AutoGenomics

INFINITI® HPV-QUAD

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 2980 Scott Street, Vista, CA USA 92081

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany

Doc EM-34018E (English) Rev. I (CO 2336) November 2012
Tel: 760-477-2248 www.autogenomics.com

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INTENDED USE

The INFINITI HPV-QUAD is indicated for use to detect the presence of 13 high risk and two (2) low risk HPV DNA types in women with abnormal Pap smear results (i.e., atypical squamous cells of undetermined significance, ASCUS). In addition, the INFINITI HPV-QUAD is designed to identify the genotype of the five high risk types 16, 18, 31, 33 and 45. Together with the patient's cytology history, other risk factors and relevant clinical information, the information from the INFINITI HPV-QUAD may be used to guide patient management.

The INFINITI HPV-QUAD results may not be used to prevent women from proceeding to colposcopy.

The INFINITI HPV-QUAD is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

Genital HPV infection is caused by human papillomavirus (HPV), a group of viruses that include more than 100 different strains or types. More than 30 of these viruses are sexually transmitted and can infect the genital area of men or women. At least 50% of sexually active men and women acquire genital HPV infection at some point in their lives. By age 50, at least 80% of women will have acquired genital HPV infection. About 6.2 million Americans get a new genital HPV infection each year. [(1)]

Human papillomaviruses are composed of an icosahedral viral particle (virion) containing an 8000 base pair double-stranded circular DNA molecule surrounded by a protein capsid. Following infection of epithelial cells, the viral DNA becomes established throughout the entire thickness of the epithelium, but intact virions are found only in the upper layers of the tissue. Thus, viral DNA can be found either in virions or as episomal or integrated HPV sequences, depending upon the type and grade of lesion.[(9,10,11,12)]

Epidemiological studies demonstrate that persistent infection with certain types of human papillomaviruses (HPVs) are a necessary risk factor for the development of invasive cervical cancer. [(2,8)] Based on such studies, genital HPV types were grouped into high and low risk HPV types, reflecting their risk potential to induce invasive cancer.

High-risk HPV are those associated with cervical intraepithelial neoplasia (CIN 2/3) and are thought to be responsible for approximately 70% of all invasive cervical cancers, although the relationship of HPV type to risk of cancer appears to vary geographically.[(2,3)] In Northern America, approximately 90% are associated with high risk types 16,18,31,33 and 45. In addition to cervical cancer, high risk HPV types may lead to cancer of the vulva, vagina or anus. The presence of certain HPV types in the female genital tract is also associated with other diseases, including Bowenoid papulosis, and cervical, vaginal and vulvar intraepithelial neoplasia. Low-risk HPV types 6 and 11 may be associated with the presence of genital warts (condyloma), but have been infrequently linked with precancerous or cancerous cervical changes.[(4,5,6,7)] It is not completely understood how HPV infection progresses to cancer.

TEST PRINCIPLE/ASSAY OVERVIEW

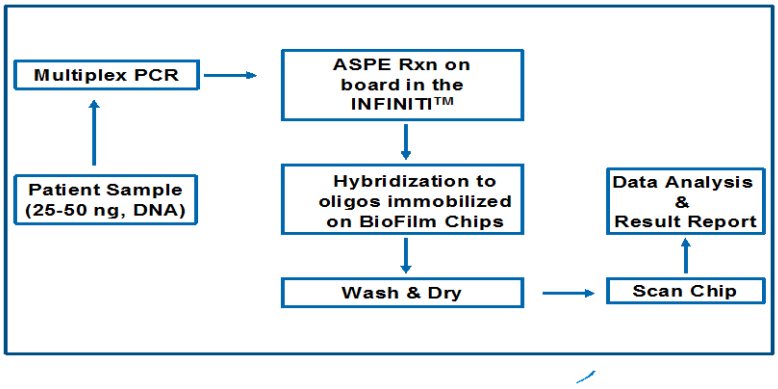
The INFINITI HPV-QUAD utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the presence of HPV genotypes in deoxyribonucleic acid (DNA) obtained from cervical specimens.

