



INFINITI[®] HPV Genotyping Assay
Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use



FOR EXPORT ONLY

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Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI HPV Genotyping Assay is indicated for use to detect and identify human papillomavirus (HPV) DNA types in women with abnormal Pap smear results (i.e., atypical squamous cells of undetermined significance, ASCUS). The INFINITI HPV Genotyping Assay is designed to genotype the HPV Types 6, 11, 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 85 in cervical specimens. Together with the patient's cytology history, other risk factors and relevant clinical information, the information from the INFINITI HPV Genotyping Assay may be used to guide patient management.

The INFINITI HPV Genotyping Assay results may not be used to prevent women from proceeding to colposcopy.

The INFINITI HPV Genotyping Assay 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 and 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) High-risk HPV types 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 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. Certain 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

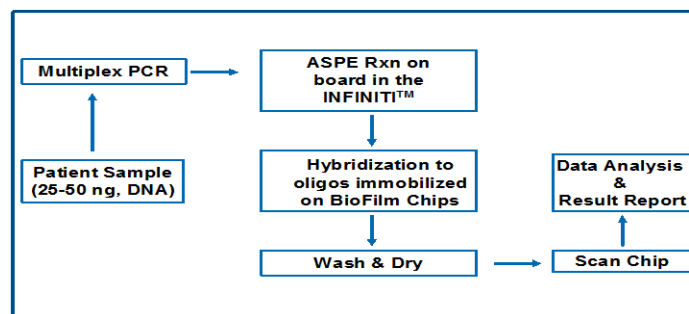
The INFINITI HPV Genotyping Assay utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the presence of HPV genotypes in deoxyribonucleic acid (DNA) obtained from cervical specimens.

The INFINITI HPV Genotyping Assay 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 CE marked INFINITI Analyzer or INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI HPV Genotyping Assay 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 and identify HPV Types 6, 11, 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 85 in cervical specimens.

Cervical specimen types may include:

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

The INFINITI HPV Genotyping Assay is comprised of the BioFilmChip[®] Microarray, the Intellipac[®] Reagent Module, and the PCR Amplification Mix. The instrumentation for the INFINITI HPV Genotyping Assay 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 Genotyping Assay uses a microarray chip (P-Chip) which has capture probes spotted on the surface of the film. 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 Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 24 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 250µl vials of PCR Amplification.

The **INFINITI Analyzer or INFINITI PLUS Analyzers** automates the INFINITI HPV 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 each of the HPV genotypes.

The Analyzers are provided with the Operator's Manuals. The Operator's Manual provides detailed description and operating principle of the Analyzer and instruction 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 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.
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service.



Sample Preparation

- Refer to the instructions provided with INFINITI® Sample Processing Kit.
- 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: 24 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® cervical specimens are recommended for use in the INFINITI HPV Genotyping Assay. 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 Genotyping Assay with other specimen types and collection devices have not been documented. Specimens for use in making ThinPrep Pap Test slides should be collected using a broom-type collection device and then placed in PreservCyt Fluid. PreservCyt Solution 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 Genotyping Assays. PreservCyt Solution specimens cannot be frozen.

REAGENTS REQUIRED AND PROVIDED BY AUTOGENOMICS

ASSAY REAGENTS (SUFFICIENT FOR 48 TESTS)

- AutoGenomics Product Number 02-1050-02 HPV Genotyping Magazine – BioFilmChip® Microarray (12 microarrays per magazine; 4 magazines per box)
- AutoGenomics Product Number 02-2050-02 HPV Genotyping Intellipac® Reagent Management Module (24 tests per Module, 2 modules per box), which contains:
 - 1.1ml Reaction Master Mix
 - dNTPs
 - Cy5-dCTP
 - Analyte Specific Primers
 - Extension Reaction Buffer
 - 2.6ml Hybridization Buffer
 - SSC
 - Hybridization Positive Control
- AutoGenomics Product Number 02-3050-02 HPV Genotyping Amp Mix: 4 x 250µl vials of Amp Mix containing:
 - dNTPs
 - Multiplex Primer Mix
 - MgCl₂
 - Reaction Buffer

REAGENTS REQUIRED BUT NOT PROVIDED WITH THE ASSAY REAGENTS

- AutoGenomics Catalog Number 12-0470-02 INFINITI Sample processing Kit
- Internal Control 1 Catalog Number 12-0170-02
- 12-0010-02: Wash buffer
- AutoGenomics Product Number 12-0040-02 HPV Type 16 DNA Template Control
- AutoGenomics Product Number 12-0050-02 HPV Type 18 DNA Template Control
- AutoGenomics Product Number 12-0060-02 HPV Type 31 DNA Template Control
- AutoGenomics Product Number 12-0070-02 HPV Type 33 DNA Template Control
- AutoGenomics Product Number 12-0080-02 HPV Type 45 DNA Template Control

Also available:

- AutoGenomics Product Number 12-0090-02 HPV Combo pack (Type 16, 18, 31, 33, 45) DNA Template Control

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- Molecular grade Water (DNase and RNase free)
- Platinum Taq DNA Polymerase (ThermoFischer, Scientific Catalog No.: 10966-018)

EQUIPMENT

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
 - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)



ASSAY PROCEDURE

DNA Extraction

The INFINITI[®] Sample Processing Kit (AutoGenomics Catalog Number 12-0470-00) has been developed for use with the HPV Genotyping Assay. Follow the instructions provided with the processing kit.

