



**THIRD WAVE  
TECHNOLOGIES**

# Cervista™ HPV HR

REF 95-438

An *in vitro* diagnostic test for the detection of DNA from 14 high-risk Human Papillomavirus (HPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in Cervical Specimens.



***In vitro* diagnostic  
medical device**



**Contains sufficient  
reagents for 96 tests**



**Temperature limitation**

**Do NOT store in frost-free freezer.**

**Protect from light.**

## **TABLE OF CONTENTS**

NAME AND INTENDED USE  
ABBREVIATIONS USED  
SUMMARY AND EXPLANATION OF THE TEST  
PRINCIPLES OF THE PROCEDURE  
REAGENTS PROVIDED  
WARNINGS AND PRECAUTIONS  
REAGENT STORAGE AND HANDLING REQUIREMENTS  
ADDITIONAL REAGENTS AND MATERIALS  
MATERIALS REQUIRED, BUT NOT PROVIDED  
SPECIMEN COLLECTION AND STORAGE FOR ANALYSIS  
TEST PROCEDURE  
PROCEDURAL NOTES AND PRECAUTIONS  
INTERPRETATION OF RESULTS  
QUALITY CONTROL  
LIMITATIONS  
EXPECTED RESULTS  
PERFORMANCE CHARACTERISTICS  
REFERENCES  
TROUBLESHOOTING GUIDE

## **NAME AND INTENDED USE**

The Cervista™ HPV HR test is an *in vitro* diagnostic test for the qualitative detection of DNA from 14 high-risk Human Papillomavirus (HPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens. The Cervista™ HPV HR test cannot determine the specific HPV type present.

The Cervista™ HPV HR 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 HR test is indicated:

- 1) To screen patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy.
- 2) In women 30 years and older the Cervista™ HPV HR test can be used with cervical cytology to adjunctively screen to assess the presence or absence of high-risk HPV types. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

Cervical specimens that may be tested with the Cervista™ HPV HR test include the following preservation system collection media and collection devices:

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

## **WARNINGS**

- The Cervista™ HPV HR test is not intended for use as a screening device for women under age 30 with normal cervical cytology.
- The Cervista™ HPV HR test is not intended to substitute for regular cervical cytology screening.
- Detection of HPV using the Cervista™ HPV HR test does not differentiate HPV types and cannot evaluate persistence of any one type.
- 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 HR 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 HR test results should not be used as the sole basis for clinical assessment and treatment of patients.

## **ABBREVIATIONS USED**

ASC-US:	Atypical squamous cells of undetermined significance
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
TWT:	Third Wave Technologies, Inc.

## **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 (HPV16, 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</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</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 more aggressively.<sup>3,6,10,11</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>4,12,13,14</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>15,16</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>7,17,15</sup> These patient management guidelines recommend testing for the presence of high-risk types of HPV as a regular screening tool, in combination with cytology, in specific instances. Principal HR HPV testing recommendations of the most recent professional practice guidelines, the *2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests*, include: 1) screening women 30 years of age and over in conjunction with cytology or other screening methods; and 2) management of women 21 years of age and over with ASC-US.<sup>15,18</sup> In all cases, patient management decisions reflect patients' overall cytology history and other risk factors in addition to the presence or absence of high-risk HPV types.<sup>7,10,18</sup>

## **PRINCIPLES OF THE PROCEDURE**

Cervista™ HPV HR is a qualitative, *in vitro* diagnostic test for the detection of DNA from 14 high-risk HPV types, namely, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

The Cervista™ HPV HR 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). 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.

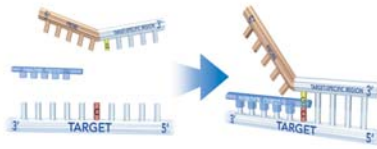
The probes are present in large molar excess and cycle rapidly on and off the target sequence so that many cleaved 5' flaps are generated per target sequence. The cleaved flaps then bind to a universal hairpin fluorescence resonance energy transfer (FRET) oligonucleotide creating another invasive structure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

quencher molecule and produces a fluorescence signal as the cleaved flaps cycle on and off. For each copy of target, the combined primary and secondary reactions result in  $10^6 - 10^7$  fold signal amplification per hour.<sup>19</sup> The flap sequences and FRET oligonucleotides are universal since they are not complementary to the targeted sequence.

