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QIAscreen® HPV PCR Test Instructions for Use (Handbook)

Σ 72

Version 1

IVD

For in vitro diagnostic use

For use with Rotor-Gene® Q MDx instrument

CE

REF 617005

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Sample to Insight

QIAGEN

Contents

Intended Use

The QIAscreen HPV PCR Test is an in vitro real-time PCR-based assay for the qualitative detection of human papillomavirus (HPV) DNA of the following 15 (probably) high-risk HPV genotypes, i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68.

Samples that may be tested with QIAscreen HPV PCR Test include DNA isolated from specimens that are collected in the following ways:

Cervical specimens collected using a brush/broom-type collection device (collected by a physician)

Vaginal specimens collected using a brush-broom or lavage device (self-collected)

Indications for use:

- As a primary test in screening of women for the risk of cervical (pre)cancer to determine the need for referral to colposcopy or other follow-up procedures
- As a follow-up test for women with Pap test results with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intra-epithelial neoplasia (lsil) to determine the need for referral to colposcopy of other follow-up procedures

This product is intended to be used by professional users, such as technicians and laboratorians, who are trained in in vitro diagnostics procedures, molecular biological techniques, and the Rotor-Gene Q MDx 5plex HRM system.

Summary and Explanation

Human papillomaviruses (HPV) belong to the family of Papillomaviridae and are small double-stranded DNA viruses. The circular genome is approximately 7.9 kilo bases in size. More than 100 types of HPV have been identified, of which certain HPV types, known as high-risk HPV (hrHPV) like HPV 16 and 18, are associated with the induction of mucosal lesions that can progress to malignancy. Cervical cancer and its precursor lesions (cervical intraepithelial neoplasia, CIN) are the most well-known complications of a persistent infection with a high-risk type of HPV (1-3).

The viral genome contains early (E) and late (L) genes, which encode proteins necessary for early and late stages of the HPV life cycle, respectively. The E6 and E7 gene products of hrHPV types have carcinogenic properties and are necessary for malignant transformation of the host cell (4). Malignant progression is often associated with viral integration into the genome of the host cell (5). Integration results in interruption of the viral genome in a region that may extend from the E1 to the L1 open reading frame (6). This may have consequences for PCR-mediated amplification of viral DNA in these regions. As not only the initiation but also the maintenance of the transformed phenotype depends on continuous expression of the viral oncoproteins (7, 8), the viral E6/E7 region is invariably retained in integrated viral genomes in cervical cancers (6). The QIAscreen HPV PCR Test targets a conserved region within the E7 gene. The assay has been clinically validated according to the international guidelines for HPV detection assays and in other studies (9, 10, 14, 15).

Principle of the Procedure

The QIAscreen HPV PCR Test is a multiplex, real-time PCR-based assay directed against the E7 gene of 15 (probable) hrHPV types that uses fluorescent probes for the detection of one or more accumulating PCR products. During each PCR cycle the fluorescent signal increases in a logarithmic manner resulting in an amplification curve. As soon as the amplification curve of the target comes above its threshold, the sample is considered positive for that target. The multiplex format allows the simultaneous detection of four different fluorescent dyes per reaction, with each fluorescent dye representing different targets. The four different targets are: **1.** HPV 16, **2.** HPV 18, **3.** the 13 other hrHPV types as a pool and **4.** the human β-globin gene. The QIAscreen HPV PCR Test separately detects HPV 16, HPV 18, and the pool of 13 other hrHPV genotypes. The human β-globin gene is used as the sample control determining both the quality of the sample DNA and the presence of potential inhibitory substances.

Materials Provided

Kit contents

QIAscreen HPV PCR Test Kit		**72 Reactions**
Catalog no.		**617005**
QIAscreen Master Mix (1 tube)	Transparent color	1080 µL
QIAscreen Positive Control (1 tube)	Transparent color	100 µL
QIAscreen Negative Control (1 tube)	Transparent color	100 µL
QIAscreen HPV PCR Test Instructions for Use (Handbook)		1

Materials Required but Not Provided

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.

