

**Cervista™ HPV 16/18**

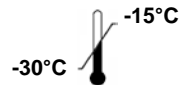
REF 92-0200

**FOR EXPORT ONLY.  
Not for sale in the United States of America.**

An *in vitro* diagnostic test for the detection of DNA from Human Papillomavirus (HPV) Type 16 and Type 18 in Cervical Specimens.



**Contains sufficient  
reagents for 96 tests**



**Temperature Limitation**

**Do not store in frost-free freezer.  
Protect from light.**

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## Abbreviations Used

ASC-US:	Atypical squamous cells of undetermined significance
LSIL:	Low-grade squamous intraepithelial lesion
CIN:	Cervical intraepithelial neoplasia
CLSI	Clinical and Laboratory Standards Institute
DNA:	Deoxyribonucleic acid
FAM:	Carboxyfluorescein dye
Red:	Redmond® red dye
FRET:	Fluorescence resonance energy transfer
FOZ:	Fold over zero (sample or control signal divided by No Target Control signal)
gDNA:	Genomic DNA
HIST2H2BE:	Human histone 2 gene, H2be gene
HPV:	Human papillomavirus
HR:	High-risk
LoB	Limit of Blank
LoD	Limit of Detection
Max.	Maximum
Min.	Minimum
NILM	Negative for intraepithelial lesion or malignancy. This category encompasses the previous categories of “within normal limits” and “benign cellular changes”.
NTC:	No target control
Oligo:	Oligonucleotide
Pap:	Papanicolaou cervical cytology test
RFU:	Relative fluorescence unit

## Intended Use

The Cervista HPV 16/18 test is an *in vitro* diagnostic test for the qualitative detection of DNA from Human Papillomavirus (HPV) Type 16 and Type 18 in cervical specimens.

The Cervista HPV 16/18 test uses the Invader™ chemistry, a signal amplification method for detection of specific nucleic acid sequences. This method uses two types of isothermal reactions: a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal (See Figure 1).

The Cervista HPV 16/18 test is indicated:

1. In women 30 years and older the Cervista HPV 16/18 test can be used adjunctively with the Cervista HPV HR test in combination with cervical cytology to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.
2. To be used adjunctively with the Cervista HPV HR test in patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results, to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.

Cervical specimens that may be tested with the Cervista HPV 16/18 test include the following preservation system and collection devices:

- ThinPrep™ Pap Test PreservCyt™ Solution
- Broom-type device (e.g. Rovers Cervex® Brush, Wallach Papette®), or Endocervical Brush/Spatula

## Warnings

- This test is not intended for use in determining the need for treatment (i.e. excisional or ablative treatment of the cervix) in the absence of high-grade cervical dysplasia. Patients who are HPV16/18 positive should be monitored carefully for the development of high-grade cervical dysplasia according to current practice guidelines.
- The Cervista HPV 16/18 test is not intended for use as a stand-alone assay. Results should be interpreted in conjunction with the Cervista HPV HR and cervical cytology test results.
- The Cervista HPV 16/18 test is not intended for use in women under age 30 with normal cervical cytology.
- The Cervista HPV 16/18 test is not intended to substitute for regular cervical cytology screening.
- The use of this test has not been evaluated for the management of women with prior cytological or histological abnormalities, hysterectomy, who are pregnant, postmenopausal, or who have other risk factors (e.g. HIV+, immunocompromised, history of STI).

The Cervista HPV 16/18 test 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.

Cervista HPV 16/18 test results should not be used as the sole basis for clinical assessment and treatment of patients.

## Summary and Explanation of the Test

Over 100 HPV types have been documented in the literature, approximately 40 of which infect the anogenital area and are transmitted sexually. Anogenital HPV is associated with virtually all cancers of the cervix.<sup>1</sup> Cervical cancer has previously been shown to be highly preventable when cytological and HPV screening programs are employed to facilitate the detection and treatment of pre-cancerous lesions.

Of the sexually transmitted types of HPV, 14 oncogenic genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), are considered high-risk (HR) HPV types due to their strong association with cervical cancers (relative to low risk HPV types, which have little or no association with cervical cancer).<sup>2,3,19</sup> Still, the vast majority of high-risk HPV infections are cleared.<sup>4</sup> Very few high-risk HPV DNA positive women develop cytologic high-grade SIL (HSIL) indicating underlying CIN2-3 or cancer.<sup>5</sup> The absolute risk of developing an incident cytologic abnormality following a HR HPV infection is known to vary in different populations.

The presence of high-risk HPV DNA in conjunction with an equivocal or ambiguous cytology result (ASC-US) places a woman at increased risk for having an underlying cervical intraepithelial neoplasia 2 or 3 (CIN2 or CIN3).<sup>6,7,8</sup> CIN3, while occurring in only approximately 5% of ASC-US cases,<sup>9</sup> is an immediate precursor to cervical cancer and consequently its detection is very important for patient management.<sup>3,7</sup> Therefore, the identification of those women with ASC-US cytology in conjunction with a high-risk HPV infection is a useful aid for clinicians to decide who should be monitored or treated more aggressively.<sup>3,8,10,11,12,13</sup>

Current scientific literature suggests that persistent infection with high-risk HPV is the main risk factor for development of high-grade cervical neoplasia and cancer.<sup>4,5,15</sup> Apparent persistence may represent continuous infection with a single HPV type, with multiple HPV types, or reinfection. Nonetheless, women with normal cervical cytology who are HR HPV negative appear to be at low risk for having or developing cervical precancerous lesions.<sup>8,10</sup>

HPV types 16 and 18 are recognized as both highly oncogenic and persistent, associated with 60% and 10% of cervical cancers, respectively, while also having the lowest clearance rates in cervical screening.<sup>10,14,15,16</sup> As a result, numerous studies report that women infected with HPV types 16 and 18 have a significantly higher risk for developing  $\geq$ CIN3 than women infected with other high-risk types.<sup>3,10,11,13</sup> Beginning in 2002, patient management guidelines have been published by various groups of U.S. healthcare professionals that recommend how women should be screened for cervical cancer according to age, the presence of cytological abnormalities in a cervical sample, and other factors.<sup>6,17,18,19</sup> These patient management guidelines consistently recommend testing for the presence of high-risk types of HPV as a regular screening tool, in combination with cytology in two instances: 1) for women age 30 and over; and 2) as an additional diagnostic tool for women 21 years of age and over with ASC-US.

According to the *2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests*, women with negative cervical cytology results who test positive for the presence of high-risk HPV DNA would benefit from type-specific HPV genotyping.<sup>1,19</sup> Studies support the validity of genotyping for HPV types 16 and 18, whether it is used as a follow-up to a high-risk HPV screening test or performed concurrently.<sup>1,2,3,10,13,19,20</sup>

## Principles of the Procedure

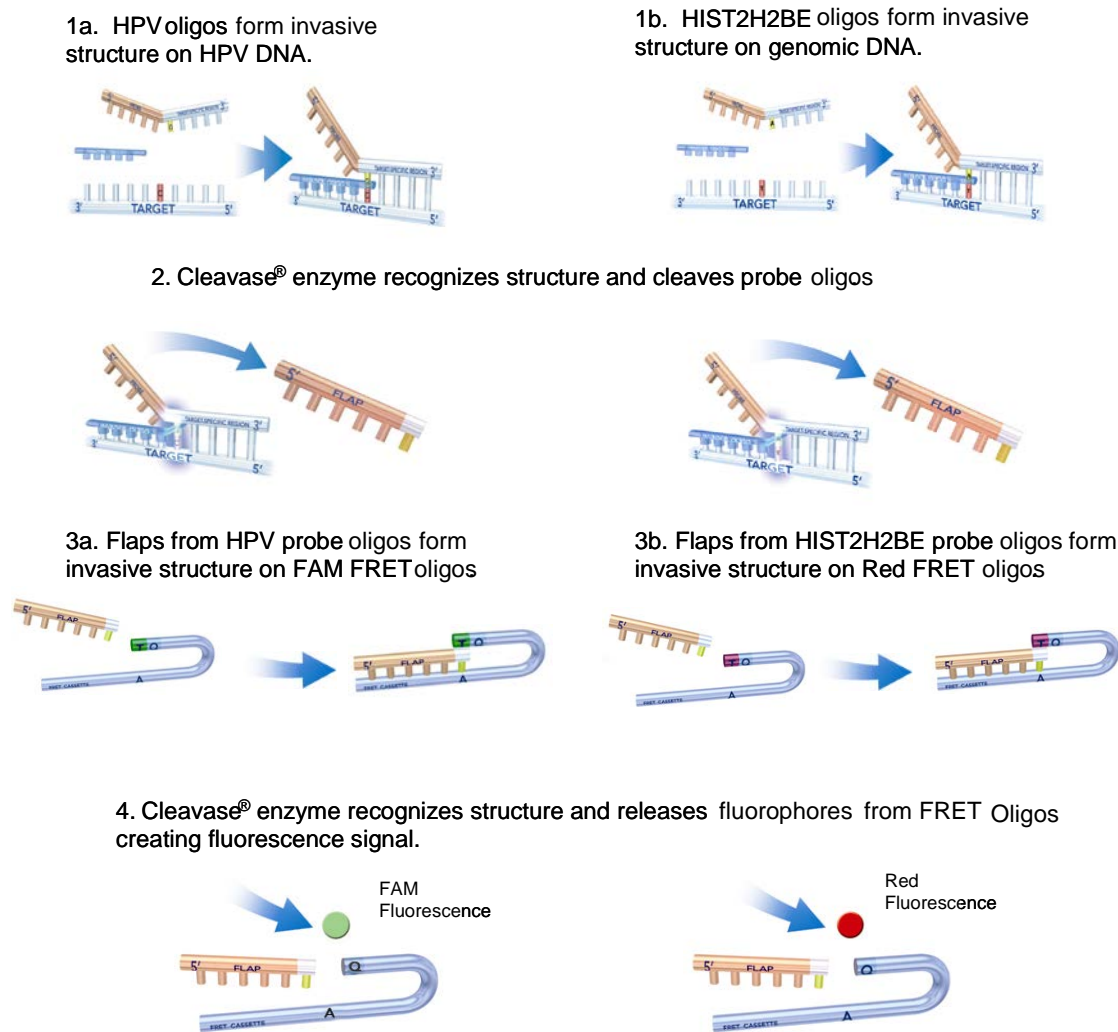
Cervista HPV 16/18 is a qualitative, in vitro diagnostic test for the detection of DNA from two high-risk HPV types: 16 and 18. The test uses the Invader chemistry, a signal amplification method for detection of specific nucleic acid sequences. The Invader technology uses two types of isothermal reactions: a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal (See Figure 1). In the primary reaction, two types of sequence specific oligonucleotides (i.e. a probe oligonucleotide and an Invader oligonucleotide) bind to the DNA target sequence. When these oligonucleotides overlap by at least one base pair on the target sequence, an invasive structure forms that acts as a substrate for the Cleavase™ enzyme. The enzyme cleaves the 5' portion (flap) of the probe at the position of the overlap.

