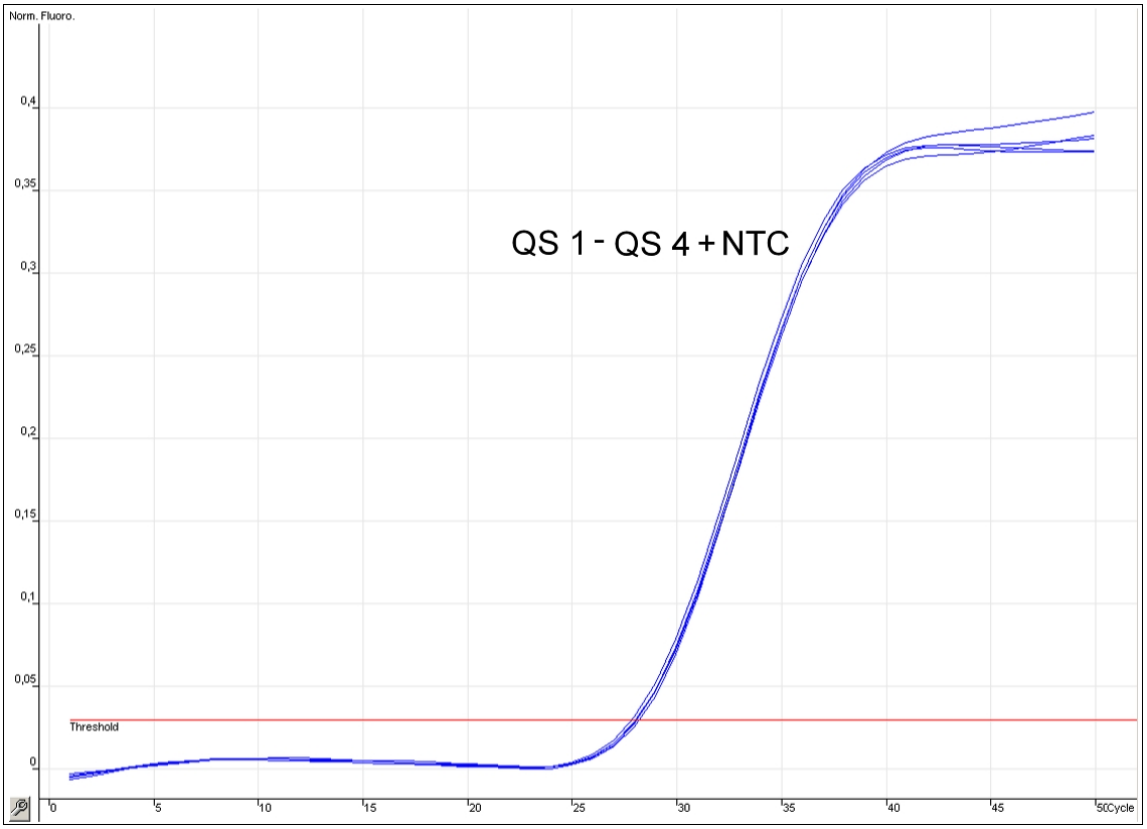


Figure 14. Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (HI Virus-1 RG QS 1–4). NTC: No template control (negative control).

Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Orange	Quantitative result (IU/ml)	Interpretation
Yes	Yes	<71.6	Valid result: HIV-1 RNA detected, <120 IU/ml* Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥71.6 and <120	Valid result: HIV-1 RNA detected, <120 IU/ml* Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes/No†	≥120 and ≤1.00 x 10^8	Valid result: HIV-1 RNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes/No†	>1.00 x 10^8	Valid result: HIV-1 RNA detected, >1.00 x 10^8 Quantitation not possible since the quantitative result is above the linear range of the assay.* †
No	Yes	–	Valid result: No HIV-1 RNA is detectable.†
Yes	No	<120	Invalid result: No result can be concluded.
No	No	–	Invalid result: No result can be concluded.‡

* If the C_T value for the internal control of a sample below the linear range or of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run ($C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$), then the sample should be treated as invalid. No result can be concluded.

† In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of HIV RNA (positive signal in the Cycling Green channel) can lead to a reduced or absent florescence signal of the internal control in the Cycling Orange channel (competition).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with positive controls (HI Virus-1 RG QS 1–4) in fluorescence channel Cycling Green	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	ⓘ For data analysis select the fluorescence channel Cycling Green for the analytical HI Virus-1 RT-PCR and the fluorescence channel Cycling Orange for the internal control RT-PCR.
b) Incorrect programming of the temperature profile of the Rotor-Gene Instrument	ⓘ Compare the temperature profile with the protocol. See "Protocol: PCR and Data Analysis", page 24.
c) Incorrect configuration of the PCR	ⓘ Check your work steps by means of the pipetting scheme, and repeat the PCR, if necessary. See "Protocol: PCR and Data Analysis", page 24.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HI Virus-1 RG RT-PCR Kit has expired	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control in fluorescence channel Cycling Orange and simultaneous absence of a signal in channel Cycling Green	
a) The PCR conditions do not comply with the protocol	ⓘ Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.

	Comments and suggestions
b) The PCR was inhibited	ⓘ Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
c) RNA was lost during extraction	ⓘ If the internal control was added to the extraction, an absent signal of the internal control can indicate the loss of RNA during the extraction. Make sure that you use the recommended isolation method (see "RNA isolation", page 20) and closely follow the manufacturer's instructions.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HI Virus-1 RG RT-PCR Kit has expired	ⓘ Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Signals with the negative controls in fluorescence channel Cycling Green of the analytical PCR

a) Contamination occurred during preparation of the PCR	ⓘ Repeat the PCR with new reagents in replicates. ⓘ If possible, close the PCR tubes directly after addition of the sample to be tested. ⓘ Make sure to pipet the positive controls last. ⓘ Make sure that work space and instruments are decontaminated at regular intervals.

	Comments and suggestions
b) Contamination occurred during extraction	ⓘ Repeat the extraction and PCR of the sample to be tested using new reagents. ⓘ Make sure that work space and instruments are decontaminated at regular intervals.

References

1. Mackay, I.M. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 10, 190.
2. Arbeitskreis Blut, V17 (09.1997), Bundesgesundheitsblatt 11/1997, p. 452–456.

Ordering Information

Product	Contents	Cat. no.
artus HI Virus-1 RG RT-PCR Kit (24)	For 24 reactions: 2 Masters, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4513253
QIAamp DSP Virus Kit — for purification of viral nucleic acids from human plasma for in vitro diagnostic purposes		
QIAamp DSP Virus Kit	For 50 preps: QIAamp MinElute® Spin Columns, Buffers, Reagents, Tubes, Column Extenders, and VacConnectors	60704
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001640
Rotor-Gene Q 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001570
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

Product	Contents	Cat. no.
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q accessories		
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Loading Block 96 x 0.2 ml Tubes	Aluminum block for manual reaction set-up in a standard 8 x 12 array using 96 x 0.2 ml tubes	9018905
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions	981005
PCR Tubes, 0.2 ml (10000)	10 x 1000 thin-walled tubes for 1000 reactions	981008

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