Consumables, reagents, and instruments for sample preparation

- Hologic PreservCyt® Solution (for self-collection sample storage)
- Standard DNA extraction kits, such as QIAamp® DSP virus spin kit (QIAGEN, cat. no. 61704) and QIAsymphony® DSP Virus/Pathogen Midi Kit (QIAGEN, cat. no. 937055) and NucleoMag 96 Tissue kit (Macherey-Nagel, cat. no. 744300)
- PBS for handling cervical specimens in PreservCyt collection medium
- AL buffer (QIAGEN, cat.no. 19075) for pretreatment of cervical specimens collected in SurePath and CellSolutions collection medium

Consumables for the Rotor-Gene Q MDx Instrument

- 0.1 mL Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or cat. no. 981106)

Equipment

- Dedicated pipets* (adjustable) for PCR (1–10 µL; 10–100 µL)
- Dedicated filter-plugged sterile DNAse-free pipette-tips
- Disposable gloves
- Benchtop centrifuge*
- Vortex mixer*

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

Equipment for Extraction and real-time PCR

- QIAsymphony SP Module (cat. no. 9001297) (for optional automation of the extraction)
- Rotor-Gene Q 5plex HRM System (cat. no. 9002033) or Rotor-Gene Q MDx 5plex HRM instrument (cat. no. 9002032) with Rotor-gene Q software version 2.3.1 or higher*
- QIAscreen run template for Rotor-Gene Q. The template is named "**QIAscreen RGQ profile v1.0.ret**".
- QIAscreen channel analysis templates for the channels green (HPV 16), yellow (HPV Other), orange (β-globin), and red (HPV 18). The templates have the file extension ".qut".

* If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn", where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

Warnings and Precautions

Safety information

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.

- The QIAscreen HPV PCR Test positive and negative controls contain sodium azide as a preservative (0.01%). Sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal through the sink, flush drains with generous amounts of cold water to prevent azide build-up.

General precautions

Use of PCR tests requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards.

Always pay attention to the following:

- Wear protective disposable powder-free gloves, a laboratory coat, and eye protection when handling specimens.
- Prevent microbial and nuclease (DNase) contamination of the specimen and the kit. DNase may cause degradation of the DNA template.
- Avoid DNA or PCR product carryover contamination, which could result in a false-positive signal.
- Always use DNase-free disposable pipet tips with aerosol barriers.

- Reagents of QIAscreen HPV PCR Test are optimally diluted. Do not dilute reagents further as this may result in a loss of performance.
- All reagents supplied in the QIAscreen HPV PCR Test are intended to be used solely with the other reagents supplied in the same kit. Do not substitute any reagent from one kit with the same reagent from another QIAscreen HPV PCR Test kit, even from the same batch, as this may affect performance.
- Refer to the Rotor-Gene Q MDx instrument user manual for additional warnings, precautions, and procedures.
- Before the first run of the day, perform a warm-up run for Rotor-Gene Q MDx 5plex HRM at 95°C for 10 minutes.
- Alteration of incubation times and temperatures may result in erroneous or discordant data.
- Do not use components of the kit that have passed their expiration date, or that have been incorrectly stored.
- Minimize exposure of the components to light: reaction mixes may be altered due to exposure.
- Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the PCR reagents.
- Discard sample and assay waste according to your local safety procedures.

Reagent Storage and Handling

Shipping conditions

The QIAscreen HPV PCR Test is shipped on dry ice. If any component of the QIAscreen HPV PCR Test is not frozen upon arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note, handbook, or the reagents, please contact one of the QIAGEN Technical Service Departments or local distributors (visit **www.qiagen.com**).

Storage conditions

The QIAscreen HPV PCR Test must be stored immediately at –30 to –15°C upon receipt in a constant-temperature freezer and protected from light.

Stability

When stored under the specified storage conditions, the QIAscreen HPV PCR Test is stable until the stated expiration date on box label.

Once opened, reagents can be stored in their original packaging at –30°C to –15°C. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze–thaw cycles.

- Gently mix by inverting the tube 10 times and centrifuge all tubes before opening.
- Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance for the stability time as long as the same batches of components are used.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Do not mix reagents from different kits, even if they are from the same lot.

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

Specimen Storage and Handling

CAUTION 	All specimens must be treated as potentially infectious material.

Cervical specimens

The QIAscreen HPV PCR Test is for use with genomic DNA samples obtained from cervical specimens (scrapes). Validated collection media for cervical specimens (scrapes) are PreservCyt, CellSolutions®, Pathtezt®, and Surepath® collection medium. Optimal storage temperature of the clinical samples is 2–8°C upon arrival at the lab. Under these storage conditions, samples in PreservCyt collection medium are stable for 3 months and in Surepath collection medium are stable for 2 weeks prior to DNA extraction.

