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digene® HPV Genotyping LQ Test, Detection Kit Handbook

Σ 96

Version 1

IVD

For detection of 18 high-risk human papillomavirus (HPV) genotypes using xMAP® technology

CE

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Contents

Kit Contents

***digene* HPV Genotyping LQ Test, Detection Kit**			**(96)**
Catalog no.			**613215**
Number of reactions			**96**
B	High-Risk HPV Beads		4.6 ml
S1	Solution 1	CONT ProClin 300	50 ml
S2	Solution 2	CONT ProClin 300	50 ml
C	Detection Conjugate, 500x		20 µl
3B	3B Buffer		400 µl
	Filter Plate (96 wells)		1
	Transparent Seals for Filter Plate		4
	Handbook	HB	1

Symbols

Contains sufficient for <N> tests

In vitro diagnostic medical device


European conformity

Catalog number


Manufacturer

Batch code

Material number


Important note


Temperature limitation


Use by


Consult instructions for use

Storage

All reagents in the *digene* HPV Genotyping LQ Test, Detection Kit should be stored immediately upon receipt at 2–8°C. Make sure to store the kit away from any source of contaminating DNA, especially amplified DNA products. All reagents are stable until their expiration date under these conditions. High-Risk HPV Beads (B) are stable until the expiration date, when stored at 2–8°C. Store the High-Risk HPV Beads (B) in the dark and do not freeze!

Intended Use

The *digene* HPV Genotyping LQ Test, Detection Kit is an in vitro hybridization assay using GP5+/6+ primers for the qualitative identification of 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 individual

High-Risk HPV types. The use of the test is indicated as a reflex test for women with a positive result obtained with the *digene* HC2 High-Risk HPV DNA Test.

Product Use Limitations

The test has been validated for use with specimens collected in Specimen Transport Medium (STM) and Hologic® ThinPrep® Pap Test PreservCyt® Solution. The product should be used by personnel trained in PCR techniques only. The recommendations on laboratory design and procedures must be followed to avoid false results and DNA contamination.

The hybridization, wash step with Solution 1 (S1), and the conjugate incubation in the procedure should be performed at exactly 50°C to prevent false positive or false negative results.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the *digene* HPV Genotyping LQ Test, Detection Kit is tested against predetermined specifications to ensure consistent product quality.

The *digene* HPV Genotyping LQ Test, Detection Kit contains a conjugate control (bead type 23) that may be used for the addition of reactive conjugate during the incubation procedure. It must always be positive and should have approximately the same intensity in each test well in the same test run. If this control has a signal below 100 Median Fluoresence Intensity (MFI), the test is invalid. The probe on bead type 44 is used as the internal control. It reacts with the co-amplified human genomic DNA present in the clinical sample. It is used as an internal control to check for PCR inhibition, adequate sample taking, and/or DNA isolation. The presence of a signal higher than or equal to 50 MFI (no background subtraction) is considered to be a positive reaction. If HPV is present in the sample, the beta-globin probe can be negative due to PCR competition as the HPV DNA is preferentially amplified

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the *digene* HPV Genotyping LQ Test, Detection Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact

us if you have any suggestions about product performance or new applications and techniques.

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

Safety Information

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

The *digene* HPV Genotyping LQ Test consists of 2 kits: the *digene* HPV Genotyping LQ Test, Amplification Kit and the *digene* HPV Genotyping LQ Test, Detection Kit. The *digene* HPV Genotyping LQ Test, Detection Kit enables easy and reliable identification of high-risk human papillomavirus (HPV) genotypes by hybridization, using xMAP technology on the LiquiChip® System. PCR products used in the reverse hybridization procedure are generated from template DNA by amplification of the highly conserved L1 sequence using the *digene* HPV Genotyping LQ Test, Amplification Kit.

Principle

xMAP technology on the LiquiChip System

The QIAGEN LiquiChip System is a flexible system for suspension arrays that uses bead-based xMAP technology. A wide variety of assay types, such as immunoassays, kinase enzyme assays, and interaction assays are performed in an aqueous, homogeneous format, both quickly and efficiently. Multiplexing of assays offers the potential for the simultaneous detection of up to 100 different analytes within a single sample.

With xMAP technology, molecular reactions take place on the surface of color-coded beads. For each pathogen, target-specific capture probes are covalently linked to a specific set of color-coded beads. Labeled PCR products are captured by the bead-bound capture probes in a hybridization suspension. A microfluidics system delivers the suspension hybridization reaction mixture to a dual laser detection device. A red laser identifies each bead (or HPV probe) by its color-coding, while a green laser detects the hybridization signal associated with each bead (indicating the presence or absence of a particular amplimer).