The INFINITI HPV-QUAD is based on the following processes:

(a) PCR amplification of purified DNA
(b) Labeling of the amplified product (analyte specific primer extension)
(c) Hybridization of the fluorescent labeled product to a microarray
(d) Scanning of the microarray
(e) Detection of fluorescence (identification of HPV types)
(f) Signal detection and analysis

Steps (b) through (f) are automated by the INFINITI Analyzer or the INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI HPV-QUAD is an *in vitro* diagnostic device that consists of reagents and instrumentation which includes polymerase chain reaction (PCR) primers, microarrays, a thermal cycler, an imager, and software designed to detect the presence of thirteen (13) high risk HPV types (Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and two low risk types (Types 6 and 11) in cervical specimens. In addition, the test performs type specific identification of high risk HPV types (Types 16, 18, 31, 33 and 45).

Cervical specimen types may include:

- Specimens collected using a broom type collection device and placed in Cytyc ThinPrep® Pap Test™ PreserveCyt® Solution.

The INFINITI HPV-QUAD is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, and the PCR Amplification Mix. The instrumentation for the INFINITI HPV-QUAD is the AutoGenomics INFINITI Analyzer or the INFINITI PLUS Analyzer with the Qmatic® Operating Software.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer `components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The INFINITI HPV-QUAD uses a microarray chip (S-Chip) which has capture probes spotted on the surface of the film. Four (4) samples can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 96 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 500µl vials of PCR Amplification.

The **INFINITI Analyzer and INFINITI PLUS Analyzer** automates the INFINITI HPV-QUAD Assay and integrates all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of HPV genotypes and provides type specific identification for high risk HPV types (Types 16, 18, 31, 33 and 45).

The Analyzers are provided with the Operator's Manuals. The Operator's Manual provides detailed description and operating principle of the Analyzers and instructions for use.

The INFINITI Analyzer and the INFINITI PLUS Analyzer are CE marked.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with cervical specimens collected in Cytyc ThinPrep Pap Test PreserveCyt Solution.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up, and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- All patient specimens are potentially hazardous, and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents. Do not mix reagents from different containers or from different lots.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Material Safety data Sheets (MSDS) are available upon request from AutoGenomics Customer Service.

Sample Preparation
- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer or INFINITI PLUS Analyzer
- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 12 months Refrigerated (2°C to 8°C)
Intellipac Reagent: 12 months Refrigerated (2°C to 8°C)
Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.
Amplification Mix: 18 months Frozen (-30°C to -15°C)

Note: Specific product expiration date is printed on the product label.

SPECIMEN COLLECTION AND STABILITY

ThinPrep (Cytyc Corp.) cervical specimens are recommended for use in the INFINITI HPV-QUAD. Specimens taken with other sampling devices or transported in other transport media have not been qualified for use with this assay. The performance characteristics of the INFINITI HPV-QUAD with other specimen types and collection devices is unknown. Specimens for use in making ThinPrep Pap Test slides should be collected using a broom-type collection device and then placed in Cytyc PreservCyt Fluid. PreservCyt Soution specimens may be held for up to three weeks at temperatures between 4°C and 37°C, following collection and prior to processing for the INFINITI HPV-QUAD. PreservCyt Solution specimens cannot be frozen.

REAGENTS REQUIRED (SUFFICIENT FOR 192 TESTS)
- AutoGenomics Product Number 02-1060-02 HPV-QUAD Magazine – BioFilmChip® Microarray: 4 magazines per box
- AutoGenomics Product Number 02-2060-02 HPV-QUAD Intellipac Reagent Management Module: 96 tests per module which contains
  - dNTPs
  - Cy5-dCTP
  - Analyte Specific Primers
  - Extension reaction Buffer
- AutoGenomics Product Number 02-3060-02 HPV-QUAD Amp Mix: 4 x 500µl vials of Amp Mix containing
  - dNTPs
  - Multiplex Primer Mix
  - $MgCl_2$
  - Reaction Buffer
- AutoGenomics Product Number 12-0030-00 Solution BF2 (Hybridization Buffer): 6 x 30ml bottles. The hybridization buffer consists of:
  - SSC
  - Sodium Azide Preservative 0.08%
  - EDTA
  - 10X Blocking Buffer