The probes are present in large molar excess and cycle rapidly on and off the target sequence so that many cleaved 5' flaps are generated per target sequence. The cleaved flaps then bind to a universal hairpin FRET oligonucleotide creating another invasive structure that the Cleavase enzyme recognizes as a substrate. The enzyme cleaves the FRET oligonucleotides between the fluorophore and quencher molecule and produces

fluorescence signal as the cleaved flaps cycle on and off. For each copy of target, the combined primary and secondary reactions result in 106 – 107 fold signal amplification per hour.<sup>21</sup> The flap sequences and FRET oligonucleotides are universal since they are not complementary to the targeted sequence.

The reagents for this test are provided as two oligonucleotide mixtures, which detect HPV16 and HPV18. Oligonucleotides that bind to the human histone 2 gene (H2be, HIST2H2BE) are also present in these two oligonucleotide mixtures. HIST2H2BE serves as an internal control producing a signal from cellular DNA present in the sample. The format of the Cervista HPV 16/18 test allows simultaneous detection of HPV DNA sequences and HIST2H2BE in a single well by utilizing two different 5'-flap sequences on the probes as well as two different FRET oligonucleotides, each with a spectrally distinct fluorophore (FAM and Red). By design, the released 5'-flaps bind only to their respective FRET oligonucleotides to generate target-specific signal (see Figure 1).

A positive result for HPV16, HPV18 or HPV16 and HPV18 is represented by a FAM fluorescent signal that lies above an empirically derived cut-off value. For each reaction, a negative result is represented by a FAM fluorescent signal that lies below the same empirically derived cut-off value. As a means to determine the relative quantity of sample DNA in each reaction, Human HIST2H2BE is measured by a Red fluorescent signal that lies above an empirically derived cut-off value in each reaction. The measure of this target serves as a quality control mechanism to confirm that a negative result is not due to insufficient sample. This internal control target also serves as an internal processing measure to ensure that the testing procedure has been adequately performed.



**Figure 1: A graphic representation of the Invader chemistry in the Cervista HPV 16/18 test.**

## Reagents Provided

**Note:** For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at [www.hologic.com/sds](http://www.hologic.com/sds).

**Table 1:** Cervista HPV 16/18 (REF 92-0200) Contents

Reagent	Vial Label Abbreviation	Vial Quantity & Reagent Volume	Description
HPV16 Oligo Mix	O16 (Red cap and red stripe)	1 x 1400 $\mu$ L	Oligonucleotides with affinity to HPV type 16 and human HIST2H2BE suspended in water and MOPS buffer (pH 7.5)
HPV18 Oligo Mix	O18 (Teal cap and teal stripe)	1 x 1400 $\mu$ L	Oligonucleotides with affinity to HPV type 18 and human HIST2H2BE suspended in water and MOPS buffer (pH 7.5)
Cleavase Enzyme Solution	E (Purple cap and purple stripe)	1 x 1100 $\mu$ L	Cleavase Enzyme suspended in 140 mM MgCl <sub>2</sub> , 10 mM Tris (pH 8.0), 25 mM KCl, 0.25% Tween 20, 0.25% Nonidet P40, 25% Glycerol and 0.05 mg/mL BSA
HPV16 Control	C16 (Clear cap and black stripe)	1 x 350 $\mu$ L	500 copies/ $\mu$ L cloned HPV type 16 DNA and 3000 copies/ $\mu$ L cloned HIST2H2BE DNA in yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer
HPV18 Control	C18 (Clear cap and black stripe)	1 x 350 $\mu$ L	500 copies/ $\mu$ L cloned HPV type 18 DNA and 3000 copies/ $\mu$ L cloned HIST2H2BE DNA in yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer
No Target Control	NTC (Clear cap and black stripe)	1 x 350 $\mu$ L	Yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer

## Warnings and Precautions

1. For *in vitro* diagnostic use.
2. Universal safety precautions should be used when handling any human tissues or fluids. Specimens should be disposed according to local requirements.
3. Product components (product residuals, packaging) can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable national, international, and regional regulations.

## Storage and Handling Requirements

- Store all reagents between -30°C and -15°C.
- Do not use reagents past expiration date indicated on outside of package.
- Do not store in a “frost-free” freezer.
- Protect from light.
- Prior to use, remove reagents from freezer and allow them to thaw at least 30 minutes at room temperature or until visual inspection indicates that no frozen material is present.
- Vortex reagents prior to each use.
- Hologic recommends no more than ten (10) freeze-thaw cycles for all Cervista HPV 16/18 test reagents.

## Additional Reagents and Materials

Invader Call Reporter™ software is a required component of this IVD test. This software is provided once with the initial order of the Cervista HPV 16/18 test and, thereafter, when incremental updates to the software are released.

The Genfind® DNA Extraction Kit is an accessory of the Cervista HPV 16/18 test. Contact Hologic to order the Genfind DNA Extraction Kit ([REF](#) 95-449).

## Materials Required, But Not Provided

### Consumable Supplies

- Pipette tips, filter barrier and nuclease-free
- 96-well polypropylene plates
- Clear plate sealers
- Mineral oil, molecular biology grade
- 2.0 mL sterile polypropylene tubes and screw caps

### Equipment

- Pipettes
- Vortex
- Fluorescence plate reader (See Table 3)
- Desktop PC with Microsoft® Windows® XP or Windows 7 operating system with Microsoft Excel® and Adobe® Reader® software
- Thermal cycler or oven capable of maintaining recommended reaction temperatures

## Specimen Collection, DNA Extraction, and Storage For Analysis

Cervical specimens should be collected in PreservCyt Solution, the ThinPrep™ Pap Test preservation system, using a broom-type device (e.g. Rovers Cervex Brush, Wallach Papette), or Endocervical Brush/Spatula.

For Cervista HPV 16/18 testing, cervical specimens can be stored at room temperature (20-30°C) in PreservCyt Solution for up to 18 weeks prior to performing the test. PreservCyt Solution specimens cannot be frozen.

DNA should be extracted from PreservCyt specimens using the Genfind DNA Extraction Kit ([REF](#) 95-449).



## Test Procedure for Cervista HPV 16/18

**Note:** Perform DNA extraction from cervical specimens collected in PreservCyt Solution using the Genfind DNA Extraction Kit (REF 95-449) prior to beginning the reaction procedure. Residual DNA extracted as part of the Cervista HPV HR test may be used for Cervista HPV 16/18 testing.

### Reaction Procedure

1. Add 10 µL of each control and sample DNA to two wells of a 96-well plate as indicated in the test plate layout (see Figure 2).

	HPV16 Mix	HPV18 Mix	HPV16 Mix	HPV18 Mix	HPV16 Mix	HPV18 Mix	HPV16 Mix	HPV18 Mix	HPV16 Mix	HPV18 Mix	HPV16 Mix	HPV18 Mix
	1	2	3	4	5	6	7	8	9	10	11	12
A	C16	C16	S06	S06	S14	S14	S22	S22	S30	S30	S38	S38
B	C18	C18	S07	S07	S15	S15	S23	S23	S31	S31	S39	S39
C	NTC	NTC	S08	S08	S16	S16	S24	S24	S32	S32	S40	S40
D	S01	S01	S09	S09	S17	S17	S25	S25	S33	S33	S41	S41
E	S02	S02	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
F	S03	S03	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
G	S04	S04	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
H	S05	S05	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45

**Figure 2: Cervista HPV 16/18 test plate layout**

2. Overlay each well with 20 µL of mineral oil and use plate-sealing tape to minimize evaporation.
3. Incubate the samples at 95°C for 5 minutes in a thermal cycler.
4. Mix the reagents and reaction mixes thoroughly and consistently prior to use.
5. Prepare the reaction mixes as indicated in the Mix Preparation sheet (printed from the Invader Call Reporter software) or according to the calculations in Table 2. Prepare one reaction mix for each of the two HPV Oligo Mixes prior to each use.

**Table 2: Reaction Mix Preparation Instructions**

Reagent	µL/Reaction	No. of Reactions Samples and Controls ( <i>k</i> )	Total Volume
HPV Oligo Mix 16 or 18	8 µL	<i>k</i>	=8 <i>k</i> (1.25) µL
Cleavase Enzyme Solution	2 µL	<i>k</i>	=2 <i>k</i> (1.25) µL
<b>Total Mix Volume</b>	<b>10 µL</b>	<b><i>k</i></b>	<b>=10<i>k</i>(1.25) µL</b>

6. Decrease thermal cycler temperature setting to 63°C.
7. Add 10 µL of the appropriate reaction mix to each well containing a control or sample (see Figure 2), taking care to pipette below the mineral oil.
8. Incubate the plate at a 63°C setting for 4 hours.

### Data Collection

1. Always bring the plate to room temperature before reading. If the plate cannot be read immediately, store it at 2-8°C (it is recommended to read the plate within 24 hours of test completion).
2. Place the 96-well plate (well A1 must be in upper left corner) in the plate holder of the fluorescence plate reader. Remove plate-sealing tape.

3. Define the plate type to set up the coordinates and probe height for the specific type of plate. Save the settings.
4. Read the entire plate. Two separate scans are required: FAM (Excitation = 485 nm, Emission = 535 nm) and Red (Excitation = 560 nm, Emission = 612 nm). To detect the HPV signal, the instrument should be set to detect the FAM dye first. To detect the sample genomic DNA, the instrument should be set to detect the Red dye.
5. Adjust the gain of the fluorescence plate reader to be in the linear dynamic range of the reader according to the manufacturer's instructions. The gain should be set so that the No Target Control (NTC) yields values that are in the background range of the reader, with a minimum RFU of 600. The NTC values do not have to be identical for the FAM and Red reads.

**Table 3: Fluorescence Plate Reader Specifications/Settings**

Multi-Labeling Measurement Parameters	Measurement 1 (FAM)	Measurement 2 (Red)
Read Mode:	Top	Top
Excitation wavelength/Bandwidth:	485/20 nm	560/20 nm
Emission wavelength/Bandwidth:	535/25 nm	612/10 nm
Number of flashes:	10	10
Integration time:	20µs	20µs

## Procedural Notes and Precautions

1. Laboratories should use good laboratory practices and comply with all applicable federal, state and local regulatory requirements.
2. Do not pool reagents from different lots or from different vials of the same lot. These components have been tested as a unit. Do not interchange components from other sources or from different lots.
3. Do not use reagents after their expiration date.
4. Mix the samples, reagents, and reaction mixes thoroughly and consistently.
5. Use nuclease-free, sterile disposable aerosol barrier pipette tips for each addition and transfer to avoid cross-contamination.
6. Use nuclease-free, disposable polypropylene tubes for preparing the reaction mixes.
7. Verify that the 96-well plate type is compatible with the specific thermal cycler and fluorescence plate reader to be used before starting the test.
8. Controls must be added to the designated positions on the test plate layout shown in Figure 2 in order for the Invader Call Reporter software to function properly.
9. Use fresh mineral oil for each reaction setup (do not transfer these reagents back to the original container once they have been dispensed).
10. Refer to the test plate layout to ensure that the correct mix is added to the appropriate column.
11. Always place the pipette tip near the bottom of the well to ensure that the reaction mix is added below the mineral oil. Mix by carefully filling and emptying the pipette tip 3-5 times.
12. The Cervista HPV 16/18 Test Procedure, Quality Controls, and the Interpretation of Results must be followed closely to obtain reliable test results.