Identification of HPV genotypes is based on a reverse hybridization procedure, using xMAP technology. Denatured biotinylated amplimers, resulting from amplification of part of the L1 region with the GP5+/6+ primer set, are hybridized with specific oligonucleotide probes, which are immobilized on specific types of beads (Table 1, page 10). After hybridization and stringent washing, streptavidin-conjugated R-Phycoerythrin is added, which binds to any biotinylated hybrid present. After incubation and additional stringent washing, the samples can be analyzed on the LiquiChip System (see flowchart on the next page). As an internal control for the presence of amplifiable DNA after isolation, a fragment from the human beta-globin gene is coamplified with the HPV DNA in the form of a multiplex PCR. Bead type 44 contains a probe capable of detecting the beta-globin amplimer.

digene HPV Genotyping LQ Detection Procedure

Hybridization product in PCR plate

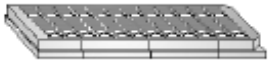
Add 3B buffer (3B), High-Risk HPV Beads (B), and amplified biotinylated product to PCR plate

Denaturation at 95°C followed by hybridization at 50°C

Add hybridization product from PCR plate to 96-well Filter Plate containing Solution 1 (S1) at 50°C

Vacuum

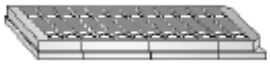
Stringent wash at 50°C (wash 1)



Filter plate

Dispense freshly prepared Conjugate solution at 50°C and incubate at 50°C

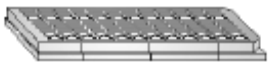
Vacuum



Dispense Solution 2 (S2) into each well at room temperature (15–25°C)

Vacuum

Wash at room temperature (wash 2)



Dispense Solution 2 (S2) into each well at room temperature and analyze on the LiquiChip System

Table 1. List of beads and coupled HPV genotyping probes

Bead type	23	24	25	26	27	28	29	30	31	32
HPV probe	CC*	16	18	26	31	33	35	39	45	51

* CC = conjugate control.

Bead type	33	34	35	36	37	38	39	40	41	42	43	44
HPV probe	52	53	56	58	59	66	68	68	73	82	82	beta-globin

Performance characteristics

Analytical sensitivity

Formal Limit of Detection (LOD) determinations have been carried out for HPV 16, HPV 18, and HPV 45. The detection limits are shown in Table 2, below.

Table 2. Analytical sensitivity Limit of Detection (LOD) for HPV 16, 18, and 45

Genotype	**LOD concentration (copies/PCR)**
HPV16	5
HPV18	8
HPV45	20

To assess the analytical sensitivity of the multiplex GP5+/6+/beta-globin PCR for HPV31, 35, 39 and 52, the testing consisted of 2 runs of 4 dilutions in duplicate.

To assess the analytical sensitivity of the multiplex GP5+/6+/beta-globin PCR for HPV26, 33, 51, 53, 56, 58, 59, 66, 68 (+ 68a subtype), 73 and 82 (MM4 and IS39), the testing consisted of 1 run of at least 4 dilutions. For the

remaining 15 types, dilutions were analyzed in replicates and the concentration with 100% positive result was determined. The results are shown in Table 3.

Table 3. Sensitivity Limit of Detection (LOD) concentration as a function of genotype

Type	Detection limit in copies per PCR	Testing method
HPV16	5	Full LOD
HPV18	8	
HPV45	20	
HPV31	10	Limited LOD
HPV35	10	
HPV39	100–1000	
HPV52	100–1000	
HPV26	100	Single dilution series
HPV33	1	
HPV51	100	
HPV53	10,000	
HPV56	10	
HPV58	100	

Table continued on next page.

Table 3. Continued

Type	Detection limit in copies per PCR	Testing method
HPV59	100	Single dilution series
HPV66	10	
HPV68	10,000	
HPV68a	1000	Single dilution series
HPV73	100	
HPV82MM4	1000	
HPV82IS39	100	

Practical analytical specificity

Amplimers from the 18 HPV genotypes and 2 subtypes targeted in the assay (i.e., HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 [68a], 73, 82MM4 [82IS39]) showed a correct result by the *digene* HPV Genotyping LQ Test, Detection Kit result, thereby confirming the identification of the correct HPV type. High amounts of amplimer (obtained from 10,000,000 HPV copies per PCR from HPV [sub] types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 56, 58, 59, 61, 66, 67, 68 [and 68a], 69, 71, 72, 73, 81, 82 [MM4 and IS39]) were used to trace any cross-reactivity. None of the probes showed any reaction with an amplimer from a non-targeted HPV type.

The specificity of the *digene* HPV Genotyping LQ Test, Detection Kit is ensured by the selection of the probes, as well as the selection of stringent reaction conditions. The probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. The detectability of all sequences of relevant strains has thus been ensured. In addition microorganisms commonly found in the female anogenital tract have been analyzed.