Cervical samples collected in PreservCyt can be stored up to 210 days after sample collection at 18–25°C, up to two and a half years at 2–8°C, and up to 2 years at <20°C. Cervical samples collected in Surepath can be stored up to 10 weeks after sample collection at 2-30°C, up to two and half years at 2–8°C, and up to 210 days at <20°C.

Self-collected vaginal brush specimens

The QIAscreen HPV PCR Test is for use with genomic DNA samples extracted from self-collected vaginal brush and self-collected cervico-vaginal lavage specimens. Self-collected vaginal brush specimens can be collected and shipped dry or in saline (0.9% w/v NaCl) and, upon arrival in the laboratory, stored in PreservCyt. Self-collected cervico-vaginal lavage specimens are collected and shipped in saline (0.9% w/v NaCl) and, upon arrival in the laboratory, stored in PreservCyt. Self-samples in PreservCyt can be stored up to 210 days after sample collection at 18–25°C, up to two and half years at 2–8°C, and up to 2 years at <20°C.

Genomic DNA samples

Once genomic DNA is extracted, it can be stored at 2–8°C for short-term storage (≤2 days) or at –30°C to –15°C for up to 12 months.

Sample Preparation

DNA extraction

Standard DNA extraction kits (e.g., column- and magnetic bead-based kits, such as QIAamp®DSP Virus spin kit, QIAsymphony® DSP Virus/Pathogen Midi Kit, and NucleoMag 96 Tissue kit, (Macherey-Nagel) are compatible with this assay. Operating details for the QIAsymphony® DSP Virus/Pathogen Midi are indicated below.

Clinical specimens in PreservCyt or PathTezt collection medium

For cervical specimens (scrapes) suspended in PreservCyt or PathTezt collection medium, the fraction of DNA to be used as input in the PCR represents 0.125% of the 20 ml PreservCyt or PathTezt cervical scrape sample. This corresponds with 25 µL of the original sample types. Since at maximum only 5 µL of extracted DNA can be used as input in the PCR, DNA extraction procedures should be executed such that 5 µL DNA extract corresponds with 25 µL cervical specimen (scrape) sample to ensure that the correct fraction of the cervical sample is used in the PCR. Equivalent media with (e.g., Surepath) or without (e.g., PreservCyt) formaldehyde should be processed similarly.

Important: PreservCyt medium can interfere with the DNA extraction process. This can be overcome in two different ways.

1. Dilute the aliquot of the PreservCyt specimen in an equal volume of PBS or lysis buffer from the DNA extraction kit and mix, before starting the DNA extraction. Make sure the total sample volume is compatible with the DNA extraction kit. If the total volume becomes too high for the extraction kit, it is advised to use method 2, outlined below.
2. Centrifuge the PreservCyt sample (≥3400 x *g* for 10 min) and remove the supernatant. The pellet is resuspended in appropriate volume of PBS or lysis buffer that is compatible with the DNA extraction kit (for the QIAamp DSP Virus spin kit: resuspend in 200 µL PBS and follow the manufacturers instruction for DNA extraction, elute in 100 µL; for the

Margery Nagel Nucleomag96 tissue kit: resuspend in 100 µL buffer T1 of that kit and follow the manufacturer's instructions, elute in 100 µL)

Equivalent media should be processed similarly.

Operating details QIAsymphony® DSP Virus/Pathogen Midi Kit

QSDSP protocol: 500 µL of cervical sample in PreservCyt is mixed with 500 µL PBS. An Integrated run performing the Complex800_V6_DSP protocol is started on the QIAsymphony following the steps described in 'QIAsymphony® SP/AS Consolidated Operating Guide – 12.3 Integrated run'. DNA is eluted in 60 µL and 5 µL is used for the QIAscreen HPV PCR Test. If you only use the QIAsymphony SP module, a sample preparation run performing the Complex800_V6_DSP protocol is performed using the QIAsymphony SP instrument. Follow the steps described in '*QIAsymphony DSP Virus/Pathogen Kit Instructions for Use (Handbook) – General Purification Protocol*'.