## Interpretation of Results

A signal to noise value (sample signal measured against signal from a No Target Control reaction well) is referred to as FOZ (Fold-Over-Zero). FOZ values are generated for both the HPV16 and HPV18 reactions. A final positive, negative or indeterminate result for any particular sample is generated based on the analysis of two separate reaction wells. When the HPV16 FOZ value and/or HPV18 FOZ value is greater than 2.13, the sample is positive for HPV16 and/or HPV18.

An indeterminate call is generated in three different scenarios 1) when the % difference between the gDNA FOZ values is  $\geq 25.0\%$  (High % difference), 2) when both HPV FOZ values are  $< 0.7$  (Low HPV FOZ) and 3) when average gDNA FOZ of a negative sample is  $< 1.5$  (low gDNA). An indeterminate call is indicative of insufficient mixing, a pipetting error or inadequate gDNA in the sample (see Troubleshooting Guide).

A summary of the sample call criteria described above is shown in Figure 3.

### Terminology

<p><b>HPV FOZ:</b> For each HPV Oligo Mix, the FAM signal of the sample divided by the FAM signal of the No Target Control.</p> <p><b>Average gDNA FOZ:</b> The average value determined from the two genomic DNA FOZ values obtained from both of the reaction mixes, calculated by dividing the Red signal of the sample by the Red signal of the No Target Control.</p> <p><b>%Difference gDNA FOZ:</b> The absolute value of the difference between the HPV16 and HPV18 genomic DNA FOZ values divided by the average genomic DNA FOZ value of the two HPV Oligo Mixes.</p>
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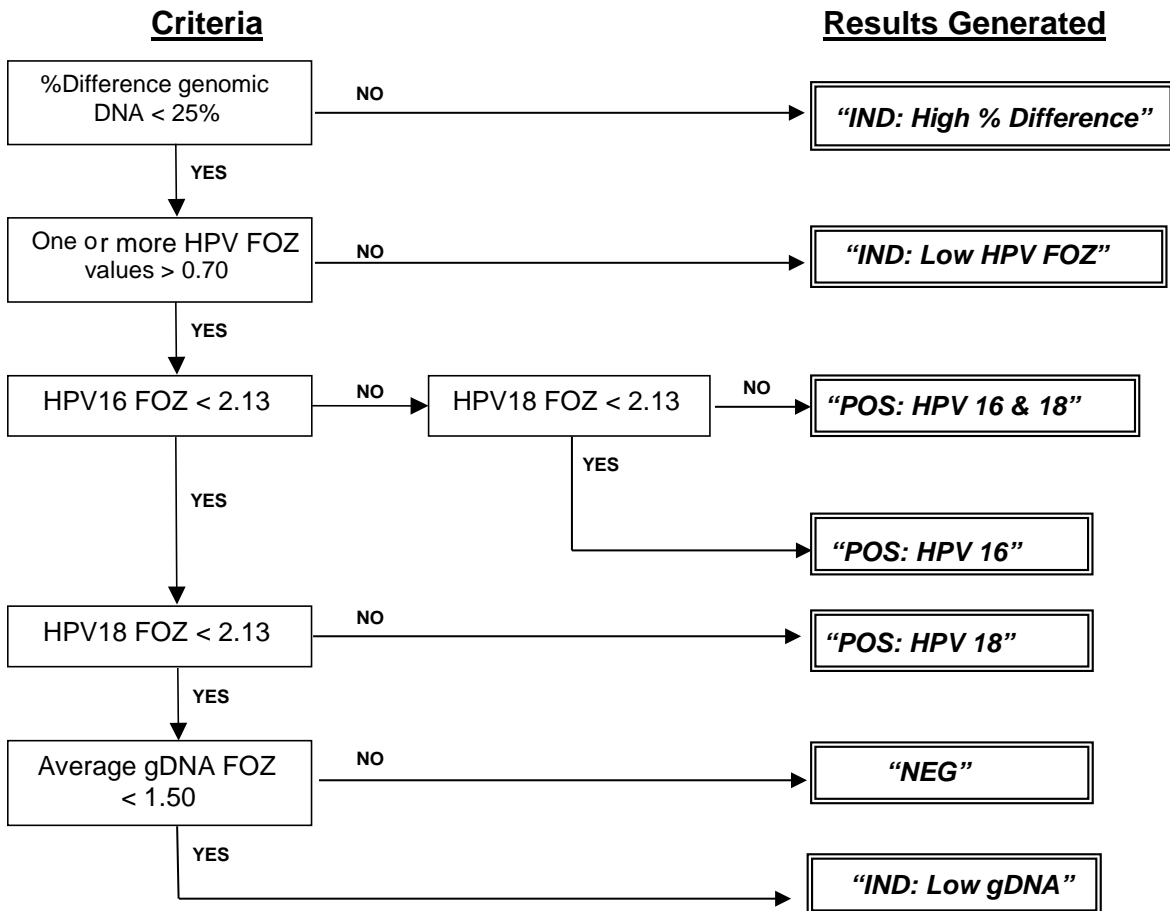


Figure 3: Cervista HPV 16/18 Sample Call Criteria Ordered Top to Bottom

**Note:** The Cervista HPV 16/18 test does not require the use of an equivocal or re-test zone.

**Table 4:** Interpretation of Cervista HPV 16/18 Test Results when High-risk (HR) HPV Results are Positive<sup>b</sup>.

Cervista HPV16/18 Test Result <sup>c</sup>	Result Report	Interpretation for patients with NILM cytology who are ≥30 years old <sup>a</sup>	Interpretation for patients with ASC-US cytology
POS:HPV16	HPV type 16 detected	Low but increased likelihood that underlying high-grade CIN will be detected at colposcopy. Medical literature suggests that progression to high-grade disease is possible. <sup>3,10,11,19</sup>	Increased likelihood that underlying high-grade CIN will be detected at colposcopy. <sup>2,6,8,9</sup>
POS:HPV18	HPV type 18 detected		
POS: HPV16 and HPV18	HPV types 16 and 18 detected		
NEG <sup>d</sup>	HPV types 16 and/or 18 not detected	Low likelihood of underlying CIN2-3 or cancer; results are not intended to prevent women from further cytology or HPV retesting. <sup>3,10,11,19</sup>	Likelihood of underlying CIN2-3 or cancer is lower, but infection with other non-16/18 high-risk HPV types still confers risk. Results are not intended to prevent women from proceeding to colposcopy. <sup>2, 6, 8, 9</sup>
IND: High % CV	Indeterminate	HPV16/18 status unknown	
IND: Low gDNA			

<sup>a</sup>According to the 2006 consensus guidelines, women 30 years and older with greater than ASC-US cytology (including ASC-H, LSIL or above) should proceed to colposcopy regardless of their HPV test results.

<sup>b</sup>In cases where HPV HR and HPV16/18 are run at the same time and a HR negative result is obtained alongside a 16/18 positive result, the 16/18 result is not interpretable. If both test results are negative, interpret the results the same as you would a HR negative result.

<sup>c</sup>The Cervista HPV 16/18 test does not determine whether high-risk HPV types other than 16/18 are present. An individual may be simultaneously infected with multiple HPV types.

<sup>d</sup>Individuals who are Cervista HPV HR positive and Cervista HPV 16/18 negative are most likely infected with a non-16/18 high-risk HPV type.

## Quality Control

### Internal Control

The Cervista HPV 16/18 test includes an internal control which determines the relative quantity of sample DNA in each reaction. The internal control, Human HIST2H2BE, is measured by a Red fluorescent signal that lies above an empirically derived cut-off value in each reaction. The measure of this target serves as a quality control mechanism to confirm that a negative result is not due to insufficient sample. This internal control target also serves as an internal processing measure to ensure that the testing procedure has been adequately performed.

### External Controls

#### Negative Control

The No Target Control must be run on each assay plate and results must meet the following criteria in order for the samples on that plate to be valid. If it does not meet these criteria, the samples and controls on that plate are invalid and must be repeated (see Table 5 for summary):

1. The minimum signal for each mix must be greater than or equal to 600 RFU ( $\geq 600$ ).
2. The % Difference between the gDNA signals from both mixes must be less than 30.0% ( $<30.0\%$ ).

**Table 5:** No Target Control Criteria

Result	Min. HPV Signal	Min. gDNA Signal	Max. % Difference (gDNA)
Valid	600	600	29.9%

## Positive Controls

HPV controls (HPV16, HPV18) must be run on each assay plate and results must meet the following criteria for the test to be valid. If controls do not meet these criteria, the samples on that plate are also invalid and testing must be repeated (see Table 6 for summary).

1. The HPV FOZ value is determined by dividing the FAM signal of the control by the FAM signal of the No Target Control for each respective mix. The HPV16 Control should yield a positive HPV FOZ value ( $\geq 2.13$ ) for only the HPV16 Oligo Mix and the HPV18 Control should yield a positive HPV FOZ value ( $\geq 2.13$ ) for only the HPV18 Oligo Mix.
2. The average gDNA FOZ of the two mixes must be greater than or equal to 1.50 ( $\geq 1.50$ ), or the control is invalid for low genomic DNA.
3. The % Difference between the gDNA FOZ values from both mixes should be less than 25.0% ( $<25.0\%$ ).

**Table 6:** HPV Control Criteria

Control	Result	HPV16 FOZ	HPV18 FOZ	Average gDNA FOZ	% Difference gDNA FOZ
HPV16 Control	Valid Control	$\geq 2.13$	$\leq 2.13$	$\geq 1.50$	$< 25.0\%$
HPV18 Control	Valid Control	$\leq 2.13$	$\geq 2.13$	$\geq 1.50$	$< 25.0\%$

**Note:** Additional external controls may be tested according to guidelines or requirements of local, state, and/or country regulations or accrediting organizations. Any additional external controls should be tested in well(s) designated for patient samples per the plate layout.

## Test Verification

1. Sample results are valid when the positive and negative controls yield the correct results. If the No Target Control (negative control) is invalid and/or any result for positive control(s) is invalid, all sample results on that plate are invalid and must be repeated. Refer to the Troubleshooting sections located in this insert and in the User Manual for Invader Call Reporter software.
2. All quality control requirements should be performed in conformance with applicable national, international, and regional regulations.