None of the tested pathogens has been reactive (see Table 4). The presence of these microorganisms is not reducing the sensitivity to detect the high-risk HPV genotypes.

Table 4. Potentially cross-reactive pathogens

Pathogen
Acinetobacter anitratus
Acinetobacter lwoffi
Bacteroides fragilis
Escherichia coli (HB101)
Escherichia coli
Klebsiella pneumoniae
Lactobacillus acidophilus
Mobiluncus curtisii
Mobiluncus mulieris

Precision

A panel of 10 different samples containing plasmids of all 20 HPV genotypes and subtypes covered by the assay was tested in triplicate at 10 times the estimated detection limit on 3 different days by 2 technicians and on another day using two different kit lots, resulting in a total of 24 data points per sample. All genotyping results were identical.

Additionally, DNA purified from 92 samples was tested by 2 technicians using 3 kit lots. Results were scored as either identical (100% matching genotypes), compatible (at least one genotype in common), or discordant (no matching genotypes). Between the 2 technicians, the identical/compatible agreement was between 98 and 100%. When comparing the different kit lots used by both technicians (i.e., comparing all six outcomes), the identical/compatible agreement was 95%.

Finally, a selection of 25 samples from the panel mentioned above was tested in duplicate by 2 technicians (inter-technician testing) at a second testing site (inter-laboratory testing). Results were again scored as identical (100% matching genotypes), compatible (at least one genotype in common), or discordant (no matching genotypes). Both technicians reached 96% identical

agreement (23/25 samples) in the duplicate testing, with 1 sample showing a compatible genotyping result.

Between the 2 technicians, the identical agreement was 88% and the compatible agreement was 12%. No discordant results were observed. When comparing all 10 genotyping results from each of the 25 samples in the inter-laboratory testing panel (first and second site), the identical/compatible agreement was 100%. This comprised 72% identical results and 28% compatible results. No discordant results were seen.

In summary, to assess the closeness of agreement between measurements, a series of artificial samples containing all HPV types covered by the assay plus 92 clinical samples was tested by multiple sampling. Each sample was assayed in replicates on different days by different technicians. Also, different lots were tested. The results showed 100% of identical genotypes in artificial samples and more than 95% identical and compatible results for clinical samples.

Accuracy

Aliquots of DNA purified from a total of 108 clinical samples comprising 50 HC2-positive specimens collected in STM, 50 HC2-positive specimens collected in PC Solution, and 8 HC2-negative specimens collected in STM were tested with the *digene* HPV Genotyping LQ Test, Detection Kit and the Free University reverse line blot (RLB) system (1).

Results were scored as either identical (100% matching genotypes), compatible (at least one genotype in common) or discordant (no matching genotypes). Discrepancies (discordant genotyping results) were resolved by repeating both assays and, in case of remaining discrepancies, by subsequent analysis of the sample with SPF10-LiPA$_{25}$ (version 1).

The comparison yielded 85% identical genotyping results, 8% compatible genotyping results, and 7% discordant genotyping results. Repeating both assays resolved 4 of the 7 discrepant samples. Subsequent analysis with the SPF10-LiPA$_{25}$ resolved another discrepant sample leaving only 2 samples discrepant. In the first sample, HPV45 was detected by the *digene* HPV Genotyping LQ Test whereas the RLB was negative. In the second sample, HPV58 was detected with the RLB and the *digene* HPV Genotyping LQ Test, Detection Kit was negative.

To assess the closeness of agreement, the *digene* HPV Genotyping LQ Test, Detection Kit was tested against the HPV RLB assay, an accepted reference value, using 108 clinical samples tested in parallel. The results were scored as identical, compatible, or discordant. Discordant samples were resolved by analysis with the SPF10-LiPA$_{25}$. The results showed a very low level of discrepant samples (2%) after resolution of initial discrepant samples (see Table 5).

Table 5. Comparison of *digene* HPV Genotyping LQ Test, Detection Kit against HPV RLB assay

Accuracy Analysis	% of Clinical Samples
Identical	85
Compatible	12
Discrepant	2

Robustness

The capacity of the *digene* HPV Genotyping LQ Test, Detection Kit to remain unaffected by deliberate variations in relevant method parameters was assessed as follows. Duplicate samples of HPV genotypes 16, 18, 45, and 52 at 10 times the detection limit of the PCR were amplified with PCR conditions 1.0°C higher or 1.0°C lower. HPV detection by the *digene* HPV Genotyping LQ Test, Detection Kit was not influenced as compared to normal cycling conditions.

A series of amplimers generated from all 18 HPV genotypes and two subtypes performed at 10 times the approximate detection limit was tested at hybridization temperatures at the upper and lower limits of the thermomixer specifications (± 2.0°C). Testing at maximum specified temperature limits of 52.0°C and 48.0°C did not change the genotyping results.

