MehrViru HPV Genotyping

Based on Real-time PCR

Detection of 12 Oncogenic HPV types (High-risk), 7 Probable/Possible Carcinogen and 11 Non-Oncogenic HPV Types (Low-risk) from patient specimens in a single assay.

The 12 High-risk HPV targeted are:

16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.

The 7 Probable/possible High-risk HPV targeted are:

26, 53, 66, 67, 68, 73, and 82.

The 11 Low-risk HPV targeted are:

6, 11, 40, 42, 43, 44, 54, 61, 62, 89, 90.

Instructions for Use – English

24 Tests

Ref: 01, 07004, 24E, 30 HPV Genotype

For use with 4 channels (FAM, HEX, ROX, Cy5) real-time devices from various manufacturers.

RUO (Research Use Only)

Background

Human papillomavirus (HPV) infection is a well-Known cause of cervical cancer and there is growing evidence of HPV being a relevant factor in other anogenital (anus, vulva, vagina and penis) as well as head and neck cancers. There are more than 200 types of HPV, which can be classified into high and low-risk categories depending upon their oncogenic potentials.

Intended Use

The MehrViru HPV PCR Test is an in vitro real-time PCR-based assay for the qualitative detection of human papillomavirus (HPV) DNA of the following 30 HPV genotypes including 12 High-risk HPV, 7 Probable/possible High-risk HPV and 11 Low-risk HPV genotypes in cervical exfoliated cells, urogenital tract secretion and FFPE (Formalin Fixed Paraffin Embedded) tissue samples. The assay to be used by trained professionals in a laboratory environment. This kit can be used on the FAM(green), HEX(yellow), ROX(orange) and Cy5(red) channels platform (i.e. Rotor-Gene Q, MIC, Azure Cielo 6, Bio-Rad CFX96, QIAquant 5plex and My Go Pro).

Technical Specification

Target Sequence E6/L1 genes. Specificity 12 High-risk HPV targeted are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 7 Probable/possible High-risk HPV targeted are 26, 53, 66, 67, 68, 73, 82, 11 Low-risk HPV targeted are 6, 11, 40, 42, 43, 44, 54, 61, 62, 89, 90 types.

The **analytical Sensitivity** (LoD) reaches up to 1.05 IU/µl (on 1st WHO International standard for Human Papillomavirus Type 16 DNA) (with the probability of 95 %), reaches up to 1.12 IU/µl (on 1st WHO International standard for Human Papillomavirus Type 18 DNA), reaches up to 1.22 IU/µl (WHO International Standards for Human Papillomavirus (HPV) DNA genotypes HPV6, HPV11, HPV31, HPV33, HPV45, HPV52, HPV58 DNA) and reaches up to 1.25 IU/µl on plasmid DNAs containing (part of) the genome of an HPV35, 39, 51, 56, 59, 26, 53, 66, 67, 68, 73, 82, 40, 42, 43, 44, 54, 61, 62, 89 and 90.

The **analytical specificity** of the primers and probes was validated with negative samples. They did not generate any signal with the specific *Human Papillomavirus* primers and probes, however there is no cross-reactivity with any other HPV type. The potential cross-reactivity of the kit was tested against the group control. It was not observed any cross-reactivity with other pathogens. Tested pathogens were *Neisseria gonorrhoeae, Chlamydia trachomatis, Gardnerella vaginalis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma* sp.*, Mycoplasma hominis, Cytomegalovirus, Streptococcus agalactiae, HSV* I, *HSV* II, *EBV, Varicella-Zoster, Streptococcus pyogenes* and *Candida*.