For cervical specimens (scrapes) suspended in SurePath or CellSolutions collection medium, the fraction of DNA to be used as input in the PCR represents 0.25% of the 10 mL SurePath or CellSolutions cervical scrape sample. This corresponds with 25 µL of the original sample. Since at maximum only 5 µL of extracted DNA can be used as input in the PCR, sample volume and DNA elution volume should be selected such that 5 µL DNA extract corresponds with 25 µL cervical specimen (scrape) sample to ensure that the correct fraction of the cervical sample is used in the PCR.

IMPORTANT: Clinical specimens collected in SurePath and CellSolutions medium must be pretreated prior to use to overcome formaldehyde-induced crosslinking using the described protocol below.

Pretreatment clinical specimens collected in SurePath and CellSolutions medium:

3. Mix the SurePath or CellSolutions specimen with a 1:1 volume of AL buffer (QIAGEN) and mix thoroughly.
4. Incubate at 90°C for 20 minutes followed by equilibration to room temperature before proceeding with DNA extraction.

Equivalent media containing formaldehyde should be processed similarly.

For self-collected vaginal brush specimens suspended in Hologic PreservCyt Solution, DNA extraction procedures should be executed, such that 5 µL DNA extract used as input in the PCR represents 0.5% of the vaginal sample. For example, the vaginal self-sample will be suspended in 2 mL PreservCyt Solution then 5 µL input DNA corresponds with 10 µL of the self-sample suspension.

For self-collected cervico-vaginal lavage specimens, the fraction of DNA to be used as input in the PCR represents 0.5% of the lavage self-sample. Thus, in the case of a total lavage volume of 3 mL, DNA extraction procedures should be executed such that 5 µL input DNA corresponds with 15 µL of the original lavage self-sample.

Protocol: QIAscreen HPV PCR Test in the Rotor-Gene Q MDx instrument

Important points before starting

Take time to familiarize yourself with the Rotor-Gene Q MDx instrument before starting the protocol. See the instrument user manual.

Before the first run of the day, perform a warm-up run for the Rotor-Gene Q MDx 5plex HRM at 95°C for 10 minutes.

A Rotor-Gene Q series software template is required to run the test. Make sure the template QIAscreen RGQ profile v1.0.ret is used.

To analyze the test for each of the four detection channels, a Rotor-Gene Q series software template is required. Make sure the correct template is used for each channel, as indicated below:

- "QIAscreen RGQ Green Channel analysis template.qut" must be used for analysis of the signals in the Green channel (HPV 16).
- "QIAscreen RGQ Orange Channel analysis template.qut" must be used for analysis of the signals in the Orange channel (β-globin).
- "QIAscreen RGQ Yellow Channel analysis template.qut" must be used for analysis of the signals in the Yellow channel (HPV Other).
- "QIAscreen RGQ Red Channel analysis template.qut" must be used for analysis of the signals in the Red channel (HPV 18).

Sample processing on Rotor-Gene Q MDx instruments with 72-tube rotor

Up to 70 genomic DNA samples can be tested within the same experiment, besides a positive and a negative control. The schematic in Table 1 provides an example of the loading block

or rotor setup for an experiment with the QIAscreen HPV PCR Test. Numbers denote positions in the loading block and indicate final rotor position.

Table 1. Plate and rotor setup for an experiment with the QIAscreen HPV PCR Test on Rotor-Gene Q MDx instrument

Strip	Tube position	Sample name	Strip	Tube position	Sample name	Strip	Tube position	Sample name
1	1	Positive Control	**7**	25	Sample 23	**13**	49	Sample 47
	2	Negative Control		26	Sample 24		50	Sample 48
	3	Sample 1		27	Sample 25		51	Sample 49
	4	Sample 2		28	Sample 26		52	Sample 50
2	5	Sample 3	**8**	29	Sample 27	**14**	53	Sample 51
	6	Sample 4		30	Sample 28		54	Sample 52
	7	Sample 5		31	Sample 29		55	Sample 53
	8	Sample 6		32	Sample 30		56	Sample 54
3	9	Sample 7	**9**	33	Sample 31	**15**	57	Sample 55
	10	Sample 8		34	Sample 32		58	Sample 56
	11	Sample 9		35	Sample 33		59	Sample 57
	12	Sample 10		36	Sample 34		60	Sample 58
4	13	Sample 11	**10**	37	Sample 35	**16**	61	Sample 59
	14	Sample 12		38	Sample 36		62	Sample 60
	15	Sample 13		39	Sample 37		63	Sample 61
	16	Sample 14		40	Sample 38		64	Sample 62
5	17	Sample 15	**11**	41	Sample 39	**17**	65	Sample 63
	18	Sample 16		42	Sample 40		66	Sample 64
	19	Sample 17		43	Sample 41		67	Sample 65
	20	Sample 18		44	Sample 42		68	Sample 66
6	21	Sample 19	**12**	45	Sample 43	**19**	69	Sample 67
	22	Sample 20		46	Sample 44		70	Sample 68
	23	Sample 21		47	Sample 45		71	Sample 69
	24	Sample 22		48	Sample 46		72	Sample 70