A series of amplimers generated from all 18 HPV genotypes and two subtypes performed at 10,000,000 HPV copies per PCR was tested at hybridization temperatures at the upper and lower limit of the thermomixer specifications (± 2.0°C). Testing at maximum specified temperature limits of 52.0°C and 48.0°C did not change the genotyping results. No cross-reactions were observed.

Interfering substances

The effect of blood and other potentially interfering substances (see Table 6) was evaluated in the *digene* HPV Genotyping LQ Test, Detection Kit. The presence of these substances does not cause false-positive results. In addition, the presence of these substances does not cause a reduced sensitivity to the HPV viruses.

Table 6. Interfering substances

Substance
Blood
Moisturizer
Hemorrhoidal anesthetic
Talcum Powder
Anti-fungal cream
Vaginal lubricant

Diagnostic evaluation

The *digene* HPV Genotyping LQ Test was compared with the established Free University RLB system (1) in a multicenter study.

For this purpose, 567 *digene* HC2 High Risk HPV DNA Test positive cervical scrapes were selected. The samples had been collected in STM and PreservCyt medium and DNA was isolated either manually with the QIAamp® MinElute® Virus Spin Kit or automated on the EZ1 Advanced with the EZ1® DSP Virus Kit.

Of the 567 cervical samples, 552 of the HC2 positives samples were also positive for one of the 18 HR HPV types by digene HPV Genotyping LQ Test.

Assay common genotypes were compared and results were scored as either identical (100% matching genotypes), compatible (at least one genotype in common), or discordant (no matching genotypes).

The percentage of identical/compatible agreement between the genotyping assays was 96.3 % (546 of 567 samples) with 80.0 % identical and 16.2 % compatible results (see Table 7). 4.5 % (21 of 567 samples) of the genotyping results were discordant.

Table 7. Comparison of genotyping findings between the *digene* HPV Genotyping LQ Test and the established Free University RLB system in 567 HC2-positive cervical smears.

Agreement	N=567 (%)
Identical typing result	454 (80.0)
Compatible typing result	92 (16.2)
Discordant typing result	21 (3.7)

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 material safety data sheets (MSDSs), available from the product supplier.

- Distilled or deionized water
- Sheath fluid (for example, LiquiChip System Fluid, cat. no. 922902 or Luminex® Sheath Fluid cat. no. 40-50000; see www.luminexcorp.com)*
- Pipets† and disposable sterile pipet tips with filters (recommended) (1–20 µl, 20–200 µl, 50–200 µl and 200–1000 µl)†
- 96-well PCR plate
- LiquiChip 200 Workstation (MDD), cat. no. 9001382, or Luminex 100 or 200 IS Total System†
- Multichannel pipet†
- Multistepper pipet and tips (for example, Gilson Repetman®; see www.gilson.com)*†
- Sonicator bath†
- Vacuum pump and plate manifold (for example, Millipore MultiScreen Vacuum Manifold; see www.millipore.com; QIAvac Vacuum Manifold and Vacuum pump (cat. no. 84010), see www.qiagen.com)*†
- Vortexer†
- Waterbath (with cover to protect the assay from light; capable of attaining 50 ± 0.5°C)†
- Incubator (with door to protect the assay from light; capable of attaining 50 ± 0.5°C) or microplate heater†
- Thermal cycler (for example, ABI™ GeneAmp® PCR System 9700; see www.appliedbiosystems.com)* †
- Calibrated thermometer
- Laboratory timer, 15 minutes (±1 minute)†
- Heater Block, XYP (Luminex, cat. no. CN-0017-01; see www.luminexcorp.com)*
- Caps or seal for 96-well PCR plate

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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

- Conical tubes for conjugate preparation (1 ml, 5ml, or 10 ml)
- Tissues
- Thermomixer, Eppendorf Thermomixer® Comfort and Adapter for MTP (Mictotiter Plates) and Adapter plate for 96 x 0.2 ml PCR tubes[††]

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

Important Notes

Preparing reagents

Conjugate Solution

To obtain a working solution of conjugate, dilute Detection Conjugate, 500x (C), 1/500, in Solution 1 (S1) preheated to 50°C. Prepare 75 µl conjugate solution for each test, with an excess of 2 tests for every per 8 tests (see Table 8). Make sure to use a new, clean, vessel. Diluted conjugate solution must be prepared just prior to use, and briefly mixed by vortexing. Place in a waterbath preheated to 50°C until use.

