GenomeMe CANADA

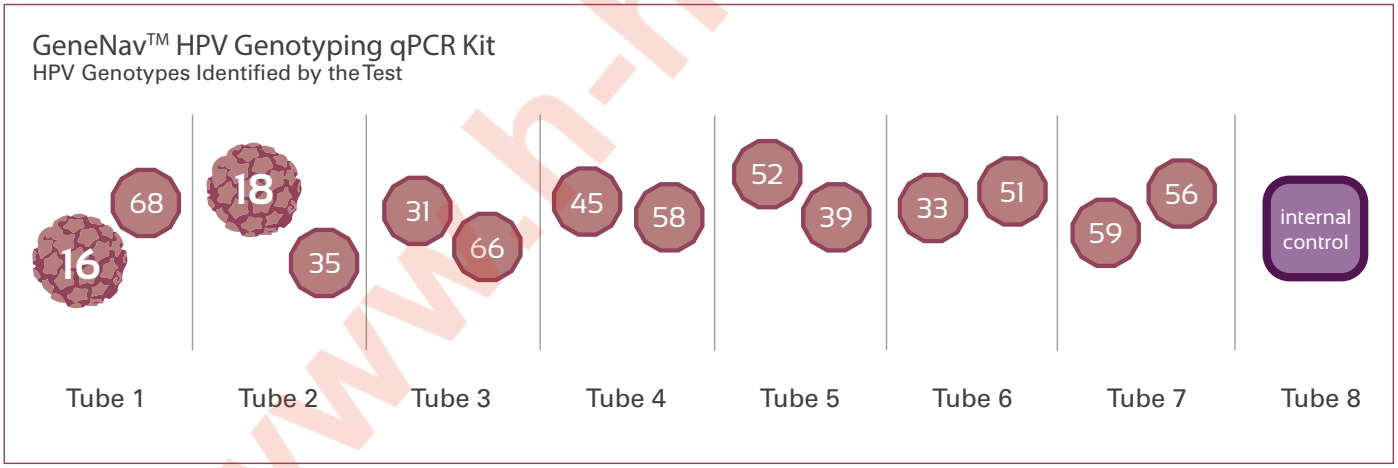
GeneNav™
HPV Genotyping qPCR Kit

IVD CE

REF E115

Introduction

Over 130 HPV types have been documented in literature, approximately 40 of which infect the anogenital area and are sexually transmitted. Persistent anogenital high risk HPV infection is associated with the majority of cervical cancers. Cervical cancer has previously been shown to be highly preventable when cytological and HPV screening programs are employed to facilitate early detection and treatment of pre-cancerous lesions. Of the sexually transmitted HPV genotypes, 14 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are considered high risk and commonly lead to cervical cancers. The GeneNav™ HPV Genotyping qPCR Kit utilizes quantitative PCR (qPCR) technology to specifically detect and simultaneously genotype each of these 14 high risk HPV types in an eight-tube assay.



Kit Components

PN Part Number	Kit Components	Quantity
E-1G	Diagnostic 2X qPCR MasterMix - No Dye	2 x 1 ml
E115-XA	GeneNav™ HPV Genotyping Primers *X*	25 µl
E115-*X*B	GeneNav™ HPV Genotyping Probes *X*	25 µl
E5	Sample Prep Solution	5 ml
E0	Nuclease-free Water	2 x 1 ml

- For 24 preps
- X = Number from 1 to 8, a total of 8 primers and 8 probes
- Store all reagents between -25°C to -15°C in a non-frost-free freezer

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Principles of the Assay

The GeneNav™ HPV Genotyping qPCR Kit uses probe-based qPCR technology to detect HPV with high specificity. Each sequence-specific probe contains a 5' fluorophore and a 3' quencher. On its own, the probe's 3' quencher effectively absorbs the emission from the nearby 5' fluorophore so that no net fluorescence is detected. During PCR amplification, the probe binds to its specific target template. While replicating the DNA sample, the PCR Taq Polymerase's 5' to 3' exonuclease activity also hydrolyzes the probe, releasing a free-floating 5' fluorophore that is detectable by the qPCR machine and can be translated into an amplification plot. In this HPV Genotyping qPCR kit, the 14 types of high risk HPV and a human ACTIN control are divided into 8 reaction tubes, with 2 fluorescent channels per tube: FAM and HEX. Detection of a positive signal in a particular channel can identify a specific genotype of HPV. An internal control, human ACTIN, indicates the quality of extracted DNA.

Sample Requirements

Specimens are limited to cervical cells collected through a cervical swab. For the GeneNav™ HPV Genotyping qPCR Kit, cervical specimens can be stored in the Specimen Transportation Medium for up to 3 days (between 2 to 8°C) or up to 6 months (at -20°C) prior to testing. Avoid repeated freeze/thawing of the samples.