## Limitations

1. The Cervista HPV 16/18 test only detects DNA of HPV types 16 and 18. This test does not detect high-risk HPV types 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Nor does it detect HPV low-risk types since there is no known clinical utility for testing Low-Risk HPV types.<sup>19</sup>
2. The Cervista HPV 16/18 test exhibits cross-reactivity to high levels of HPV High-Risk type 31. An HPV16 positive result was observed with  $10^7$  copies/reaction of HPV type 31.
3. A negative result does not exclude the possibility of HPV16 and/or 18 infection because very low levels of infection or sampling error may cause a false-negative result.
4. The test has been validated for use with cervical cytology specimens collected in PreservCyt Solution using a Rovers Cervex Brush, Wallach Papette, or Endocervical Brush/Spatula.
5. The performance of the Cervista HPV 16/18 test was established using DNA extracted with the Genfind DNA Extraction Kit.
6. The performance of the Cervista HPV 16/18 test was established using cervical cytology PreservCyt specimens processed on the ThinPrep 2000 processor, it has not been established using other processors.

7. The performance of the Cervista HPV 16/18 test has not been adequately established for HPV vaccinated individuals.
8. Interference was observed in cervical specimens contaminated with high levels (2%) of contraceptive jelly and/or anti-fungal creams when DNA was isolated with the Genfind DNA Extraction Kit. Under these conditions, false-negative results may be obtained.
9. The Cervista HPV 16/18 DNA Test for human papillomavirus types 16 and 18 is not recommended for evaluation of suspected sexual abuse.
10. 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.
11. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2-3 or cancer.
12. A negative HPV 16/18 result does not exclude the possibility of future cytologic HSIL or underlying CIN2-3 or cancer.
13. PreservCyt Solution specimens containing volumes less than 2 mL after the ThinPrep Pap Test slides are prepared are considered inadequate for the Cervista HPV 16/18 test.

## Expected Results

### High-Risk HPV16 and HPV18 Prevalence

The reported prevalence of HPV infection in women ranges widely, from 14% to more than 90%.<sup>22</sup> Several factors can affect the HPV prevalence among patient populations due to heterogeneity in geographic location, age, number of sexual partners, history of abnormal cervical cytology, coupled with differences in sampling techniques and testing methods and the intermittent nature of the infection. The Cervista HPV 16/18 multi-center prospective clinical study enrolled women from 89 clinical sites across 23 states throughout the United States which produced a demographically diverse patient population. Tables 7 and 8 show the prevalence of HPV16 and HPV18 observed in the study stratified by age.

**Table 7:** Prevalence of HPV16 and HPV18 Among Women with ASC-US Cytology Stratified by Age

Age Group	Prevalence of HPV16	Prevalence of HPV18	Prevalence of HPV16 and 18 Positive
18 < 21	31% (40/129)	7.8% (10/129)	1.6% (2/129)
21 < 30	23.7% (117/493)	6.3% (31/493)	1.6% (8/493)
30 < 39	11.9% (37/312)	2.6% (8/312)	0.6% (2/312)
39 < 49	8.3% (22/266)	1.9% (5/266)	0% (0/266)
49 < 59	5.9% (7/118)	1.7% (2/118)	0% (0/118)
≥ 59	13.3% (6/45)	2.2% (1/45)	0% (0/45)
All	16.8% (229/1363)	4.2% (57/1363)	0.9% (12/1363)

**Table 8:** Prevalence of HPV16 and HPV18 Among Women with NILM Cytology Stratified by Age

Age Group	Prevalence of HPV16	Prevalence of HPV18	Prevalence of HPV16 and 18 Positive
30 < 40	3.4% (21/616)	0.8% (5/616)	0.2% (1/616)
40 < 50	4.0% (27/674)	0.7% (5/674)	0% (0/674)
50 < 60	5.1% (25/486)	1.0% (5/486)	0.2% (1/486)
60 < 70	3.9% (6/154)	0% (0/154)	0% (0/154)
≥70	0% (0/30)	0% (0/30)	0% (0/30)
All	4.0% (79/1960)	0.8% (15/1960)	0.1% (2/1960)

## Performance Characteristics

### Clinical Sensitivity and Specificity of Cervista HPV 16/18 Among Women with ASC-US Cervical Cytology Results

A multi-center prospective clinical study was conducted to evaluate the performance of the Cervista HPV 16/18 test among patients with ASC-US cytology results to determine the need for referral to colposcopy. All clinical performance characteristics were established using ThinPrep liquid cytology specimens. Initial ThinPrep cervical specimens were classified according to the 2001 Bethesda System Classification. All women (18 years or older) with cytology results of ASC-US during routine cervical cancer screening procedures were invited to participate in the study prior to learning their HPV status. For women who consented, their initial residual ASC-US ThinPrep specimens were subsequently obtained for Cervista HPV 16/18 testing. All patients who consented to the study underwent colposcopic examination. Investigators and patients remained blinded to the patient's HPV status until after completion of the colposcopic procedures, to avoid bias. Colposcopically directed histological specimens were examined by pathologists who were also blinded to the patient's HPV status. 1,514 women age 18 and over with ASC-US results were ultimately enrolled in the study from 89 clinical sites across the United States.

The clinical performance of the Cervista HPV 16/18 test was measured against colposcopy and histology results. Biopsy samples were collected from the women with ASC-US cytology as warranted by standard of care guidelines at each participating clinical site. Consensus histology results provided by a central pathologist review panel served as the "gold standard" for determining the presence or absence of disease. In the absence of histology data, the lack of colposcopically visible cervical lesions and no biopsy equated to the absence of disease.

There were 1,312 ASC-US subjects with known disease status (central histology or negative colposcopy) and Cervista HPV HR and Cervista HPV 16/18 results. The clinical performance characteristics of the Cervista HPV 16/18 test are shown in Tables 9-15.

**Table 9: Cervista HPV 16/18 Results as Compared to Colposcopy/Central Histology Results among Women with ASC-US Cytology**

Cervista HPV HR Result	Cervista HPV 16/18 Result	Disease(Central Histology)					Total
		Neg Colposcopy No Biopsy	No CIN	CIN1	CIN2	CIN3	
HPV HR Positive	HPV16 Positive	39	83	40	25	14	201
	HPV18 Positive	11	22	9	0	1	43
	HPV16 & 18 Positive	1	3	5	2	2	13
	HPV16 & 18 Negative	109	273	98	15	5	500
HPV HR Negative	HPV16 and/or 18 Positive	3	3	1	0	0	7
	HPV16 & 18 Negative	210	304	29	5	0	548
<b>Total</b>		<b>373</b>	<b>688</b>	<b>182</b>	<b>47</b>	<b>22</b>	<b>1312</b>

Among those with Cervista HPV HR determinate results and disease status data, percent of Indeterminate Cervista HPV 16/18 results in the clinical study of women with ASC-US cytology was 0% (0/1312) with 95% CI: 0% to 0.3%.

**Table 10:** Cervista HPV 16/18 versus Colposcopy /Consensus Histology Results ( $\geq$ CIN2), among Women with ASC-US Cytology

Cervista HPV HR Result	Cervista HPV 16/18 Result	$\geq$ CIN2		Total
		Positive	Negative	
HPV HR Positive	HPV16 Positive	39	162	201
	HPV18 Positive	1	42	43
	HPV16 & 18 Positive	4	9	13
	HPV16 & 18 Negative	20	480	500
HPV HR Negative	HPV16 and/or 18 Positive	0	7	7
	HPV16 & 18 Negative	5	543	548
Total		69	1243	1312

**Table 11:** Risks of  $\geq$  CIN2 for Different Outcomes of Cervista HPV HR and Cervista HPV16/18 TestsPrevalence of  $\geq$  CIN2: 5.3%

Cervista HPV HR Result	Cervista HPV 16/18 Result	Risk	95% CI		Likelihood Ratio	95% CI	
HPV HR Positive	HPV16 and/or 18 Positive	17.1% (44/257)	13.0%	22.2%	3.72	2.93	4.54
	HPV16 & 18 Negative	4.0% (20/500)	2.6%	6.1%	0.75	0.51	1.06
HPV HR Negative	HPV16 & 18 Negative	0.9% (5/548)	0.4%	2.1%	0.17	0.07	0.36

**Table 12:** Performance of the Cervista HPV16/18 Test for Women with Cervista HPV HR Positive Results:Prevalence of  $\geq$ CIN2 among Women with Cervista HPV Positive Results: 8.5%

		95% CI
Sensitivity	68.8% (44/64)	56.6% to 78.8%
Specificity	69.3% (480/693)	65.7% to 72.6%

**Table 13:** Cervista HPV 16/18 versus Colposcopy / Consensus Histology Results ( $\geq$  CIN3), among Women with ASC-US Cytology

Cervista HPV HR Result	Cervista HPV 16/18 Result	$\geq$ CIN3		Total
		Positive	Negative	
HPV HR Positive	HPV16 Positive	14	187	201
	HPV18 Positive	1	42	43
	HPV16 & 18 Positive	2	11	13
	HPV16 & 18 Negative	5	495	500
HPV HR Negative	HPV16 and/or 18 Positive	0	7	7
	HPV16 & 18 Negative	0	548	548
Total		22	1290	1312



**Table 14: Risks of ≥CIN3 for Different Outcomes of Cervista HPV HR and Cervista HPV16/18 Tests**

Prevalence of ≥CIN3: 1.7%

Cervista HPV HR Result	Cervista HPV 16/18 Result	Risk	95% CI		Likelihood Ratio	95% CI	
HPV HR Positive	HPV16 and/or 18 Positive	6.6% (17/257)	4.2%	10.3%	4.15	2.99	5.08
	HPV16 & 18 Negative	1.0% (5/500)	0.4%	2.3%	0.59	0.26	1.14
HPV HR Negative	HPV16 & 18 Negative	0.0% (0/548)	0.0%	0.7%	0.00	0.00	0.37

**Table 15: Performance of the Cervista HPV16/18 Test for Women with Cervista HPV HR Positive Results:**

Prevalence of ≥CIN3 among the Subjects with Cervista HPV Positive Results: 2.9%

		95% CI
<b>Sensitivity</b>	77.3%(17/22)	56.6% to 89.9%
<b>Specificity</b>	67.3%(495/735)	63.9% to 70.6%

**Table 16:** Clinical Performance of the Cervista HPV 16/18 Test Stratified by Age for Women with Cervista HPV HR Positive Results