Principles of the Procedure

This kit is designed for a specific amplification of E6 and L1 genes in HPV DNA. The targeted region of HPV DNA is amplified by several specific primers and detected by fluorescence probes. A non-rivalry internal control is added in the HPV DNA detection system to ensure proper PCR procedure. 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 surpasses 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 in 8 HPV Reaction Mixes are:

Reaction Mix 1. HPV 16(FAM), HPV 18(HEX) and internal control (ROX)

Reaction Mix 2. HPV 31 (FAM), HPV 45 (HEX), HPV 51 (ROX) and HPV 73 (Cy5)

Reaction Mix 3. HPV 33 (FAM), HPV 52 (HEX), HPV 59 (ROX) and HPV 68 (Cy5)

Reaction Mix 4. HPV 35 (FAM), HPV 39 (HEX), HPV 56 (ROX) and HPV 58 (Cy5)

Reaction Mix 5. HPV 26 (FAM), HPV 53 (HEX), HPV 66 (ROX) and HPV 82 (Cy5)

Reaction Mix 6. HPV 6 (FAM), HPV 40 (HEX), HPV 44 (ROX) and HPV 61 (Cy5)

Reaction Mix 7. HPV 11 (FAM), HPV 42 (HEX), HPV 67 (ROX) and HPV 89 (Cy5)

Reaction Mix 8. HPV 43 (FAM), HPV 54 (HEX), HPV 62 (ROX) and HPV 90 (Cy5).

Kit contents

The kit is composed of **8 HPV Reaction Mixes**, **HPV Positive Control.**

* The **HPV Reaction Mix 1~8** includes a HPV DNA detection system. It contains primers and fluorescent probes specific for HPV DNA.

* The **HPV Reaction Mix 1** includes a HPV detection and internal control system. It contains primers and FAM-labeled probes specific for HPV 16 DNA, HEX-labeled probe specific for HPV18 DNA and ROX-labeled probe specific for internal control. The internal control system is designed to detect a housekeeping gene as reference gene to assess the presence of inhibitors and confirm the validity of each experiment.

* The **HPV Positive Control** contains recombinant gene with HPV plasmid DNA.

Tube No.	Quantity	Main Ingredients	Contents
①	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 1
②	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 2
③	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 3
④	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 4
⑤	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 5
⑥	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 6
⑦	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 7
⑧	500 µL/tube ×1	Master mix, Primers, Probes	HPV Reaction Mix 8
PC	150 µL/tube ×1	Plasmid DNA	Positive Control

Storage Conditions and Product Stability

* All kit components should be stored at -20°C upon arrival.

* Repeated thawing and freezing (More than 5 times) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.

* All reagents can be stored for 1 year at -20°C without showing any reduction in performance.

Materials Required but Not Provided

* Consumables, reagents and instruments for sample preparation
* Dedicated pipets (adjustable) for PCR (1–10 µl; 10–100 µl)
* Dedicated filter-plugged sterile DNAse-free pipette-tips
* Disposable gloves
* Liquid-based cytology medium
* Standard DNA extraction kits, such as DSP Virus Kits (QIAGEN)

Equipment

* Benchtop centrifuge
* Vortex mixer
* 4 channels (FAM, HEX, ROX, Cy5) real-time devices from various manufacturers.

Specimen Storage and Handling Cervical specimens

The MehrViru HPV PCR Test is for use with genomic DNA samples obtained from cervical specimens (scrapes), 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 preservative medium. Self-collected cervico-vaginal lavage specimens are collected and shipped in saline (0.9% NaCl) and, upon arrival in the laboratory, stored in preservative medium.

Optimal storage temperature of the clinical samples is 2–8°C upon arrival at the lab. Under these storage conditions, samples in preservative medium are stable for 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 –15°C to –30°C for up to 12 months.

Sample Preparation and DNA extraction

Standard DNA extraction kits (e.g., column- and magnetic bead-based kits) are compatible with this assay.

For cervical specimens (scrapes) suspended in liquid-based cytology medium, the fraction of DNA to be used as input in the PCR represents 200 ul of the 10 ml or 300ul of the 15 ml cervical scrape sample. 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 200 µl cervical specimen (scrape) sample to ensure that the correct fraction of the cervical sample is used in the PCR. Equivalent media with or without formaldehyde should be processed similarly.

For self-collected vaginal brush specimens suspended in liquid-based cytology Solution, DNA extraction procedures should be executed, such that 5 µl DNA extract used as input in the PCR represents 200 µl of the vaginal sample. For example, the vaginal self-sample will be collected in 2 ml liquid-based cytology Solution then 5 µl input DNA corresponds with 40 µl of the self-sample suspension.