Table 8. Preparation of conjugate solution

No. of tests	Volume of Solution 1 (S1) (µl)	Volume of Detection Conjugate, 500x (C) (µl)	Total volume (µl)
1	100	0.20	100.0
8	750	1.5	751.5
24	2250	4.5	2254.5
96	9000	18.0	9018.0

Analysis of multiplex results

To use the *digene* HPV Genotyping LQ Test, Detection Kit, the QIAplex® MDD Software must be updated with the QIAplex MDD HPV Genotyping LQ Software. To update your QIAplex MDD Software, run the MDD software installer included on the QIAplex MDD HPV Genotyping LQ Software CD.

For technical help call QIAGEN Technical Services (see back cover of QIAGEN handbooks and user guides).

Protocol 1: Preparation of the Filter Plate

Important points before starting

- Do not use reagents that have passed their expiration date.
- Do not mix reagents from different lot numbers.

Things to do before starting

- Allow all reagents and the High-Risk HPV Beads (B) to equilibrate to room temperature (15–25°C) before use.
- Set a waterbath to 50°C. Make sure that the temperature of the water is 50 ± 0.5°C. Check the temperature using a calibrated thermometer.
- Place Solution 1 (S1) in the waterbath at 50°C for at least 30 minutes.
- Set an incubator to 50°C. Make sure that the temperature of the incubator is 50 ± 0.5°C. Check the temperature using a calibrated thermometer.
- Place the Heater Block, XYP, in the incubator preheated to 50°C.
- Set the thermomixer to 50°C for at least 10 minutes before use.

Procedure:

1. **Check that the Heater Block, XYP, is placed in the incubator preheated to 50°C ± 0.5°C.**
2. **To equilibrate the Filter Plate, dispense 200 µl of water into each test well of the Filter Plate.**
3. **Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold. Dry the bottom of the Filter Plate using clean tissue.**
4. **Repeat steps 2 and 3.**

   ⓘ It is essential that the bottom of the Filter Plate is dry to prevent the next solution (Solution 1 [S1]) from immediately flowing through the Filter Plate.
5. **Dispense 200 µl of Solution 1 (S1) preheated to 50°C into each test well. Place the Filter Plate on the Heater Block, XYP, in the incubator preheated to 50°C. Keep the Filter Plate in the incubator until use.**

Protocol 2: Hybridization and Denaturation

Important points before starting

- Do not use reagents that have passed their expiration date.
- Do not mix reagents from different lot numbers.
- The vial containing High-Risk HPV Beads (B) should be closed immediately after use; prolonged exposure to light could lead to poor bead classification.
- Place all reagents and the High-Risk HPV Beads (B) at 2–8°C immediately after use.
- Prevent prolonged exposure of the tests including beads or detection conjugate to light. Prolonged exposure will lead to poor bead classification and/or conjugate deterioration.
- Protect the PCR plate and Filter Plate containing beads from direct light, but take care to avoid bad heat transfer (for example, do not wrap the plates in aluminum foil).
- Ensure that the temperature from the hybridization procedure until the second wash procedure does not drop below 50°C.

Procedure

1. **Dispense 4 µl of 3B Buffer (3B) into each well of a 96-well PCR plate (not supplied) for the required number of tests.**
2. **Before each use, gently vortex the vial of High-Risk HPV Beads (B) for 5 s. Next, sonicate the vial of beads for 20 s. Then, gently vortex the vial of beads again for 5 s.**
3. **Dispense 46 µl High-Risk HPV Beads (B) into each well of the 96-well PCR plate containing 3B Buffer (3B) (3B Buffer together with High-Risk HPV Beads (B) are referred to as "hybridization mix").**
4. **Add 4 µl of biotinylated PCR product, amplified using the *digene* HPV Genotyping LQ Test, Amplification Kit, to each well of the 96-well PCR plate containing hybridization mix. Use a fresh pipet tip with filter for each sample. Mix gently by pipetting up and down a few times.**
5. **Seal the wells of the 96-well PCR plate using caps or a seal.**

6. **To denature the biotinylated PCR amplicons and enable hybridization to single-stranded oligo probes on the High-Risk HPV Beads (B), place the 96-well PCR plate in a thermal cycler (preferably with a heated lid with pressure) and program the thermal cycler as follows:**
   - **Heat to 95°C with maximum ramping speed**
   - **3 min at 95°C**
   - **Cool to 60°C with maximum ramping speed**
   - **Maintain indefinitely at 60°C**

   This step enables complete denaturation of the biotinylated PCR amplicons and efficient hybridization to single-stranded oligo probes on the High-Risk HPV Beads (B).
7. **When the thermal cycler has cooled down to 60°C, immediately place the 96-well PCR plate in the thermomixer and hybridize at 50°C for 15 min at 500 rpm.**

   ⓘ It is important to immediately transfer the 96-well PCR plate in order to prevent the temperature from dropping below that required for hybridization. If the temperature drops below the hybridization temperature, this may result in nonspecific binding.