		Central Histology ≥ CIN2		
<b>Age: 18 to &lt;21</b>		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
HPV HR Positive	HPV16 and/or 18 Positive	7	39	46
	HPV16 & 18 Negative	2	54	56
HPV HR Negative		0	23	23
Total		9	116	125
<b>Disease Prevalence<sup>a</sup>:</b>	8.8% (9/102)	95% CI		
<b>Sensitivity:</b>	77.8% (7/9)	40.0% to 97.2%		
<b>Specificity:</b>	58.1% (54/93)	47.4% to 68.22%		
<b>Age: 21 to &lt;30</b>		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
HPV HR Positive	HPV16 and/or 18 Positive	21	117	138
	HPV16 & 18 Negative	9	197	206
HPV HR Negative		0	138	138
Total		30	452	482
<b>Disease Prevalence<sup>a</sup>:</b>	8.7% (30/344)	95% CI		
<b>Sensitivity:</b>	70.0% (21/30)	50.6% to 85.3%		
<b>Specificity:</b>	62.7% (197/314)	57.1% to 68.1%		
<b>Age: 30 to &lt;39</b>		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
HPV HR Positive	HPV16 and/or 18 Positive	7	30	37
	HPV16 & 18 Negative	3	126	129
HPV HR Negative		3	125	128
Total		13	281	294
<b>Disease Prevalence<sup>a</sup>:</b>	6.0% (10/166)	95% CI		
<b>Sensitivity:</b>	70.0% (7/10)	34.8% to 93.3%		
<b>Specificity:</b>	80.8% (126/156)	73.7% to 86.6%		
<b>Age: 39 or older</b>		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
HPV HR Positive	HPV16 and/or 18 Positive	9	27	36
	HPV16 & 18 Negative	6	103	109
HPV HR Negative		2	264	266
Total		17	394	411
<b>Disease Prevalence<sup>a</sup>:</b>	10.3% (15/145)	95% CI		
<b>Sensitivity:</b>	60.0% (9/15)	32.3% to 83.7%		
<b>Specificity:</b>	79.2% (103/130)	71.2% to 85.8%		

<sup>a</sup>Prevalence of ≥ CIN2 among women with Cervista HPV HR Positive Results

## Performance of the Cervista HPV 16/18 Test as a Reflex HPV Test

### Performance of the Cervista HPV 16/18 Test as a Reflex HPV Test to Help Guide Patient Management In Women 30 Years and Older With NILM Cytology

A longitudinal 3 year post-approval study has been initiated to support the use of the Cervista HPV 16/18 test as a reflex test in women 30 years of age and older with normal cytology and positive HPV HR test results. The study design is described below, along with preliminary analytical data obtained from the study population at enrollment. This analytical study was used for evaluation of agreement of the Cervista HPV16/18 test with DNA sequencing as a comparator for HPV detection in the ASC-US and NILM ≥30 populations. Approval for this indication is being given prior to completion of the longitudinal studies in light of the analytical study results. Additionally, consistent data obtained from multiple cross-sectional and prospective cohort studies conducted

with a variety of cell sampling methods and utilizing a variety of HPV DNA testing methods (both FDA approved, and research grade) provide strong evidence that a negative HPV DNA test implies very low risk of prevalent or incipient CIN2-3 or cancer when cervical cytology are normal.<sup>5,8,12,23</sup> Furthermore, the absence of HPV16 and 18 in this population of women further reduces the risk of developing cervical disease and conversely the presence of HPV16 or HPV18 augments the relative risk of cervical disease among women  $\geq 30$  years of age regardless of NILM cytological findings.<sup>10,11,13,24</sup>

## Description of NILM $\geq 30$ clinical study

Approximately 2,000 qualified subjects with normal Pap test results (NILM) have been enrolled from 26 active clinical centers throughout the United States. It is anticipated that not less than 1,000 subjects will have 3-year follow-up data. The subject retention rate at the end of the first year of follow-up has been nearly 80%. Subjects will be followed for 3 years and have annual study visits. At each follow-up visit, a cervical cytology test is performed. Women who have ASC-US or higher grade cytology results will have a colposcopy performed, and subsequently a biopsy if needed. Analysis of these data will focus on the three-year risk of cervical disease associated with NILM subjects positive for Cervista HPV 16/18 as compared to those negative for the test at the time of enrollment ( $T_0$ ) and also the three-year risk of cervical disease associated with NILM subjects positive for Cervista HPV 16/18 as compared to those negative for any HPV high-risk type at the time of enrollment ( $T_0$ ). The presence or absence of HPV at  $T_0$ , will be compared against the presence or absence of (a)  $>$  CIN2 and (b)  $>$  CIN3 throughout the study. The presence of CIN2, CIN3 or cervical cancer will be ascertained by central histology. Negative results will be defined by colposcopy unless central histology results are available to supersede an initial positive colposcopic indication. All histological interpretation will be conducted by a central pathology review panel.

## Comparison of DNA Sequencing and Cervista HPV16/18 for the ASC-US and NILM $\geq 30$ Populations

Residual DNA samples from both the ASC-US and NILM subjects were used for PCR amplification and sequencing. DNA samples were amplified using consensus primers for the HPV L1 gene. A portion of the human beta-globin gene was also amplified as an internal control. Purified amplicons were used as templates in multiple sequencing reactions for 14 high-risk types of HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The sequencing data was analyzed using various sequence alignment software.

Below is a comparison between Cervista HPV 16/18 and DNA sequencing for the detection of HPV16 and 18 in both ASC-US and NILM  $\geq 30$  populations:

**Table 17: Performance of Cervista HPV16/18 and PCR Sequencing Results, NILM  $\geq 30$**

Cervista Result	PCR Sequencing										Total
	HR Indeterminate	HR Negative	One HR Type			Two HR Types			Mult. HR Types		
			16	18	Other	16&18	Other	18&Other	Others	16&Other	
<b>HPV HR Positive</b>											
HPV16 Positive	1	48	8	0	6	0	2	0	0	1	66
HPV18 Positive	0	2	0	6	0	0	0	0	0	0	8
HPV16 and 18 Positive	0	1	0	0	1	0	0	0	0	0	2
HPV16 and 18 Negative	12	203	1	0	60	0	0	0	3	0	279
<b>HPV HR Negative</b>											
HPV16 Positive	0	12	1	0	0	0	0	0	0	0	13
HPV18 Positive	0	6	0	0	0	0	0	0	0	0	6
HPV16 and 18 Negative	39	1512	2	0	6	0	0	0	0	0	1559
<b>Total</b>	<b>52</b>	<b>1784</b>	<b>12</b>	<b>6</b>	<b>73</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>3</b>	<b>1</b>	<b>1933</b>

Among those with Cervista HPV HR determinate results and PCR Sequencing samples, percent of Indeterminate Cervista HPV 16/18 results for women with NILM cytology was 0% (0/1933) with 95% CI: 0% to 0.2%.

**Table 18:** Comparison of Cervista HPV16 and/or HPV18 Results vs PCR Sequencing Results for Women with Cervista HPV HR Positive Results, NILM  $\geq 30$

Cervista HPV HR Positive:	PCR Sequencing HR Positive HPV16 and/or HPV18		Total
	Positive	Negative	
Cervista HPV 16 and/or 18 Positive	17	58	75
Cervista HPV 16 & 18 Negative	1	266	267
<b>Total</b>	<b>18</b>	<b>324</b>	<b>342</b>

Positive Percent Agreement and Negative Percent Agreement			
Agreement	Percent	95% CI	
Positive % Agreement	94.4% (17/18)	74.2%	99.0%
Negative % Agreement	82.1% (266/324)	77.6%	85.9%

**Table 19:** Performance of Cervista HPV16/18 and PCR Sequencing Results, ASC-US

Cervista Result	HR IND	HR NEG	PCR Sequencing											Total	
			One HR Type			Two HR Types			Multiple HR Types						
			16	18	Other	16 & 18	16 & Other	18 & Other	Others	16 & 18 & Other	16 & Other	18 & Other	Other s		
<b>HPV HR Positive</b>															
HPV16 Positive	7	25	96	0	41	0	29	0	1	0	6	0	0	205	
HPV18 Positive	0	0	0	27	7	0	0	9	0	0	0	0	0	43	
HPV16 and 18 Positive	0	1	2	0	1	2	1	1	0	3	0	1	1	13	
HPV16 and 18 Negative	32	95	6	2	335	0	1	0	27	0	2	0	3	503	
HPV16 and/or 18 Indeterminate	2	1	0	0	7	0	0	0	2	0	0	0	0	12	
<b>HPV HR Negative</b>															
HPV16 Positive	1	3	2	0	0	0	0	0	0	0	0	0	0	6	
HPV16 and 18 Negative	35	510	9	0	10	0	0	0	1	0	0	0	0	565	
HPV16 and/or 18 Indeterminate	1	5	0	0	1	0	0	0	0	0	0	0	0	7	
<b>Total</b>	<b>78</b>	<b>640</b>	<b>115</b>	<b>29</b>	<b>402</b>	<b>2</b>	<b>31</b>	<b>10</b>	<b>31</b>	<b>3</b>	<b>8</b>	<b>1</b>	<b>4</b>	<b>1354</b>	

Among those with Cervista HPV HR determinate results and PCR Sequencing samples, the percent of Indeterminate Cervista HPV 16/18 results for women with ASC-US cytology was 1.4% (19/1354) with 95% CI: 0.9% to 2.2%.

**Table 20:** Comparison of Cervista HPV16 and/or HPV18 Results vs PCR Sequencing Results for Women with Cervista HPV HR Positive Results, ASC-US

Cervista HPV HR Positive:	PCR Sequencing, HR Positive HPV16 and/or HPV18		Total
	Positive	Negative	
Cervista HPV 16 and/or 18 Positive	177	77	254
Cervista HPV 16 & 18 Negative	11	460	471
<b>Total</b>	<b>188</b>	<b>537</b>	<b>725</b>

**Positive Percent Agreement and Negative Percent Agreement**

Agreement	Percent	95% Score CI	
Positive % Agreement	94.1% (177/188)	89.8%	96.7%
Negative % Agreement	85.7% (460/537)	82.4%	88.4%

**Analytical Sensitivity**

Cloned HPV plasmid DNA, representing the HPV types 16 and 18 detected by the Cervista HPV 16/18 test, was tested to determine the individual analytical sensitivity for each specific type.

Nine HPV-negative characterized DNA samples isolated from cervical specimens were tested in replicates of eight (9 samples x 8 replicates/sample = 72 data points) to determine the Limit of Blank (LoB). The LoB values (FAM FOZ) were 1.18 and 1.21 from HPV16 and HPV18 respectively.

Limit of Detection (LoD) is the lowest amount of analyte in a sample that the sample has the test results "HPV16 or HPV18 detected" at least 95% of the time (results of the test are above the analytical cut-off 95% of the time). Individual Limit of Detection (LoD) values were calculated for both HPV types (16, 18). Each HPV plasmid DNA was tested at concentrations of 5000, 2500, 1250, and 625 copies per reaction, each in a background of three genomic DNA concentrations isolated from an HPV-negative cell line (10 ng, 100 ng, and 1 µg per reaction). All positive samples were tested in replicates of eight resulting in 24 replicates per HPV plasmid DNA concentration.

The LoB and LoD were evaluated according to the CLSI document EP17-A.<sup>25</sup>

The Limit of Detection for each HPV type is referenced in Table 21. Limits are described in terms of the FAM FOZ and as a copy number range. Copy number per reaction LoD values were reported as the copy number range in which 95% of the observed FAM FOZ values were above the LoB.