Note: Fill all unused positions with empty tubes.

PCR on Rotor-Gene Q MDx instruments with 72-tube rotor

1. Set up the QIAscreen HPV PCR Test.

   Note: To minimize the risk for PCR reaction contamination, it is strongly recommended that you use a PCR-cabinet with UV-irradiation capability.
   Important: Dispensing of the QIAscreen Master Mix must be performed in an area separate from that in which the DNA extraction is performed.

   1a. Clean the bench area, pipets, and tube rack prior to use with a DNA-degrading solution to prevent template or nuclease contamination.
   Note: Change tips between each tube to avoid any nonspecific template or reaction mix contamination which may lead to false-positive results.
   1b. Mix gently by inverting 10 times, then briefly centrifuge before use to collect the solution at the bottom of the tube.
   1c. Dispense 15 µL of the QIAscreen Master Mix into the appropriate tubes of the tube-strips (at maximum 72 tubes per Rotor-gene Q MDx run). Reaction setup can be done at room temperature.
   1d. Return the QIAscreen Master Mix to the freezer to avoid any material degradation. Transport tubes to separate area to dispense the QIAscreen Positive Control and sample DNA.
   1e. Add 5 µL of the negative control to tube position 2, mix by pipetting up and down or by flicking the tube, and close the tube by pressing the cap on the tube.
   1f. Add 5 µL of the QIAscreen Positive Control to tube position 1, mix by pipetting up and down or by flicking the tube, and close the tube.
   Note: Change tips between each tube to avoid any nonspecific template or reaction mix contamination, which may lead to false-positive results.
   1g. Add 5 µL of sample DNA to the appropriate tubes containing the QIAscreen Master Mix, mix by pipetting up and down or by flicking the tubes, and close the tubes by pressing the caps on the tubes.
   1h. Once a set 4 tubes have been filled, cap the tubes.

Note: The PCR tubes can be stored for 30 minutes between pipetting samples into the PCR tubes and start of the experiment in the machine at 2-8°C in the dark.

2. Prepare the Rotor-Gene Q MDx and start experiment as follows:

Important: Before the first run of the day, perform a warm-up run for the Rotor-Gene Q MDx 5plex HRM at 95°C for 10 minutes.

2a. Place a 72-well rotor on the rotor holder.

2b. Fill the rotor with strip tubes according to the assigned positions, starting at position 1, as shown in Table 1, with empty capped strip tubes placed into all unused positions.

Note: Make sure the first tube is inserted into position 1 and the strip tubes are placed in the correct orientation and positions as shown in Table 1.

2c. Attach the locking ring.

2d. Load the Rotor-Gene Q MDx instrument with the rotor and locking ring, and close the instrument lid.

2e. Go to New Run window and click Open a template in another folder….

2f. Select the QIAscreen run template named QIAscreen RGQ profile v1.0.ret.

2g. Select Rotor type: 72-well rotor and Locking ring attached and click Next.

2h. At operator, enter initials, and click Next.

2i. In the following window, click Next.

2j. Click Start run.

To enter sample names, click Edit samples (this can also be done after the run is completed).

Table 2. Target and channel settings*

Target	Detection Channel
β-globin	Orange
HPV 16	Green
HPV 18	Red
HPV Other*	Yellow

* HPV Other comprises the pool of 13 non-16/18 HPV types.

3. Analyze the data.

3a. Select the tubes to be used for analysis.

3b. Go to Analysis tool window, select Cycling A. Green and click Show. Click Import under Imported Settings (right bottom of window) and select the file QIAscreen RGQ Green Channel analysis template.qut. Select Cycling A. Green and click Hide.

3c. Select Cycling A. Orange and click Show. Click Import under Imported Settings and select the file QIAscreen RGQ Orange Channel analysis template.qut. Select Cycling A. Orange and click Hide.