   ⓘ It is also important to immediately transfer the 96-well PCR plate when the thermal cycler has cooled down to 60°C in order to prevent bead sedimentation. If beads sediment too long, this may result in low signals.
8. **During the last 3 min of the hybridization incubation, prepare a fresh working solution of conjugate (see Table 8, page 20). Place the diluted conjugate solution in the waterbath at 50°C and keep it there until ready to use. Protect the diluted conjugate solution from light.**
9. **With the 96-well PCR plate still in the thermomixer, transfer the entire volume of sample–hybridization mix (54 µl) from each well of the 96-well PCR plate to the Filter Plate containing 200 µl Solution 1 (S1) preheated to 50°C. During this step, make sure the Filter Plate remains on the preheated Heater Block, XYP.**

Protocol 3: Wash 1

Important points before starting

- Apply gentle vacuum to avoid sticking of the beads in the filter, resulting in a lowered bead count.
- After aspiration using the vacuum manifold, the bottom of the Filter Plate must be dried using clean tissue. It is essential that the bottom of the Filter Plate is dry otherwise the next solution will immediately flow through the Filter Plate. Do not dry by wiping, but by pressing the plate on the tissue. Do not leave the Filter Plate on the tissue when adding the next solution. Place it on a flat bench otherwise the dispensed solution will immediately flow through.

Procedure

1. **Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold.**

   The bead-bound targets will remain in the Filter Plate and the liquid will flow through.

2. **Dry the bottom of the Filter Plate using a clean tissue.**
3. **Return the Heater Block, XYP, to the incubator set to 50°C for later use.**
4. **Dispense 100 µl of Solution 1 (S1) preheated to 50°C into each well of the Filter Plate. Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold.**
5. **Dry the bottom of the Filter Plate using a clean tissue.**

Protocol 4: Conjugate Incubation

Procedure

1. **Dispense 75 µl of conjugate solution preheated to 50°C into each test well (freshly prepared in step 8, "Protocol 2: Hybridization and Denaturation."**
2. **Incubate the Filter Plate at 50°C in the thermomixer at 500 rpm for 2 min. Protect the Filter Plate from light.**
3. **Place the Filter Plate on the preheated Heater Block, XYP, and incubate in the incubator at 50°C for 13 min. Protect the Filter Plate from light.**
4. **Remove the Filter Plate from the incubator. Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold.**
5. **Dry the bottom of the Filter Plate using a clean tissue.**

Protocol 5: Wash 2

Procedure

1. **Dispense 100 µl of Solution 2 (S2) [at room temperature (15–25°C)] into each well of the Filter Plate. Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold.**
2. **Dry the bottom of the Filter Plate using a clean tissue.**
3. **Dispense 100 µl of Solution 2 (S2) (room temperature) into each well. Place the Filter Plate onto the vacuum manifold and switch on the vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum and remove the Filter Plate from the vacuum manifold.**
4. **Dry the bottom of the Filter Plate using a clean tissue.**
5. **Dispense 100 µl Solution 2 (S2) (room temperature) into each test well.**

Protocol 6: Readout

Procedure

1. **Incubate the Filter Plate at a temperature between room temperature (15–25°C) and 50°C in the thermomixer at 500 rpm for 1 min. Protect the Filter Plate from light until use in step 2.**

   ⓘ Thorough mixing is essential to ensure resuspension of the High-Risk HPV Beads (B).

2. **Place the Filter Plate on the XY-platform of the LiquiChip System and start analysis. QIAplex MDD HPV Genotyping LQ Software will allow for specimen entry and data analysis. Reference the QIAplex MDD HPV Genotyping LQ Software Manual for details. For analysis directly using the LiquidChip IS software use the settings listed in Table 1and Table 9. For more information, see "Appendix A: Interpretation of Results," page 32.**

Table 9. Settings required for analysis on the LiquiChip System

Item	Setting
Sample volume (µl)	50
Sample time-out (s)	60
Minimal counts/bead type	100
DD gate low	8000
DD gate high	18500
Data type	Median MFI

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Long measuring time (>60 s)	
a) No appropriate settings on the LiquiChip System	ⓘ Enter the appropriate settings for the LiquiChip System; see Tables 1 and 9.
b) Not enough Solution 2 (S2) in test well	ⓘ Switch on vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum. Repeat procedure from "Protocol 5: Wash 2."
c) High-Risk HPV Beads (B) not properly mixed	ⓘ Repeat the test. Use the same amplified material.
d) Sampling needle clogged	ⓘ Clean the needle, aspirate, and repeat from "Protocol 5: Wash 2."
Weak (<100 MFI) or no signals within a test series including the conjugate control	
Incorrect amount of conjugate solution used	ⓘ Switch on vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum. Repeat the procedure from "Conjugate incubation," using the same series of test wells. Prepare freshly diluted conjugate solution.
High background signal (overall 10–100 MFI)	
a) Excess conjugate not properly washed off	ⓘ Switch on vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum. Repeat the procedure from "Protocol 4: Conjugate Incubation."
b) Excess, unbound PCR product not properly washed off	ⓘ Switch on vacuum. Apply vacuum to the Filter Plate until transfer is complete. Switch off the vacuum. Repeat the procedure from "Protocol 3: Wash 1."