**Table 21:** Cervista HPV 16/18 Test Analytical Sensitivity Summary

HPV DNA Type	LoD (Copy Number/Reaction)	LoD (FAM FOZ)	SDs
16	625-1250	1.34	0.10
18	625-1250	1.33	0.07

In addition to the analytical sensitivity study described above, cell line dilutions were prepared to evaluate the performance of the HPV16/18 assay using two HPV positive cell lines (HeLa and SiHa) diluted with a HPV negative cell line (Jurkat) to a final concentration of 100,000 cells/mL in PreservCyt media. DNA was isolated from the cell line samples using the Genfind DNA Extraction Kit. Using a clinical HPV16 and HPV18 FOZ cut-off of 2.13, concentrations of approximately 2,500 cells/mL for both SiHa and HeLa cells were above the clinical cut-off 95% of the time.

## Clinical Cut-off of the Cervista HPV 16/18 test

The clinical cut-off was evaluated based on HPV16/18 test results targeting a 5% positive rate in the NILM  $\geq 30$  population from a multi-center clinical study. The 95th percentile of the maximum HPV16 and HPV18 FOZ values was determined for NILM  $\geq 30$  subjects and based on this analysis, a FOZ value of  $\geq 2.13$  was selected as the positive cut-off value for the Cervista HPV 16/18 test.

## Precision

Repeatability and within-laboratory precision of the Cervista HPV 16/18 test was demonstrated in a 21-day study with three alternating operators, each performing two runs per day on individually assigned sets of equipment. Each run consisted of one plate. Different plate layouts were used for the runs within a day. The procedure followed CLSI EP5-A2.

Each run consisted of genomic DNA samples isolated from two HPV positive cell lines (SiHa – Type 16 and HeLa – Type 18), a HPV negative cell line (Jurkat) and contrived samples containing HPV16 or HPV18 plasmid DNA and Jurkat DNA. Each sample was tested in duplicate. The total number of measurements per sample was 84 (21 days, 2 runs per day, 2 replicates per run).

The repeatability and within-laboratory precision values were calculated for each target at each concentration. The precision values for HPV16 FOZ are shown in Table 22 and the HPV18 FOZ values are shown in Table 23. A summary of positive HPV16 and positive HPV18 results are shown in Tables 24 and 25 respectively.

**Table 22: HPV16 Precision Values for Each Target and Concentration**

Target	Copies/Reaction <sup>a</sup> or Cells/mL <sup>b</sup>	N	Mean HPV16 FOZ	Within-Run (repeatability)		Between-Run		Between- Day		Between- Operator		Total (Within-lab precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV16	5,000 <sup>a</sup>	84	3.708	0.196	5%	0.238	6%	0.348	9%	0.364	10%	0.411	11%
	20,000 <sup>a</sup>	84	7.397	0.697	9%	0.460	6%	0.390	5%	0.331	4%	0.708	10%
HPV18	5,000 <sup>a</sup>	84	1.021	0.028	3%	0.042	4%	0.031	3%	0.027	3%	0.047	5%
	20,000 <sup>a</sup>	84	1.024	0.041	4%	0.069	7%	0.045	4%	0.048	5%	0.073	7%
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat <sup>b</sup>	84	2.430	0.160	7%	0.115	5%	0.138	6%	0.135	6%	0.196	8%
	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	5.465	0.220	4%	0.360	7%	0.384	7%	0.324	6%	0.486	9%
HeLa/Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	0.784	0.029	4%	0.047	6%	0.049	6%	0.048	6%	0.063	8%
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	0.893	0.037	4%	0.037	4%	0.039	4%	0.036	4%	0.053	6%
Jurkat	10,000 <sup>b</sup>	84	0.886	0.111	12%	0.074	8%	0.064	7%	0.029	3%	0.114	13%
	20,000 <sup>b</sup>	84	0.870	0.029	3%	0.035	4%	0.030	3%	0.023	3%	0.044	5%
	100,000 <sup>b</sup>	84	0.917	0.066	7%	0.042	5%	0.042	5%	0.039	4%	0.070	8%

<sup>a</sup> HPV16 or HPV18 plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

<sup>b</sup> Genomic DNA isolated from HPV positive cells (SiHa and HeLa) and/or HPV negative cells (Jurkat) at the indicated concentration (cells/mL).

**Table 23: HPV18 Precision Values for Each Target and Concentration**

Target	Copies/Reaction or Cells/mL <sup>b</sup>	N	Mean HPV18 FOZ	Within-Run (repeatability)		Between- Run		Between- Day		Between- Operator		Total (Within- lab precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV16	5,000 <sup>a</sup>	84	0.978	0.041	4%	0.055	6%	0.059	6%	0.050	5%	0.076	8%
	20,000 <sup>a</sup>	84	0.990	0.055	6%	0.068	7%	0.062	6%	0.043	4%	0.087	9%
HPV18	5,000 <sup>a</sup>	84	3.620	0.243	7%	0.255	7%	0.265	7%	0.230	6%	0.363	10%
	20,000 <sup>a</sup>	84	8.483	0.396	5%	0.613	7%	0.595	7%	0.378	4%	0.787	9%
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat <sup>b</sup>	84	0.874	0.051	6%	0.035	4%	0.045	5%	0.043	5%	0.062	7%
	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	0.858	0.023	3%	0.052	6%	0.043	5%	0.044	5%	0.059	7%
Hela/Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	2.988	0.163	5%	0.174	6%	0.175	6%	0.064	2%	0.243	8%
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	7.918	0.427	5%	1.466	19%	1.757	22%	0.463	6%	2.062	26%
Jurkat	10,000 <sup>b</sup>	84	0.927	0.055	6%	0.054	6%	0.055	6%	0.043	5%	0.077	8%
	20,000 <sup>b</sup>	84	0.920	0.035	4%	0.038	4%	0.035	4%	0.027	3%	0.051	6%
	100,000 <sup>b</sup>	84	0.951	0.054	6%	0.042	4%	0.036	4%	0.031	3%	0.060	6%

<sup>a</sup> HPV16 or HPV18 plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

<sup>b</sup> Genomic DNA isolated from HPV positive cells (SiHa and HeLa) and/or HPV negative cells (Jurkat) at the indicated concentration (cells/mL).



**Table 24:** Summary of Positive HPV16 Results for Precision Study.

Target	Copies/Reaction <sup>a</sup> or Cells/mL <sup>b</sup>	N	Mean HPV16 FOZ	HPV16 Positive % (n)			Total
				Operator 1	Operator 2	Operator 3	
HPV16	5,000 <sup>a</sup>	84	3.708	100% (28)	100% (28)	100% (28)	100% (84)
	20,000 <sup>a</sup>	84	7.397	100% (28)	100% (28)	100% (28)	100% (84)
HPV18	5,000 <sup>a</sup>	84	1.021	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 <sup>a</sup>	84	1.024	0% (0)	0% (0)	0% (0)	0% (0)
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat <sup>b</sup>	84	2.430	82% (23)	100% (28)	100% (28)	94% (79)
	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	5.465	100% (28)	100% (28)	100% (28)	100% (84)
Hela/Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	0.784	0% (0)	0% (0)	0% (0)	0% (0)
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	0.893	0% (0)	0% (0)	0% (0)	0% (0)
Jurkat	10,000 <sup>b</sup>	84	0.886	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 <sup>b</sup>	84	0.870	0% (0)	0% (0)	0% (0)	0% (0)
	100,000 <sup>b</sup>	84	0.917	0% (0)	0% (0)	0% (0)	0% (0)

<sup>a</sup> HPV16 or HPV18 plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

<sup>b</sup> Genomic DNA isolated from HPV positive cells (SiHa and HeLa) and/or HPV negative cells (Jurkat) at the indicated concentration (cells/mL).

**Table 25:** Summary of Positive HPV18 Results for Precision Study

Target	Copies/Reaction <sup>a</sup> or Cells/mL <sup>b</sup>	N	Mean HPV18 FOZ	HPV18 Positive % (n)			Total
				Operator 1	Operator 2	Operator 3	
HPV16	5,000 <sup>a</sup>	84	0.978	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 <sup>a</sup>	84	0.990	0% (0)	0% (0)	0% (0)	0% (0)
HPV18	5,000 <sup>a</sup>	84	3.620	100% (28)	100% (28)	100% (28)	100% (84)
	20,000 <sup>a</sup>	84	8.483	100% (28)	100% (28)	100% (28)	100% (84)
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat <sup>b</sup>	84	0.874	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	0.858	0% (0)	0% (0)	0% (0)	0% (0)
Hela/Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	2.988	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	7.918	100% (28)	100% (28)	86% (24)	95% (80)
Jurkat	10,000 <sup>b</sup>	84	0.927	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 <sup>b</sup>	84	0.920	0% (0)	0% (0)	0% (0)	0% (0)
	100,000 <sup>b</sup>	84	0.951	0% (0)	0% (0)	0% (0)	0% (0)

<sup>a</sup> HPV16 or HPV18 plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

<sup>b</sup> Genomic DNA isolated from HPV positive cells (SiHa and HeLa) and/or HPV negative cells (Jurkat) at the indicated concentration (cells/mL).

## Reproducibility

Reproducibility of the Cervista HPV 16/18 test was assessed at three external sites using a panel of HPV positive and negative cultured cells and HPV positive and negative cervical specimens. DNA was extracted from 2 mL of cervical specimen or cultured cells suspended in PreservCyt Solution. The DNA was extracted using the Genfind DNA Extraction Kit. Sixteen samples were extracted for DNA and tested with Cervista HPV 16/18 at three locations on five non-consecutive days within a two-week time period. Two lots of Cervista HPV 16/18 kits and three lots of Genfind DNA Extraction Kits were used across the 3 sites for the study. The total number of measurements for each sample was 15 = (3 sites x 5 days x 1 run per day). A summary of the percent agreement between the expected and observed results combined for all sites is shown in Table 26. A summary of individual sample results across sites with a cumulative mean, standard deviation and 95% confidence interval for the HPV16 and HPV18 FOZ values are presented in Table 27 and Table 28.