DNA Isolation

1. Transfer 500 µl of freshly re-suspended Specimen Transport Media/Sample mixture into an Eppendorf tube. Centrifuge the mixture at 12000 rpm for 5 minutes. Discard the supernatant.
2. Thoroughly re-suspend the Sample Prep Solution (E5) and immediately transfer 200 µl of the freshly re-suspended Sample Prep Solution (E5) to the collected cell pellet.
3. Vortex the mixture thoroughly. Heat the mixture at 100°C for 10 minutes then cool the mixture on ice for 5 minutes. Centrifuge the mixture at 12000 rpm for 5 minutes. The resulting supernatant is used directly as the template in the
4. ensuing analyses and will be referred to as the "extracted DNA".

note If the supernatant is transferred into another tube, it may be stored up to one year at -20°C.

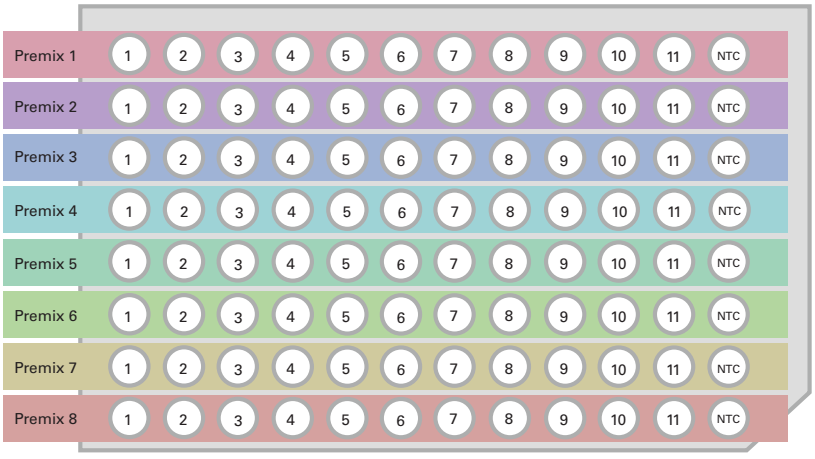
Quantitative PCR (qPCR) Reaction Setup

1. Thaw the following reagents on ice: Diagnostic 2X qPCR MasterMix - No Dye (E-1G), the eight GeneNav™ HPV Genotyping Primers X (E115-XA), and the eight GeneNav™ HPV Genotyping Probes X (E115-XB). Gently and evenly mix each individual reagent, then briefly centrifuge the reagents to collect the contents.
2. A premix solution is needed for each of the eight reaction tubes (i.e. Premix *X* for tube *X*). Each premix solution should contain the corresponding primers and probes for tube *X*. Each premix *X* volume = (number of sample preps + 1) x the total volume of premix reagents for tube *X* as listed below:

Reagents for Premix X	Volume
Diagnostic 2X qPCR MasterMix - No Dye (E-1G)	10 µl
GeneNav™ HPV Genotyping Primers X (E115-XA)	1 µl
GeneNav™ HPV Genotyping Probes X (E115-XB)	1 µl
Nuclease-free Water (E0)	6 µl
Total Volume	18 µl

X ⇒ Number from 1 to 8, a total of 8 primers and 8 probes

Figure 1: 96-well qPCR Setup Guide. You may adjust this setup to suit your own needs.



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3. Please refer to **Figure 1** as a guide for this step. Evenly aliquot each premix into its corresponding qPCR tube labelled Premix 1 to 8. Add 2 µl of each extracted DNA solution (refer to Step 4 of DNA Isolation protocol) into all eight qPCR tubes. Do not add more than one sample of extracted DNA into a single qPCR tube.
4. Use Nuclease-free Water (E0) instead of extracted DNA for the No Target Control (NTC). A single 96-well qPCR plate can run 11 patient samples and 1 NTC, as shown in **Figure 1**.

Control: The NTC must be run on each assay plate, and the cycle threshold (CT) for NTC must be more than 35 for the samples on the plate to be valid. If this criteria is not met, the samples and controls on that plate are invalid and must be repeated.

qPCR Cycling Condition

Transfer the reaction setup into a qPCR machine and set up the following thermal cycling program. It is recommended to use Applied Biosystem's 7500, QuantStudio's 7 Flex system, or BioRad's CFX96 instrument.

DNA Pre-denaturing	95°C, 10 min	1 Cycle
Denaturing	95°C, 15 sec	40 Cycles
Anneal / Extension	61°C, 60 sec (Collect FAM and HEX signals)	

note Signals are collected at the end of each extension step through FAM and HEX channels.

Detection Channels

Two detection channels (FAM and HEX) are used in each of the eight tubes. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM and HEX channels for all 8 tubes containing samples to be tested with GenomeMe™'s GeneNav™ HPV Genotyping qPCR Kit.