	Comments and suggestions
c) Inappropriate amount of 3B buffer (3B) in test well	ⓘ Repeat the test. Use the same amplified material.
d) Poor heat transfer during hybridization and/or conjugate incubation	ⓘ Repeat the test. Use the same amplified material.
Weak signals (<100 MFI) with all the bead types, except for the conjugate control	
a) Inappropriate amount of amplified material used for hybridization	ⓘ The concentration of amplified material is too low due to inefficient amplification. Check the amount of amplified product by running a 10 µl aliquot on a 2% agarose gel. The length of the amplified HPV product varies between 139 and 148 bp, depending on the HPV type. See "Inefficient amplification" below for more information. The beta-globin amplimer has a size of 258 bp, but will probably not be visible on gel.
b) Solution 1 (S1) was not correctly preheated	ⓘ Before use, warm Solution 1 (S1) to at least 50± 0.5°C in a waterbath. Make sure that the temperature does not exceed 50± 0.5°C.
c) Temperature exceeded 50°C during hybridization, wash 1, or conjugate incubation	ⓘ Make sure to set the waterbath and incubator to the correct temperature.
d) Thermal cycler incorrectly programmed for denaturation	ⓘ Check that the correct cycling conditions were used. If necessary, calibrate the thermal cycler.

Inefficient amplification

a) Too much DNA used	ⓘ Check the PCR by agarose gel electrophoresis. If too much DNA was added, a smear of DNA is often visible on the gel. Due to the low annealing temperature of the PCR some background bands on the gel are normal. Dilute the DNA and repeat the amplification by adding 10 times less DNA.
b) Insufficient amount of DNA used	ⓘ Repeat the amplification reaction (see digene *HPV Genotyping LQ Test, Amplification Kit Handbook*) and add more DNA.
c) Problems with DNA	ⓘ If necessary, repeat the DNA purification. Repeat the amplification reaction (see digene *HPV Genotyping LQ Test, Amplification Kit Handbook*).
d) Thermal cycler incorrectly programmed	ⓘ Check that the correct cycling conditions were used. If necessary, calibrate the thermal cycler.

False-positive signals

a) Temperature during hybridization, wash 1, or conjugate incubation too low	ⓘ Nonspecific signals can occur if the temperature for hybridization, wash 1, or conjugate incubation is too low. The temperature should be 50 ± 0.5°C. Make sure to set the temperature of the waterbath and of the incubator correctly. Always close the door of the incubator during incubation.
b) Too much DNA used	ⓘ Check the PCR by agarose gel electrophoresis. If too much DNA was added, a smear of DNA is often visible on the gel. Due to the low annealing temperature of the PCR, some background bands on the gel are normal. Dilute the DNA and repeat the amplification by adding 10 times less DNA.

c) Contamination	ⓘ If a similar nonspecific pattern occurs with most of the tests, contamination may be present. Contamination may be introduced during handling of clinical samples, DNA purification, or PCR setup. Therefore, repeat the amplification reaction using freshly prepared solutions and/or repeat the DNA purification procedure. If the Negative DNA Control result is negative but the samples remain positive for the HPV type, it is most likely that contamination has occurred during handling of the clinical samples.
d) Solution 1 (S1) not properly heated	ⓘ Before use, warm Solution 1 (S1) to at least 50± 0.5°C in a waterbath. Make sure that the temperature does not exceed 50± 0.5°C.
e) Thermomixer incorrectly programmed	ⓘ Check that the correct conditions were used. If necessary, calibrate the thermomixer.

Weak signals with the bead for beta-globin

The PCR was designed to optimally detect HPV DNA.	ⓘ It is possible that for some DNA preparation procedures or sample types the beta-globin signal will always be low and the samples will not necessarily be invalid. Please contact technical support.

Appendix A: Interpretation of Results

Positive result

The presence of a signal higher than or equal to 100 MFI (no background subtraction) is considered to be a positive reaction. The interpretation of the test result is directly linked to the probe name of the HPV type (for example, a signal with bead type 24, to which the HPV 16 probe is linked, indicates the presence of HPV type 16). Multiple HPV types can be present in a single sample.