**Table 26:** Data Summary for a Multi-center Reproducibility Study of the Cervista HPV 16/18 Test.

Expected Result	Number of Results	Results in Agreement	Percent Agreement	Lower Limit of 95% CI
Positive	150	150	100.0%	97.5%
Negative	90	90	100.0%	95.9%

**Table 27:** Summary of Cervista HPV16 Results from a Multi-Center Reproducibility Study

Sample	Sample Type and Concentration (cells/mL)	N	HPV16 FOZ		HPV16 Positive % (n)			Total (n)
			Mean	SD	Site 1	Site 2	Site 3	
1. Neg	100,000 Jurkat	15	0.899	0.048	0 (0)	0 (0)	0 (0)	0
2. Pos: HPV18	10,000 HeLa 90,000 Jurkat	15	0.883	0.076	0 (0)	0 (0)	0 (0)	0
3. Pos: HPV18	5,000 HeLa 95,000 Jurkat	15	0.847	0.083	0 (0)	0 (0)	0 (0)	0
4. Pos: HPV18	2,500 HeLa 97,500 Jurkat	15	0.833	0.073	0 (0)	0 (0)	0 (0)	0
5. Pos: HPV16	20,000 SiHa 80,000 Jurkat	15	6.345	0.553	100 (5)	100 (5)	100 (5)	15
6. Pos: HPV16	10,000 SiHa 90,000 Jurkat	15	4.933	0.598	100 (5)	100 (5)	100 (5)	15
7. Pos: HPV16	5,000 SiHa 95,000 Jurkat	15	3.049	0.473	100 (5)	100 (5)	100 (5)	15
8. Pos: HPV18 zand HPV16	5,000 SiHa 2,500 HeLa 12,500 Jurkat	15	3.047	0.387	100 (5)	100 (5)	100 (5)	15
9. Neg	Cervical Pool	15	0.905	0.078	0 (0)	0 (0)	0 (0)	0
10. Neg	Cervical Pool	15	0.888	0.097	0 (0)	0 (0)	0 (0)	0
11. Pos: HPV18	Cervical Pool	15	0.958	0.154	0 (0)	0 (0)	0 (0)	0
12. Neg	Cervical Pool	15	0.865	0.127	0 (0)	0 (0)	0 (0)	0
13. HPV16	Cervical Pool	15	9.769	0.658	100 (5)	100 (5)	100 (5)	15
14. Neg	Cervical Pool	15	0.919	0.093	0 (0)	0 (0)	0 (0)	0
15. HPV16	Cervical Pool	15	2.782	0.611	100 (5)	100 (5)	100 (5)	15
16. Neg	Cervical Pool	15	1.049	0.130	0 (0)	0 (0)	0 (0)	0

**Table 28:** Summary of Cervista HPV18 Results from a Multi-Center Reproducibility Study

Sample	Sample Type and Concentration (cells/mL)	N	HPV18 FOZ		HPV18 Positive % (n)			
			Mean	SD	Site 1	Site 2	Site 3	Total
1. Neg	100,000 Jurkat	15	0.927	0.042	0 (0)	0 (0)	0 (0)	0
2. Pos: HPV18	10,000 HeLa 90,000 Jurkat	15	9.322	0.831	100 (5)	100 (5)	100 (5)	15
3. Pos: HPV18	5,000 HeLa 95,000 Jurkat	15	6.121	1.105	100 (5)	100 (5)	100 (5)	15
4. Pos: HPV18	2,500 HeLa 97,500 Jurkat	15	3.645	0.455	100 (5)	100 (5)	100 (5)	15
5. Pos: HPV16	20,000 SiHa 80,000 Jurkat	15	0.831	0.043	0 (0)	0 (0)	0 (0)	0
6. Pos: HPV16	10,000 SiHa 90,000 Jurkat	15	0.963	0.043	0 (0)	0 (0)	0 (0)	0
7. Pos: HPV16	5,000 SiHa 95,000 Jurkat	15	0.927	0.031	0 (0)	0 (0)	0 (0)	0
8. Pos: HPV18 and HPV16	5,000 SiHa 2,500 HeLa 12,500 Jurkat	15	3.815	0.435	100 (5)	100 (5)	100 (5)	15
9. Neg	Cervical Pool	15	0.896	0.049	0 (0)	0 (0)	0 (0)	0
10. Neg	Cervical Pool	15	0.892	0.053	0 (0)	0 (0)	0 (0)	0
11. Pos: HPV18	Cervical Pool	15	10.413	1.945	100 (5)	100 (5)	100 (5)	15
12. Neg	Cervical Pool	15	1.146	0.121	0 (0)	0 (0)	0 (0)	0
13. HPV16	Cervical Pool	15	0.861	0.053	0 (0)	0 (0)	0 (0)	0
14. Neg	Cervical Pool	15	0.927	0.029	0 (0)	0 (0)	0 (0)	0
15. HPV16	Cervical Pool	15	0.921	0.035	0 (0)	0 (0)	0 (0)	0
16. Neg	Cervical Pool	15	0.921	0.050	0 (0)	0 (0)	0 (0)	0

## Interfering Substances

Three cell-line samples (one HPV negative, one HPV16 positive, one HPV18 positive) described in Table 29 were tested with interferents that could potentially be present in the cervical specimen or transferred inadvertently during sample extraction using the Genfind DNA Extraction Kit (Table 30). Concentration levels were chosen to represent extreme conditions that could potentially occur during specimen collection if the cervix was not cleared prior to obtaining the specimen. DNA was isolated from pure and impure samples using the Genfind DNA Extraction Kit and was tested with the Cervista HPV 16/18 test to assess interference caused by the introduced substances.

**Table 29:** Interfering Substances Sample Descriptions

Sample	Description
Jurkat	Cell line sample stored in PreservCyt solution containing 100,000 cells/mL Jurkat (HPV Negative) cells
SiHa/Jurkat	Cell line sample stored in PreservCyt solution containing 7,500 cells/mL SiHa cells (HPV16 Positive) and 92,500 cells/mL Jurkat cells
HeLa/Jurkat	Cell line sample stored in PreservCyt solution containing 2,500 cells/mL HeLa cells (HPV18 Positive) and 97,500 cells/mL Jurkat cells

**Table 30: Interference Results**

Source	Interferent		Concentrations Tested	Interference Observed?
	Type			
Cervical Specimen	Blood		Visually Detectable	No
	Mucous		Visually Detectable	No
	Blood/Mucous		Visually Detectable	No
	Vaginal Douche		0.5%, 2%	No
	Contraceptive Jelly		0.5%, 2%	Yes <sup>a</sup>
	Anti-fungal Cream containing 2% clotrimizole		0.5%, 2%	Yes <sup>a</sup>
	Anti-fungal Cream containing 4% miconazole		0.5%, 2%	Yes <sup>a</sup>
Genfind DNA Extraction Kit Sample Processing	PreservCyt Solution		0.5%, 2%	No
	70% Ethanol		5%, 10%	No
	Magnetic Beads		5%, 10%	No

<sup>a</sup>The levels of interferent required to cause testing failures (2%) are unusually high and should not be encountered in actual clinical specimens.

During DNA extraction, the contraceptive jelly showed visually detectable interference with the magnetic bead separation in the 10 mM Tris buffer, causing low DNA recovery and insufficient DNA sample for testing.

The levels of interferent required to cause testing failures are unusually high and should not be encountered in actual clinical specimens if the clinician follows the proper cervical cytology sampling procedure of clearing the cervix before obtaining the cell sample for cervical cytology.

## Cross-Reactivity

A panel of bacteria, fungi, and viruses commonly found in the female anogenital tract, as well as several Human papillomavirus types of high, low, or undetermined risk were tested with the Cervista HPV 16/18 test to assess potential cross-reactivity.

**Table 31: Organisms Added to PreservCyt**

The organisms listed below were added to PreservCyt Solution at concentrations of approximately  $1 \times 10^5$  cfu/mL and  $1 \times 10^7$  cfu/mL. DNA from these organisms and a negative cell line (Jurkat,  $1 \times 10^5$  cells/mL) was extracted using the Genfind DNA Extraction Kit. All samples yielded negative results with the Cervista HPV 16/18 test.

<i>Candida albicans</i>	<i>Proteus vulgaris</i>
<i>Corynebacterium pseudodiphtheriticum</i>	<i>Staphylococcus aureus</i>
<i>Enterococcus faecalis</i>	<i>Staphylococcus epidermidis</i>
<i>Escherichia coli</i>	<i>Streptococcus mitis</i>
<i>Lactobacillus acidophilus</i>	<i>Streptococcus pyogenes</i>

**Table 32: Purified DNA**

Purified DNA obtained from the organisms listed below was tested at concentrations of  $1 \times 10^5$  copies/reaction and  $1 \times 10^7$  copies/reaction using the Cervista HPV 16/18 test. All samples yielded negative results.

Herpes simplex virus, type 1 (HSV-1)	<i>Chlamydia trachomatis</i>
Herpes simplex virus, type 2 (HSV-2)	<i>Neisseria gonorrhoeae</i>
Human Immunodeficiency Virus type 1 (HIV-1, pol and env regions)	<i>Neisseria meningitidis</i>
	<i>Mycoplasma hominis</i>

**Table 33: Cloned DNA**

Cloned DNA or PCR amplicons for the following samples were tested at concentrations of  $1 \times 10^5$  copies/reaction and  $1 \times 10^7$  copies/reaction unless noted, using the Cervista HPV 16/18 test. All samples yielded negative results.

Human papillomavirus type 1a	Human papillomavirus type 51
Human papillomavirus type 6	Human papillomavirus type 52
Human papillomavirus type 11	Human papillomavirus type 53
Human papillomavirus type 31 <sup>a</sup>	Human papillomavirus type 58
Human papillomavirus type 35	Human papillomavirus type 59
Human papillomavirus type 39	Human papillomavirus type 66
Human papillomavirus type 42	Human papillomavirus type 67
Human papillomavirus type 43	Human papillomavirus type 68
Human papillomavirus type 44	Human papillomavirus type 70
Human papillomavirus type 45	Human Internal Control gene

<sup>a</sup>Human papillomavirus type 31 yielded positive HPV16 results with the Cervista HPV 16/18 test at  $1 \times 10^7$  copies/reaction. Upon further titration of the HPV 31 sample, negative results were obtained with the Cervista HPV 16/18 test at  $\leq 1 \times 10^6$  copies/reaction.

An additional cross-reactivity study was conducted for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Mycoplasma hominis* utilizing whole organisms spiked into PreservCyt Solution containing HPV-negative Jurkat Cells (100,000 cells/mL). Three lots of each organism were prepared and DNA was isolated from all samples using the Genfind DNA Extraction kit. This study demonstrated that the Cervista HPV 16/18 test does not cross-react with DNA isolated from PreservCyt® samples containing up to containing up to  $1.0 \times 10^7$  cfu/mL of *Neisseria meningitidis* and *Mycoplasma hominis*,  $5 \times 10^6$  cfu/mL of *Neisseria gonorrhoeae* and  $1.0 \times 10^6$  cfu/mL *Chlamydia trachomatis*.

## Troubleshooting Guide

Observation	Probable Cause	Solutions
Insufficient volume made for reaction mixes	Number of samples entered in "Assay Selection" tab of software is less than samples added to the plate.	Manually recalculate the required amount of reaction mix needed to complete the entire plate.
		Recreate software printouts using correct number of samples.
	Excess reaction mix volume added to 96-well microplate.	Verify the correct reaction mix volumes were added to each well.
		Verify the calibration information on equipment is current.
No Target Control displays the following results: <ul style="list-style-type: none"> <li>• Increase gain for scan 1</li> <li>• Increase gain for scan 2</li> <li>• Increase gain for both scans</li> </ul>	Fluorescence microplate reader gain settings are too low causing the raw fluorescent signal values to fall below the minimum requirement.	Increase the fluorometer gain settings for the designated scan(s) so that the No Target Control produces a minimum signal of 600 RFU and re-read the plate.
Errors occur during data import: <p>"Check FAM &amp; Red gain settings and read the whole plate again. (Partial plate reads are not allowed.)"</p> <p>"Check FAM gain setting and read the whole plate again. (Partial plate reads are not allowed.)"</p> <p>"Check Red gain setting and read the whole plate again. (Partial plate reads are not allowed.)"</p>	Fluorometer issues	See Troubleshooting Guide in the Invader Call Reporter Software User Manual for fluorometer issues that may contribute to this error.
	Thermal incubation period was longer than the specified length of time recommended	Confirm that the incubation was performed for the specified length of time and at the specified temperature.