3d. Select Cycling A. Red and click Show. Click Import under Imported Settings and select the file QIAscreen RGQ Red Channel analysis template.qut. Select Cycling A. Red and click Hide.

3e. Select Cycling A. Yellow and click Show. Click Import under Imported Settings and select the file QIAscreen RGQ Yellow Channel analysis template.qut.

3f. Click Save.

3g. OPTIONAL: For interpretation of the results, the data can be exported as a .csv file. Go to File > Save as > Excel Analysis Sheet and save the export file.

4. Unload the Rotor-Gene Q MDx instrument and discard the strip tubes according to your local safety regulations.

Interpretation of Results

The run and sample validation criteria are indicated below under A and B, respectively. Appropriate measures are indicated in case one (or more) criteria are not met.

A. Validation criteria of QIAscreen HPV PCR Test controls

Targets in the QIAscreen Positive Control should give C_T values that are lower than 29 for β-globin, lower than 30 for HPV 16 and HPV 18, and lower than 32 for HPV Other. If this is not the case and analysis settings are correct, the experiment should be repeated.

None of the targets in the QIAscreen Negative Control should give a signal above the threshold till the end of the PCR run (i.e., cycle 40 or not defined). If a signal is seen before cycle 40, and analysis settings are correct, the experiment should be repeated.

Note: If the controls do not comply with the established limits and repetition excludes errors in technique, check the following items:

- Expiration date on reagent package
- Temperature of the reagents
- Settings of the PCR system and of the software
- Contamination

If controls are still invalid, contact the manufacturer's customer service or your local distributor.

B. Interpretation of sample results

The result for a sample is determined as follows (Table 3).

Table 3. Interpretation of results

	C_T value HPV target(s)	C_T value β-globin	Interpretation
1	HPV 16 and/or HPV 18 <36 and/or HPV Other <33.5	Any	HPV positive
2	HPV 16 and HPV 18 ≥36 or not defined and HPV Other ≥33.5 or not defined	≤30	HPV negative
3	HPV 16 and HPV 18 ≥36 or not defined and HPV Other ≥33.5 or not defined	>30	Invalid

1. HPV positive. When C_T value(s) of HPV 16 and/or HPV 18 is (are) <36 and/or Other HPV is <33.5 (irrespective of C_T value of β-globin). The channel indicates the type(s) present. **2. HPV negative.** When C_T value for β-globin is ≤30 and C_T values for HPV 16 and HPV 18 are ≥36 or show no signal and HPV Other is ≥33.5 or show no signal. **3. Invalid.** When C_T value of β-globin is >30 and C_T values of HPV 16 and HPV 18 are ≥36 or show no signal and Other HPV is ≥33.5 or show no signal.

Limitations

- For the indicated intended use the test should be performed on cervical scrape specimens or self-collected (cervico-)vaginal specimens. However, the QIAscreen HPV PCR Test has also been evaluated for use with DNA extracted from formalin-fixed paraffin-embedded (FFPE) biopsy specimens.
- Specimen collection, transport, and storage may affect the number of copies of a target in the specimen, causing a potential false positive or false negative result.
- These instructions only apply to the Rotor-Gene Q MDx 5plex HRM instrument.
- Poor DNA extraction performance may lead to invalid test results. Consult your local distributor or the manufacturer customer service for technical advice on DNA extraction protocol if this persists.
- Samples with equivocal results due to low copy number of the targets can be confirmed by repeat analysis.
- In rare cases, cervical lesions can be induced by natural HPV variants or HPV types that are not targeted by the QIAscreen HPV PCR Test.
- QIAscreen HPV PCR Test reagents may exclusively be used for in vitro diagnostics.
- Use of PCR tests requires good laboratory practices, including maintenance of equipment, that are dedicated to molecular biology and is compliant with applicable regulations and relevant standards.
- Reagents and instructions supplied for the QIAscreen HPV PCR Test have been validated for optimal performance.
- The QIAscreen HPV PCR Test is to be used by laboratory professionals trained in the use of the Rotor-Gene Q MDx instruments.
- The product is to be used by personnel specially instructed and trained in the techniques of real-time PCR and in the in vitro diagnostic procedures only. Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.
- Strict compliance with the Instructions for Use (handbook) is required for optimal QIAscreen HPV PCR Test results.

- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- All reagents supplied in the QIAscreen HPV PCR Test are intended to be used solely with the other reagents supplied in the same kit. This may otherwise affect performance.
- Any off-label use of this product and/or modification of the components will void Self-screen B.V.'s liability.
- It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the performance studies.

Performance Characteristics

Limit of Detection (LoD)

The limit of detection (LoD) was determined using gBlocks (i.e. double-stranded genomic DNA blocks) containing part of the E7 gene of an HPV genotype. Serial 3-fold gBlock dilutions series of the 15 targeted HPV types (i.e. 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68) were prepared in a background of 50 ng human DNA, and tested in 8-fold. For β-globin, the LoD was assessed on a 3-fold serial dilution series in water of a gBlock containing part of the β-globin gene which was tested in 8-fold.

Table 4. Limit of detection (LoD) of the QIAscreen HPV PCR Test assay of 15 HPV types and β-globin gene

Target	LoD (copies per PCR)
HPV 16	206
HPV 18	69
HPV 39, 45	617
HPV 31, 33, 35, 51, 56, 59, 66, 67	1852
HPV 52, 58, 68	5556
β-globin	617

Analytical specificity*

Analytical specificity was determined against plasmid DNAs of non-targeted HPV genomes (i.e., HPV 6, 11, 26, 40, 42, 43, 53, 61, and 70) at a concentration of at least 46,000 copies/test and against the 3 most potentially pathogenic vaginal microorganisms *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Candida albicans* at a concentration of at least 10,000 copies/test. The test did not show any cross-reactivity with the non-targeted HPV types 6, 11, 26, 40, 42, 43, 53, and 61, or the micro-organisms. Only for HPV 70, a positive signal was observed in the 'HPV Other' channel (i.e., the channel that detects the pool of 13 non-16/18 HPV types), which after further diluting could be detected at >17,000 copies/test. HPV 70 is considered probably carcinogenic on the basis of epidemiological, phylogenetic, and functional studies (11-13).

Clinical performance on cervical specimens (scrapes)

The clinical sensitivity and specificity of the test for cervical intraepithelial neoplasia grade 2 or higher (CIN 2+) in cervical specimens (scrapes) stored in PreservCyt have been validated in two different studies by a non-inferiority analysis relative to the high-risk HPV GP5+/6+ PCR(10) or the Hybrid Capture 2 (14) following the international guidelines for HPV test requirements for cervical cancer screening (9). The clinical sensitivities for CIN 2+ were 96.8% (61/63) and 92.9% (91/98) and the clinical specificities for CIN 2+ were 95.1% (783/823) and 94.2% (933/990), respectively. The clinical sensitivity and specificity were non-inferior to that of the reference assays GP5+/6+ PCR (10) or Hybrid Capture 2 (14), indicating a very good clinical performance. For women with ASC-US or LSIL, the clinical sensitivity and specificity values for CIN2+ were 97.4% (37/38; 95%CI 83.5–99.6) and 59.8% (52/87; 95%CI: 49.2–69.5), respectively.[14]

* Performance characteristics are indicated for test version ABI7500. Equivalence analysis demonstrated similar performance and validation for QIAscreen HPV PCR Test for the Rotor-Gene Q MDx 5plex HRM.

Reproducibility*

The intra-laboratory reproducibility and inter-laboratory agreement of the test were validated according to the international guidelines for HPV test requirements for cervical cancer screening (9). The intra-laboratory reproducibility on cervical specimens (scrapes) over time was 99,5% (544/547) with a kappa value of 0.99 and the inter-laboratory agreement was 99,2% (527/531) with a kappa value of 0.98, indicating very good agreement (10).

Performance on self-collected (cervico-)vaginal specimens*

The performance of the test in self-collected (cervico-)vaginal specimens has been validated for two different sampling methods: 1) self-collected lavage specimens, and 2) self-collected brush specimens. For self-collected lavage specimens, the agreement with the reference assay GP5+/6+ PCR was 96.7% (59/61) with a CIN 2+ sensitivity of 91.4% (21/23) (10). For self-collected brush specimens, the agreement with GP5+/6+ PCR was 92.9% (104/112) with a CIN 2+ sensitivity of 93.9% (31/34) (10).