Quality control

The *digene* HPV Genotyping LQ Test, Detection Kit contains a conjugate control (bead type 23) that may be used for the addition of reactive conjugate during the incubation procedure. It must always be positive and should have approximately the same intensity in each test well in the same test run. If this control has a signal below 100 MFI, the test is invalid The probe on bead type 44 is used as the internal control. It reacts with the co-amplified human genomic DNA present in the clinical sample. It is used as an internal control to check for PCR inhibition and adequate sample taking and/or DNA isolation. If HPV is present in the sample the beta-globin probe can be negative due to PCR competition, as the HPV DNA is preferentially amplified.

Table 10. Comments on results

Conjugate Control	HPV result	Beta-globin	Result	Comment
+	+	+	valid	
+	+	–	valid	HPV DNA is preferentially amplified, resulting in an absent beta-globin signal (PCR competition)
+	–	+	valid	

Table continued on next page.

Table 10. Continued

Conjugate Control	HPV result	Beta-globin	Result	Comment
–	+/–	+/–	In-valid	Repeat detection assay with the same amplimer
+	–	–	In-valid	Either the DNA purification or the PCR failed. Repeat the entire assay starting with DNA purification through PCR and reverse hybridization using the original sample. Possibly no cells were present in the cervical smear. If the same result is obtained, contact Technical Support.

Appendix B: Control of Contamination in PCRs

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination. For the negative control, only the conjugate control should be positive.

Recommendations for laboratory design and procedures

The following sequence of operations is recommended:

1. Preparation and aliquoting of PCR mixes
2. Preparation of samples (DNA isolation)
3. Amplification
4. Analysis of the biotinylated PCR products by xMAP technology

Personnel involved in steps 3 and 4 should not participate in subsequent work for steps 1 and 2 on the same day. Similarly, after being involved in step 2, personnel should not participate in subsequent work for step 1 on the same day.

To prevent contamination (for example, with amplification products) of specimens and to avoid false-positive results, the procedure should be performed in three physically separated rooms, each with its own set of supplies and pipets. One room is needed for reagent preparation, another for sample preparation, and a third room for amplification and PCR product detection. All equipment should be kept in the room where it is used and should not be transferred between rooms. Filter tips should be used for pipetting to minimize cross-contamination between specimens. In addition, wear disposable gloves and change them frequently.

Room 1: This room should be used only for storage and preparation of PCR reagents. This room and its equipment must be kept free of amplification products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room. Disposable gloves should be worn at all times.

Room 2: This room is used for sample preparation and must be kept free of amplification products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room. During sample preparation, disposable gloves should be changed frequently. Carefully uncap vials containing processed sample to avoid cross-contamination. Avoid opening more than one reaction vial containing sample at the same time.

Room 3: This room is used for amplification and detection of PCR products. Laboratory personnel should wear a clean laboratory coat, which must not be worn outside this room and must be changed daily. When working with amplification products, disposable gloves should be worn.

The *digene* HC2 High-Risk HPV Test and the *digene* HPV Genotyping LQ Test, Detection Kit can be performed in the same room. When doing so, perform the specimen processing, denaturation, and transfer to the hybridization plate for the HC2 Test prior to entering the HC2 and Genotyping LQ Detection testing laboratory (Room 3). This prevents the original specimen, which should be processed in Room 2, from exposure to amplification products used in Room 3.

In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with DNA AWAY and RNase AWAY (Molecular BioProducts) or a 1/10 dilution of a commercial bleach solution.*† Afterwards, the benches and pipets should be rinsed with RNase-free water.

General chemical precautions

We recommend storing PCR stock solutions in small aliquots and using fresh aliquots for each PCR.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp, or contact QIAGEN Technical Services or your local distributor.

Cited References

1. van den Brule, A. J., Pol R., Fransen-Daalmeije, N., Schouls, L. M., Meijer, C. J., and Snijders, P. J. (2002) GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol **40**, 779.

* Most commercial bleach solutions are approximately 5.25% sodium hypochlorite. Sodium hypochlorite is an irritant and should be handled with caution.

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

Ordering Information

Product	Contents	Cat. no.
digene HPV Genotyping LQ Test (96)	*digene* HPV Genotyping LQ Test, Amplification Kit, *digene* HPV Genotyping LQ Test, Detection Kit	613215
QIAplex® MDD HPV Genotyping LQ Software	Software package that accompanies the *digene* HPV Genotyping LQ Test, Detection Kit	9020145
Related products		
QIAamp MinElute Virus Spin Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
EZ1® DSP Virus Kit (48)	For 48 viral nucleic acid preps: Pre-filled Reagent Cartridges, Disposable Filter-Tips, Disposable Tip Holders, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724

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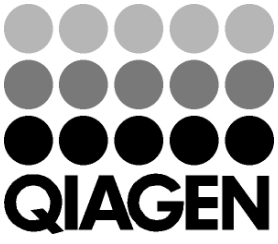

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