- **FOR INFINITI Analyzer***:* AutoGenomics Product Number 12-0020-00 Solution BF1
  OR
  FOR INFINITI PLUS Analyzer*:* Product Number 12-0330-00: Buffer Solution BF1

- AutoGenomics Product Number 12-0040-00 HPV Type 16 DNA Template Control
- AutoGenomics Product Number 12-0050-00 HPV Type 18 DNA Template Control
- AutoGenomics Product Number 12-0060-00 HPV Type 31 DNA Template Control
- AutoGenomics Product Number 12-0070-00 HPV Type 33 DNA Template Control
- AutoGenomics Product Number 12-0080-00 HPV Type 45 DNA Template Control

Also available:

- AutoGenomics Product Number 12-0090-00 HPV Combo pack (Type 16, 18, 31, 33, 45) DNA Template Control

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - Commercially available DNA extraction kits may be used in association with the INFINITI HPV-QUAD. The DNA sample may be re-suspended in water or TE pH 8. The minimum final DNA concentration should be ≥ 10 ng/µl. The purified DNA should have an A260/280 ≥ 1.60.
- Distilled Water (DNAse and RNAse free)
- Platinum Taq DNA Polymerase (Invitrogen, Catalog No:10966-018)

EQUIPMENT AND CONSUMABLES

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates and Product Number 11-0110-00: 48 Well Plate Lid

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ASSAY PROCEDURE

DNA Extraction

Follow the instructions provided with the DNA extraction kit used. The DNA sample may be resuspended in water or TE pH 8. The minimum final DNA concentration should be ≥ 1 ng/µl. The purified DNA should have an A260/280 ≥ 1.60.

DNA Controls

It is recommended that positive controls and a no HPV template control (i.e., molecular grade water or HPV free human DNA) are included in each test run. The no HPV template control serves as a contamination control. If this control is positive, then samples should be tested again taking appropriate measures to prevent contamination.

The following positive controls from AutoGenomics are recommended for use with the HPV QUAD (refer to section on REAGENTS REQUIRED for Product Number):

(a) HPV Type 16 DNA Template Control
(b) HPV Type 18 DNA Template Control
(c) HPV Type 31 DNA Template Control
(d) HPV Type 33 DNA Template Control
(e) HPV Type 45 DNA Template Control
or
(f) HPV Combo pack (Type 16, 18, 31, 33, 45) DNA Template Control

Note: Please use proper PCR technique to prevent contamination of reagents with HPV controls. Sealing the well plate containing sample HPV DNA and "no template control" samples with caps **before** adding the HPV controls is recommended to prevent cross contamination.

Amplification Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling specimens and controls.
- Ensure that tubes are properly sealed to avoid evaporation or spillage.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All amplification reaction volumes should be about 15 µl. Otherwise, do not proceed with the assay.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix.

Amplification mix	9.75 µl
Platinum Taq polymerase	0.25 µl
Total volume of PCR Master mix	10.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 10.0 µl of master mix into wells of the well plate.

3. Add 5.0 µl of sample or control DNA to each well.

PCR master mix	10.0 µl
Sample or control DNA	5.0 µl
Total volume of amplification reaction	15.0 µl

Note: **This is a QUAD assay**. When loading samples, always load the samples in multiples of four and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. **Do not** load the B wells**.**

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Thermocyling Conditions for the HPV-QUAD Assay:

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2a 2b 2c	94 58 (-0.8/cycle) 72	5 60 40	10
3	94 50 72	5 60 40	30
4	4	hold	1

Note: After each cycle in step 2 the temperature is decreased by 0.8°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour and 53 minutes (± 5 min). If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Catalog # 11-0110-00, reusable).
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Buffer Solution 1 and 2 should be placed in the INFINITI bottle holders. Buffer Solution 1 goes in the left holder (near the magazine) and Buffer Solution 2 in the right holder (near the Intellipac).
   - **FOR INFINITI PLUS Analyzer***:*
     Buffer Solution 2 should be placed in the INFINITI bottle holders. Buffer Solution 2 goes in the right holder (near the Intellipac).