DNA Controls

It is required to run known positive controls and a negative control in each test run.

The following positive controls from AutoGenomics are recommended for use with the HPV Genotyping assay (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 plates 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 Platinum Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix tube 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 20 μ l. Otherwise, do not proceed with the assay.

Note:

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

1. Prepare the PCR master mix.	
Amplification mix	14.75 μ l
Platinum Taq polymerase	0.25 μ l
Internal Control 1	1.00 μ l
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Total volume of PCR master mix	16.0 μ l

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

2. Gently vortex the PCR master mix then dispense 16.0 μ l of master mix into wells of the well plate.

3. Add 4 µl of sample or control DNA to each well.
- | | |
|--|---------|
| PCR master mix | 16.0 µl |
| Sample or control DNA | 4.0 µl |
| <hr/> | |
| Total volume of amplification reaction | 20.0 µl |

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.

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2a	94	5	10x
2b	58 - 50 (-0.8/cycle)	60	
2c	72	40	
3a	94	5	30x
3b	50	60	
3c	72	40	
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 48 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:**
Wash Buffer should be placed in the INFINITI bottle holders. The Wash Buffer goes in the left holder (near the magazine).
 - **FOR INFINITI PLUS Analyzer:**
Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Wash buffer.

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)

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 Genotyping Assay 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 Genotyping Assay are reported to the user as "Positive" or "Negative" for each of the HPV DNA types detected.

The Internal Control 1 is intended to identify specimens that contain polymerase inhibitors. INFINITI HPV Genotyping Assay results are interpreted as follows:

HPV Result	Internal Control (IC) 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 Genotyping Assay 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)

Analytical sensitivity studies were performed on the two (2) most prevalent high risk HPV types (16, 18) related to cervical cancer (Reference: John Schiller, Ph.D.- National Cancer Institute, US Dept. of Health and Human Services).

HPV DNA samples for Types 16 (Siha) and 18 (Hela) were used to establish the level of detection for the INFINITI HPV Genotyping Assay. The following table provides the results of the study. Serial dilutions were made in molecular grade water for the study.

HPV Type	Lowest detectable level		Highest detected level	
	Concentration of samples tested in the study	Copy number of DNA	Concentration of samples tested in the study	Copy number of DNA
Type - 16	0.0625 ng DNA/ μ l	62.5 - 125	100 ng DNA/ μ l	100,000 - 200,000
Type - 18	0.25 ng DNA/ μ l	2,500 - 7,500	100 ng DNA/ μ l	1,000,000 - 5,000,000



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 2.2×10^7 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 Assay. HPV Type 16 Plasmid and HPV Type 31 Plasmid samples were used. Water was used as “no template control”. All calls made were 100% correct, and all water calls were as expected (NTC). No carry-over contamination was observed.

Potential Interference from Blood and other Substances

The presence of whole blood, douche, anti-fungal cream and contraceptive jelly (agents that may commonly be found in cervical specimens) at concentrations that may be found in cervical specimens, does not affect the INFINITI HPV Genotyping Assay.

Assay Interference

Interference studies in which different assays were run on the same Analyzer demonstrated that there is no interference when different assays run on the same Analyzer.

Clinical Feasibility

The INFINITI HPV Genotyping Assay was evaluated by an independent clinical reference laboratory to determine if the assay can be performed in the clinical use environment, following the instruction for the assay.

The clinical feasibility study demonstrated that the INFINITI HPV Genotyping Assay can be performed in the clinical environment (independent of AutoGenomics) following the procedures established for the assay.

The evaluation also provided information on reproducibility (using cell controls) for the INFINITI HPV Genotyping Assay.

Within Run Reproducibility (5 replicates each per run)

	% agreement	% CV within chip signals	% CV between chip signals	% CV between chip ratios
CaSki (HPV16)	100%	0.5 to 5.8% (5 chips, n=2)	20.7% (5 chips)	35.9% (5 chips)
HeLa (HPV18)	100%		9.8% (5 chips)	17.7% (5 chips)

Between Run Reproducibility

	% agreement	% CV between run signals	% CV between run ratios
12 runs CaSki (HPV16)	100%	21.4% (n=24)	34% (n=12)
17 runs HeLa (HPV18)	100%	24.4% (n=34)	32% (n=17)

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