Result Analysis

1. If the internal control channel (FAM in Tube 8) shows a CT value of greater than 37, the test has failed due to insufficient amounts of DNA or poor quality DNA. Re-extract DNA and repeat the assay.
2. If the internal control shows a CT value of less than 37, and all other HPV types (FAM and HEX in Tubes 1 to 7) show a CT value of greater than 37, the sample is negative for all 14 types of HPV that this kit detects.
3. If both the internal control (FAM in Tube 8) and any one of the HPV types (FAM or HEX in Tubes 1 to 7) show a CT value of less than 37, the sample is positive for the indicated HPV type.
4. If the internal control (FAM in Tube 8) and two or more of the HPV types (FAM or HEX in Tubes 1 to 7) show a CT value of less than 37, the sample is positive for all indicated HPV types (Chimeric Infection).

Refer to the table below for HPV genotyping information per tube and channel.

Tube #	Channel	HPV Type
Tube 1	FAM	16
	HEX	68
Tube 2	FAM	18
	HEX	35
Tube 3	FAM	31
	HEX	66
Tube 4	FAM	58
	HEX	45

Tube #	Channel	HPV Type
Tube 5	FAM	52
	HEX	39
Tube 6	FAM	33
	HEX	51
Tube 7	FAM	59
	HEX	56
Tube 8	FAM	Internal Control

note Sample results are only valid when negative controls yield no amplification. If the NTC is invalid in an assay, all sample results in the same assay are therefore also invalid and the assay must be repeated.

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Performance Characteristics

The following performance characteristics of the GeneNav™ HPV Genotyping qPCR Kit have been established following the procedure described in this datasheet.

Analytical Sensitivity: 130 copies/reaction for all 14 types.

Analytical Specificity: Cross reaction among these 14 HPV types was not observed. A panel of bacteria, fungi, and viruses commonly found in the female anogenital tract were tested with the GeneNav™ HPV Genotyping qPCR Kit to assess potential cross-reactivity. The pathogens tested include: *Candida albicans, Proteus vulgaris, Corynebacterium pseudodiptheriticum, Staphylococcus aureus, Enterococcus faecalis, Staphylococcus epidermidis, Escherichia coli, Streptococcus mitis, Lactobacillus acidophilus, Streptococcus pyogenes, Herpes simplex virus, type 1 and 2 (HSV-1 and 2), Chlamydia trachomatis, Neisseria gonorrhoeae, Human Immunodeficiency Virus type 1 (HIV-1 pol and env regions), and Mycoplasma hominis.* Negative results were obtained from all above-mentioned organisms.

Reproducibility: Reproducibility of the GeneNav™ HPV Genotyping qPCR Kit was assessed at two external sites using a panel of HPV positive and negative cultured cells and HPV positive and negative cervical specimens. DNA was extracted from 500 µl of cervical samples or cultured cells suspended in Specimen Transportation Medium. The DNA was extracted using the sample prep solution included in this Kit. DNA was extracted from sixteen samples and tested with GeneNav™ HPV Genotyping qPCR Kit at two sites on five non-consecutive days within a two-week time period. Two lots of GeneNav™ HPV Genotyping qPCR Kits were used across the two sites for the study. The total number of measurements for each sample was 20 (2 sites x 5 days x 2 lots x 1 run per day). The results showed that the coefficient of variation (CV) for the kit performance was less than 5% among different product lots and different operators.

Limitations

1. The GeneNav™ HPV Genotyping qPCR Kit detects DNA of 14 HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
2. A negative result does not exclude the possibility of HPV infection because very low levels of infection or sampling error may cause a false-negative result.
3. The test has been validated for use only with cervical swab specimens collected in specimen transportation medium.
4. The performance of the GeneNav™ HPV Genotyping qPCR Kit was established exclusively using DNA extracted with the enclosed sample prep solution.
5. The performance of the GeneNav™ HPV Genotyping qPCR Kit has not been adequately established for HPV vaccinated individuals.
6. Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.
7. Infection with HPV is not an indicator of cytologic High-grade squamous intraepithelial lesion (HSIL) or underlying high-grade Cervical intraepithelial neoplasia (CIN), nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more HPV types do not develop CIN2-3 or cancer.
8. A negative High-Risk HPV result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.

Disclaimer

INTENDED USE:

1. To diagnose and identify the exact type of HPV infection in patients. Detection of HPV using the GeneNav™ HPV Genotyping qPCR Kit can differentiate between HPV types and evaluate persistence of one type.
2. In women 30 years and older, the GeneNav™ HPV Genotyping qPCR Kit can be used with a cervical cytology to adjunctively assess the presence or absence of HPV infection. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

WARNINGS:

1. The GeneNav™ HPV Genotyping qPCR Kit is not intended for use as a screening device for women under age 30 with normal cervical cytology.
2. The GeneNav™ HPV Genotyping qPCR Kit is not intended to substitute for regular cervical cytology screening.
3. The use of this test has not been evaluated for the management of women with prior cytological or histological abnormalities, have had a hysterectomy, who are pregnant, postmenopausal, or who have other risk factors (e.g., HIV+, immunocompromised, history of STI).
4. The GeneNav™ HPV Genotyping qPCR Kit is designed to enhance existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.
5. The GeneNav™ HPV Genotyping qPCR Kit results should not be used as the sole basis for clinical assessment and treatment of patients.

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