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

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QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from the INFINITI HPV-QUAD should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are made.

INTERPRETATION OF RESULTS

Results from the INFINITI HPV-QUAD are reported to the user as "Positive" or "Negative" for the presence of high risk HPV DNA types. In addition, the INFINITI HPV-QUAD will report state if any of the following HPV types present: 16, 18, 31, 33, 45.

The beta globin gene control is intended to identify specimens that have inadequate sample integrity. INFINITI HPV QUAD results are interpreted as follows:

HPV Result	Beta globin (BG) Result	INTERPRETATION
ALL HPV Types Negative	Positive	HPV Negative
ALL HPV Types Negative	Negative	Invalid Test Result
ANY HPV Type Positive	Positive or Negative	Valid Test Result

If the analyzer detects a problem or an error (e.g., assay parameters not met), no results will be reported. Instead, a description of the problem or error code will be displayed. The Trouble Shooting section of the INFINITI Analyzer Operator's Manual provides an explanation of the errors. The assay needs to be repeated.

DISPOSAL

Waste materials for the INFINITI HPV-QUAD are common waste materials generated in clinical laboratories, and should be handled/disposed of in accordance with the policies/procedures in place in the clinical laboratory.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

Analytical Sensitivity (Level of Detection)
Plasmids containing HPV genomic DNA for Types 16, 18, 31, 33, and 45 were used as samples to establish the level of detection for the INFINITI HPV-QUAD. The following table provides the results of the study.

HPV Type	Lowest detectable copy number of plasmid DNA	Highest detectable copy number of plasmid DNA
Type - 16	2,000	2,000,000
Type - 18	2,000	2,000,000
Type - 31	2,000	2,000,000
Type - 33	2,000	2,000,000
Type - 45	2,000	2,000,000

***Note*:** In clinical specimens, HPV 16 is present in the highest copy number. Over 55% of HPV 16 positive clinical specimens contain more than 10^8 copies/µg of extracted DNA. Normal cytology for Type 16 is 2.2 x 10^7 copies/µg. Median DNA copy number varies by $>10^4$ among the viral types.[15]

Sample Carry-over
Sample carryover studies demonstrated that there is no sample carry-over with the INFINITI HPV-QUAD. Two Plasmid DNA samples were used (Type 16 and Type 18). No carry-over was detected when a series of 25pg/rxn of Type 16 was followed by 0.025pg/rxn of Type 18, followed by 25pg/rxn of Type 16, followed by a "No Template Control" or water, was run six times.

Potential Interference from Blood and other Substances
The effect of blood and other potentially interfering substances was evaluated for the INFINITI HPV-QUAD. Whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) were added to samples at concentrations that may be found in cervical specimens. Two HPV Plasmid samples were used in the study: HPV Type 16 and HPV Type 18. The study demonstrated that the presence of these substances does not affect the INFINITI HPV-QUAD.

Assay Interference
Interference from other assays run on the INFINITI Analyzer was evaluated. The assay interference studies demonstrated that running the INFINITI HPV-QUAD and the INFINITI FII/FV/MTHFR Assay on the same instrument does not affect the results of each assay; i.e., one assay does not interfere with the results of the other.

Clinical Feasibility
The INFINITI HPV-QUAD was evaluated by an independent clinical reference laboratory to determine if the assay can be performed in the clinical use environment following the instructions for the assay.

The clinical feasibility study demonstrated that the INFINITI HPV-QUAD can be performed in the clinical environment (independent of AutoGenomics) following the procedures established for the assay.
DNA was extracted using the Qiagen's QIAmp MiniElute Media Kit. PCR was performed using the HPV Amp Mix and following the conditions specified for the INFINITI HPV-QUAD. The PCR product was placed in the INFINITI Analyzer for the primer extension, hybridization to the microarray chips, wash steps and signal detection using the S-Chip and the HPV-QUAD Intellipac.

Assay Reproducibility evaluated using CaSki and HeLa cell controls was:
- 100% within run; n=3 on 3 different chips using HeLa (HPV18) and 3 clinical specimens
- 100% between runs (n=3); 4 clinical specimens

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