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QIAscreen HPV PCR Test Instructions for Use (Handbook)



Version 1



For in vitro diagnostic use

For use with Rotor-Gene® Q MDx instrument



617005



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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 or 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 (9, 10).

Principle of the Procedure

The QIAscreen HPV PCR Test is a multiplex, real-time PCR-based assay directed against the E7 gene of 15 (probably) 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 |
| Catalog no. | | 617005 |
| Number of reactions | | 72 |
| 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® MinElute® Media Kits and QIASymphony® DSP Virus/Pathogen Kits (QIAGEN, cat. no. 57414 or cat. no. 937036)

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 μ l; 10–100 μ l)
- Dedicated filter-plugged sterile DNase-free pipette-tips
- Disposable gloves
- Benchtop centrifuge*
- Vortex mixer*

Equipment for real-time PCR

- 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”.

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

[†] 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 “mmyynn”, 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.

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- 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 5-plex 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 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

| | |
|---|--|
| <p>CAUTION</p>  | <p>All specimens must be treated as potentially infectious material.</p> |
|---|--|

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.

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. Samples in PreservCyt collection medium may be stored at 2–8°C for no more than 3 months.

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 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 MinElute Media Kits and QIAasymphony DSP Virus/Pathogen Kits) are compatible with this assay.

For cervical specimens (scrapes) suspended in Surepath, PreservCyt, CellSolutions, or PathTezt collection medium, the fraction of DNA to be used as input in the PCR represents 0.25% of the 10 ml Surepath or CellSolutions sample or 0.125% of the 20 ml PreservCyt or PathTezt cervical scrape sample. This corresponds with 25 μ l of the sample types. Since at maximum only 5 μ l of extracted DNA can be used as input in the PCR, DNA extraction procedures should be executed such that 5 μ l DNA extract corresponds with 25 μ l 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.

For self-collected vaginal brush specimens suspended in Hologic PreservCyt Solution, DNA extraction procedures should be executed, such that 5 μ l 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 μ l input DNA corresponds with 10 μ l 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 μ l input DNA corresponds with 15 μ l 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 5-plex 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 5-plex 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) were validated by a non-inferiority analysis relative to high-risk HPV GP5+/6+ PCR following international guidelines for HPV test requirements for cervical cancer screening (9). The clinical sensitivity for CIN 2+ was 96.8% (61/63) and the clinical specificity for CIN 2+ was 95.1% (783/823). The clinical sensitivity and specificity were non-inferior to that of the reference assay GP5+/6+ PCR (10), 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 5-plex 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 5-plex 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 17 | 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 17 | 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 $2-8^{\circ}\text{C}$ 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 17 | Check pipetting scheme and the reaction setup. Repeat the sample. |
| b) | Cross-contamination | Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination. |
| c) | Reagents contaminated | Replace all critical reagents. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination. |
| d) | Strip tube inversion | Check the pipetting scheme and the reaction setup. |
| e) | Time-delay between pipetting samples and start of the run | The PCR mixes can be stored 30 minutes between preparation of the mixes and start of the run in the machine at 2-8°C in the dark. |
| f) | Probe degradation | Keep reaction mixes protected from light. Check for false positive on the fluorescence curve. |

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 17 | 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 |
|  | In vitro diagnostic medical device |
|  | CE-IVD marked symbol |
|  | Catalog number |
|  | Lot number |
|  | Material number |
|  | Components |
|  | Contains |
|  | Number |
| Rn | R is for revision of the Instructions for Use (Handbook) and n is the revision number |
|  | 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 |
| 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 2019 | Updated Warnings and Precautions section; Added CellSolutions® in Specimen Storage and Handling section and Trademarks; 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 |

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