Interfering substances*

Traces of EDTA (0.5M), HCl (1N), Silica beads (1 µL), Blood (1 µL), Ureum (40 g/100 mL), and lysis buffer inhibited the performance of the test. ETOH 96% (1 µL) and DMSO 4 % (v/v) had no inhibitory effect on the performance of the test. Inhibition is monitored by the sample control (e.g., β-globin target).

* Performance characteristics are indicated for test version ABI7500. Equivalence analysis demonstrated similar performance and validation for QIAscreen HPV PCR Test for the Rotor-Gene Q MDx 5plex HRM.

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

		Comments and suggestions
Sample is scored invalid: the amplification of β-globin is too low or absent		
a)	Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q MDx instruments with 72-tube rotor" on page 20	Check pipetting scheme and the reaction setup. Repeat the sample.
b)	Check the DNA eluate	Repeat DNA extraction.
Positive control is scored invalid: the amplification is too low or absent for one or more of the targets		
a)	Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q MDx instruments with 72-tube rotor" on page 20	Check pipetting scheme and the reaction setup. Repeat the sample.
b)	Partial degradation	Store kit contents at –15 to –30°C. Avoid repeated freezing and thawing to a maximum of five cycles.
c)	PCR reagents partially degraded	Store kit contents at –15 to –30°C and keep the reaction mixes protected from light. Avoid repeated freezing and thawing.
d)	Strip tube inversion	Check the pipetting scheme and the reaction setup.
e)	Expiry date	Check the expiry date of the used kit.
f)	Time-delay between pipetting samples and start of the run	The PCR mixes can be stored 30 minutes between pipetting samples in the PCR and start of the run in the machine at 2-8°C in the dark.

		Comments and suggestions
No template control (NTC) is invalid		
a)	Pipetting error or omitted reagents. See "PCR on Rotor-Gene Q MDx instruments with 72-tube rotor" on page 20	Check pipetting scheme and the reaction setup. Repeat the sample.
Absent or low signals in sample, but the control run ok		
a)	Inhibitory effects	Always check if there are no remains of buffers during DNA extraction. Repeat DNA extraction.
b)	Pipetting error. See "PCR on Rotor-Gene Q MDx instruments with 72-tube rotor" on page 20	Check pipetting scheme and the reaction setup. Repeat the PCR run.

If the problem persists, contact QIAGEN Technical Service.

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
	Use by
IVD	In vitro diagnostic medical device
CE	CE-IVD marked symbol
REF	Catalog number
LOT	Lot number
MAT	Material number
COMP	Components
CONT	Contains
NUM	Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number
GTIN	Global Trade Item Number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Keep away from sunlight
	Consult instructions for use
	Caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
QIAscreen HPV PCR Test	For 72 reactions, includes: Master Mix, Positive Control, Negative Control, Instructions for Use	617005
QIAsymphony SP	QIAsymphony sample prep module (optional for extraction)	9001297
Rotor-Gene Q MDx		
Rotor-Gene Q MDx 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: includes 1-year warranty on parts and labor, installation and training	9002035
Rotor-Gene Q MDx 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: includes 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx Accessories		
Loading Block 72 x 0.1 mL Tubes	Aluminum block for manual reaction set up with a single-channel pipet in 72 x 0.1 mL tubes	9018901

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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
R2, August 2018	Updated Warnings and Precautions section; Added CellSolutions® in Specimen Storage and Handling section and Trademaks; Revised Sample Preparation section to replace fraction representations with percentages; Updated Protocol: QIAscreen HPV PCR Test for RGQ MDx; Revised column 3 of Table 1 in Protocol: QIAscreen HPV PCR Test for RGQ MDx; Updated PCR on RGQ MDx with 72-tube rotor section to add Important note and change New experiment to New Run window; Updated Performance Characteristics section; Corrected catalog number for QIAscreen HPV PCR Test; Layout updates
R3, June 2023	Updated the specimen storage and handling section; Updated the sample preparation section with pretreatment of samples stored in SurePath and instructions for DNA extraction with the QIAamp DSP Virus Spin kit and DNA extraction with the QIAsymphony using the QIAsymphony DSP Virus/Pathogen Midi kit; Updated the clinical performance for samples stored in SurePath and added the reference for the validation of samples stored in SurePath.

Self-screen B.V. is the legal manufacturer of the QIAscreen HPV PCR Test.

The QIAscreen HPV PCR Test is manufactured for QIAGEN by Self-screen B.V.