Observation	Probable Cause	Solutions
No Target Control displays the following results: High %Difference (gDNA NTC)	Insufficient or inconsistent mixing of reagents	<ul style="list-style-type: none"> <li>• Be sure all samples, reagents and reaction mixes are mixed thoroughly.</li> <li>• When adding reaction mix to each well, place tips at the bottom of the well (beneath mineral oil) and slowly pipette up and down 3-4 times.</li> </ul>
	Incorrect preparation of reaction mixes	<ul style="list-style-type: none"> <li>• Verify all liquid is expelled from the pipette tip during additions.</li> <li>• Verify the correct reagent was added to each well.</li> <li>• Verify the correct reagent volumes were added to each well.</li> </ul>
	Inconsistent addition of the No Target Control or reaction mix to the microplate	<ul style="list-style-type: none"> <li>• Verify the calibration information on equipment is current.</li> <li>• Visually inspect plate for consistent volumes between wells.</li> </ul>
	Suspected contamination during sample addition or reaction mix preparation	Use nuclease-free aerosol barrier tips and sterile tubes when making the reaction mixes.
		Wear gloves when setting up the test.
		Since nucleases may be present, make sure that pipette tips do not touch any other surfaces except the solution being dispensed
		Do not touch pipette tips with hands Clean lab surfaces using appropriate materials
	Sample evaporation	Verify mineral oil addition to each well.
Bubbles in the reaction wells	If possible, spin down plates prior to fluorescence scanning.	
Prepared reaction mixes were not used within recommended time period	Use reaction mixes within 30 minutes of preparation.	

Observation	Probable Cause	Solutions
Control(s) displays "Invalid Control" result	Insufficient or inconsistent mixing of controls	<ul style="list-style-type: none"> <li>• Be sure all controls and reagents are mixed thoroughly and consistently.</li> <li>• When adding reaction mix to each well, place tips at the bottom of the well (beneath mineral oil) and slowly pipette up and down 3-4 times.</li> </ul>
	Inconsistent addition of reaction mix	<ul style="list-style-type: none"> <li>• Make sure that all liquid is expelled from the pipette tip during additions.</li> <li>• Verify that the correct control was added to each well.</li> </ul>
	Insufficient or inconsistent addition of control	<ul style="list-style-type: none"> <li>• Verify that the correct control volume was added to each well.</li> <li>• Verify the calibration information on equipment.</li> <li>• Visually inspect plate for consistent volumes between wells.</li> </ul>
	Correct control(s) was not added to the plate or was not added to the correct plate position	Verify the correct controls were added to the correct plate positions.
	Incubation period was shorter or longer than the specified length of time recommended	Confirm that the incubation was performed for the specified length of time and at the specified temperature.
	Suspected contamination during sample addition	Use nuclease-free aerosol barrier tips and sterile tubes during set up.
		Wear gloves when setting up the test.
		Make sure that pipette tips touch only the solution being dispensed.
		Do not touch pipette tips with hands. Clean lab surfaces using appropriate materials.
	Sample evaporation	Verify mineral oil addition to each well.
The plate may not be orientated properly	When scanning the plate, orient the plate so well A1 is in the upper left-hand corner.	
Bubbles in the reaction wells	If possible, spin down plates prior to fluorescence scanning.	
Prepared reaction mixes were not used within recommended time period	Use reaction mixes within 30 minutes of preparation.	



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Observation	Probable Cause	Solutions
Sample displays "IND: High %Difference" result	Insufficient or inconsistent mixing of samples	<ul style="list-style-type: none"> <li>• Be sure all samples and reagents are mixed thoroughly.</li> <li>• When adding reaction mix to each well, place tips at the bottom of the well (beneath the mineral oil) and slowly pipette up and down 3-4 times.</li> </ul>
	Inconsistent addition of reaction mix	<ul style="list-style-type: none"> <li>• Verify all liquid is expelled from the pipette tip during additions.</li> <li>• Verify the correct sample was added to each well.</li> <li>• Verify the correct sample volume was added to each well.</li> </ul>
	Inconsistent addition of sample	<ul style="list-style-type: none"> <li>• Verify the calibration information on equipment is current.</li> <li>• Visually inspect plate for consistent volumes between wells.</li> </ul>
	Suspected contamination during sample addition	Use nuclease-free aerosol barrier tips and sterile tubes during set up.
		Wear gloves when setting up the test.
		Make sure that pipette tips touch only the solution being dispensed.
		Do not touch pipette tips with hands.
	Sample evaporation	Verify mineral oil addition to each well.
	Bubbles in the reaction wells	If possible, spin down plates prior to fluorescence scanning.
Prepared reaction mixes were not used within recommended time period	Use reaction mixes within 30 minutes of preparation.	

Observation	Probable Cause	Solutions
Sample displays "IND: Low gDNA" result	Insufficient number of cells in specimen	<ul style="list-style-type: none"> <li>• Mix the specimen well and repeat DNA extraction from the specimen.</li> </ul>
	Suspected error during DNA extraction	<ul style="list-style-type: none"> <li>• Verify the correct sample volume was added to each well.</li> </ul>
	Insufficient amount of DNA was used in the test	<ul style="list-style-type: none"> <li>• Verify that proper procedure was followed for DNA extraction.</li> </ul>
	DNA sample inhibition	Repeat DNA extraction from the specimen.
		Refer to the Package Insert, Performance Characteristics (Interfering Substances) section.
The DNA sample(s) may not have been completely denatured	Verify that the sample was denatured at the correct temperature and for an appropriate amount of time.	
Sample displays "IND: Low HPV FOZ" result	Suspected error during DNA extraction	<ul style="list-style-type: none"> <li>• Repeat DNA extraction from the specimen.</li> <li>• Verify that proper procedure was followed for DNA extraction.</li> </ul>
	DNA sample inhibition	<ul style="list-style-type: none"> <li>• Refer to the Package Insert, Performance Characteristics (Interfering Substances) section.</li> </ul>
Insufficient Sample DNA volume	Insufficient elution volume during DNA extraction	<ul style="list-style-type: none"> <li>• Repeat DNA extraction from the specimen.</li> <li>• Verify that proper procedure was followed for DNA extraction.</li> </ul>
High number of DNA samples with positive FAM FOZ values in both reaction mixes	Suspected error during DNA extraction	<ul style="list-style-type: none"> <li>• Repeat DNA extraction from the specimen.</li> </ul>
	Suspected DNA extraction reagent contamination	<ul style="list-style-type: none"> <li>• Verify that proper procedure was followed for DNA extraction.</li> </ul>

## Bibliography

1. American Society for Colposcopy and Cervical Pathology website: [www.asccp.org](http://www.asccp.org), 2008.
2. National Cancer Institute website: [www.cancer.gov](http://www.cancer.gov), 2008.
3. Meijer CJ, Snijders PJ, and Castle PE. 2006. Clinical utility of HPV genotyping. *Gynecol Oncol* 103: 12-17.
4. Winer RL, N Kiviat, J Hughes, D Adams, S Lee, J Kuypers, L Koutsky. 2005. Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis* 191: 731-738.
5. Schlecht NF, Kulaga S, Robitaille J, et al. 2001. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 286: 3106-3114.
6. Wright TC, Jr., Cox JT, Massad LS, Twigg LB, Wilkinson EJ. 2002. 2001 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 287: 2120-2129.
7. Solomon D, Schiffman M, and Tarone R. 2001. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst* 93(4): 293-299.
8. Sherman ME, Schiffman M, and Cox TJ. 2002. Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS). *J Natl Cancer Inst* 94(2): 102-107.
9. Davey DD, Neal MH, Wilbur DC, Colgan TJ, Styer PE, and Mody DR. 2004. Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the college of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch Path Lab Med* 128: 1224-1229.
10. Khan MJ, Castle PE, Lorincz AT, et al. 2005. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 97:1072-1079.
11. Castle PE et al 2005. Human Papillomavirus Type 16 Infections and 2-Year Absolute Risk of Cervical Precancer in Women With Equivocal or Mild Cytologic Abnormalities. *J Natl Cancer Inst* 97: 1066-1071.
12. Mayrand MH, E Duarte-Franco, I Rodrigues, SD Walter, J Hanley. 2007. A Ferenczy, S Ratnam, F Coutlée, EL Franco. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 357(16): 1579-1588.
13. Wheeler CM, WC Hunt, M Schiffman, PE Castle. 2006. Human papillomavirus genotypes and the cumulative 2-Year risk of cervical cancer. *J Infect Dis* 194: 1291-1299.
14. Bosch X and Harper D. 2006. Prevention strategies of cervical cancer in the HPV vaccine era. *Gynecol Oncol* 103(1): 21-24.
15. Bulkman NW, Berkhof J, Bulk S, Bleeker MC, van Kemenade FJ, Rozendaal L, Snijders PJ, Meijer CJ; POBASCAM Study Group. 2007. High-risk HPV type-specific clearance rates in cervical screening. *Br J Cancer* 96(9): 1419-1424.
16. Sandadi S, RL Flyckt, KM Zanotti. 2008. Cost effectiveness of a triage strategy for atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion cytology using human papillomavirus 16/18 genotyping. *Gynecological Oncol* 108(S96): 218.
17. Saslow D, Runowicz CD, Solomon D, Moscicki A-B, Smith RA, Eyre HJ, Cohen C. 2002. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Can Jour Clin* 53: 342-362.
18. Wright TC, Jr., Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, et al. 2004. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol* 103: 304-309.
19. Wright TC, Jr., Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, and Solomon D. 2007. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 197(4): 346-355.
20. Castellsague X et al. 2006. Worldwide Human Papillomavirus Etiology of Cervical Adenocarcinoma and Its Cofactors: Implications for Screening and Prevention. *J Natl Cancer Inst* 98: 303-315.
21. Hall JG, Eis PS, Law SM, Reynaldo LP, Prudent JR, Marshall DJ, Allawi HT, Mast AL, Dahlberg JE, Kwiatkowski RW, de Arruda M, Neri BP, and Lyamichev VI. 2000. Sensitive detection of DNA polymorphisms by the serial invasive signal amplification reaction. *PNAS* 97(15): 8272-8277.
22. Revzina N, DiClemente R. 2005. Prevalence and incidence of human papillomavirus infection in women in the USA: a systematic review. *Int J of STD & AIDS* 528-537.
23. Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. 2001. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* 357(9271): 1831-1836.
24. Woodman CB, Collins S, Rollason TP, Winter H, Bailey A, Yates M, Young LS. 2003. Human papillomavirus type 18 and rapidly progressing cervical intraepithelial neoplasia. *361(9351)*: 40-43.
25. CLSI document EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

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