Protocol

Assay Profile

Program the thermocylcer according to the program shown in Table below.

Step	Temperature	Time	Acquisition	Cycle
Hold	95°	10'		1
PCR	95°	10''		40
	58°	30''	FAM, HEX, ROX, Cy5	
	70°	15''		

1. Gently vortex and briefly centrifuge the Master Mix and Positive Control tubes.

2. Add 20 µL of Master Mix into PCR tubes.

3. Add 5 µl of the isolated nucleic acid sample or 5 µl of Positive Control into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 25 µl. The negative clinical material can be used as negative isolation control. The customer has to use his own negative control.

4. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following Assay profile.

Be very careful when handling the Positive Control or the clinical material; incorrect handling could result in contamination and the consequent Impairment of the kit components.* The recommended amount of sample DNA to be used is 5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Adjust the final volume of the PCR reaction to 25 µL using the Nuclease-Free Water provided.

Target and channel setting:

G1	G2	G3	G4	G5	G6	G7	G8
16 FAM High-risk	**31** FAM High-risk	**33** FAM High-risk	**35** FAM High-risk	**26** FAM possible High-risk	**6** FAM Low-risk	**11** FAM Low-risk	**43** FAM Low-risk
18 HEX High-risk	**45** HEX High-risk	**52** HEX High-risk	**39** HEX High-risk	**53** HEX possible High-risk	**40** HEX Low-risk	**42** HEX Low-risk	**54** HEX Low-risk
INC ROX Human Gene	**51** ROX High-risk	**59** ROX High-risk	**56** ROX High-risk	**66** ROX possible High-risk	**44** ROX Low-risk	**67** ROX possible High-risk	**62** ROX Low-risk
	73 Cy5 possible High-risk	**68** Cy5 possible High-risk	**58** Cy5 High-risk	**82** Cy5 possible High-risk	**61** Cy5 Low-risk	**89** Cy5 Low-risk	**90** Cy5 Low-risk

Data Analysis

The run and sample validation criteria are indicated below.

Appropriate measures are indicated in case one (or more) criteria are not met.

Validation criteria of MehrViru HPV PCR Test controls

Targets in the MehrViru Positive Control should give CT values that are lower than 35 for HPV 16 and HPV 18, lower than 35 for Other HPV types and lower than 30 for INC. If this is not the case and analysis settings are correct, the experiment should be repeated.

None of the targets in the MehrViru 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:

CT value HPV target(s)	CT value INC	Interpretation
HPV 16 and/or HPV 18 **High Risk HPV** **< 35** **Low Risk HPV**	**< 30**	**Positive**
HPV 16 and/or HPV 18 **High Risk HPV** **> 35** **Low Risk HPV**	**< 30**	**Negative**
HPV 16 and/or HPV 18 **High Risk HPV** **>35** **Low Risk HPV** **or show no signal**	**> 35**	**Invalid**

HPV positive. When CT value(s) of HPV 16, HPV 18 and other HPV types is (are) <35 (irrespective of CT value of INC). The channel indicates the type(s) present.

HPV negative. When CT value for INC is <30 and CT values for HPV 16, HPV 18 and other HPV types are >35 or show no signal.

Invalid. When CT value of INC is >35 and CT values of HPV 16, HPV 18 and other HPV types are >35 or show no signal.

MehrViru HPV PCR Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR. Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated. Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test. The presence of PCR inhibitors may cause false negative or invalid results. Potential mutations within the target regions of the HPV (High Risk) genome covered by the primers in this kit may result in failure to detect the presence of the pathogen. The respective user is liable for any and all damages resulting from application of MehrViru HPV PCR Kit for use deviating from the intended use as specified in the user manual.
All products sold by Mehrbio are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

MehrViru HPV PCR Test Detection was proved

Its excellent performance in 2019 WHO evaluation

100% proficiency in all tests performed by participant.

Contact Information

For technical assistance and more information, please call our Technical Support Center at **Teb Abzar Asia** (Tabas Med) Co.

Support: 021-88334041 (105)