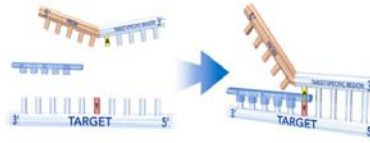
The reagents for this test are provided as three oligonucleotide mixtures, which detect the 14 types of HPV grouped according to phylogenetic relatedness, i.e. viral types with similar DNA sequences (A5/A6, A7, A9 HPV groups). Oligonucleotides that bind to the human histone 2 gene (H2be, HIST2H2BE) are also present in these three oligonucleotide mixtures. HIST2H2BE serves as an internal control producing a signal from genomic DNA present in the sample. The format of the Cervista™ HPV HR 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 indicates that at least one of the 14 high-risk types is present in the DNA sample. This result 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 an 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 a processing measure to ensure that the testing procedure has been adequately performed.

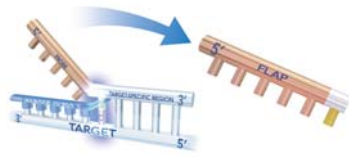
1a. HPV oligos form invasive structure on HPV DNA.



1b. HIST2H2BE oligos form invasive structure on genomic DNA.



2. Cleavase® enzyme recognizes structure and cleaves probe oligos



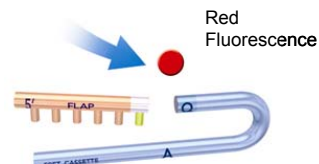
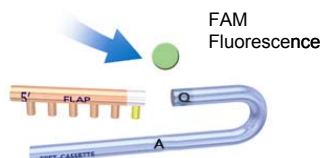
3a. Flaps from HPV probe oligos form invasive structure on FAM FRET oligos



3b. Flaps from HIST2H2BE probe oligos form invasive structure on Red FRET oligos



4. Cleavase® enzyme recognizes structure and releases fluorophores from FRET Oligos creating fluorescence signal.



**Figure 1:** A Graphic Representation of the Invader® Chemistry in Cervista™ HPV HR

## **REAGENTS PROVIDED**

**Table 1: Cervista™ HPV HR (REF 95-438) Contents**

<b>Reagent</b>	<b>Vial Label Abbreviation</b>	<b>Vial Quantity &amp; Reagent Volume</b>	<b>Component Description</b>
HPV Oligo Mix 1	O1 (Blue cap and blue stripe)	1 x 1400 $\mu$ L	Oligonucleotides with affinity to HPV types 51, 56, 66 and human HIST2H2BE suspended in water and MOPS buffer (pH 7.5)
HPV Oligo Mix 2	O2 (Yellow cap and yellow stripe)	1 x 1400 $\mu$ L	Oligonucleotides with affinity to HPV types 18, 39, 45, 59, 68 and human HIST2H2BE suspended in water and MOPS buffer (pH 7.5)
HPV Oligo Mix 3	O3 (Orange cap and orange stripe)	1 x 1400 $\mu$ L	Oligonucleotides with affinity to HPV types 16, 31, 33, 35, 52, 58 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
HPV Control 1	C1 (Clear cap and black stripe)	1 x 200 $\mu$ L	1000 copies/ $\mu$ L cloned HPV type 51 DNA and 3000 copies/ $\mu$ L cloned HIST2H2BE DNA in yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer
HPV Control 2	C2 (Clear cap and black stripe)	1 x 200 $\mu$ L	1000 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
HPV Control 3	C3 (Clear cap and black stripe)	1 x 200 $\mu$ L	1000 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
No Target Control	NTC (Clear cap and black stripe)	1 x 200 $\mu$ L	Yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer

## **WARNINGS AND PRECAUTIONS**

**For *in vitro* diagnostic use.**

### **Safety and Handling Precautions**

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

## **REAGENT 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.
- Third Wave Technologies recommends no more than six (6) freeze-thaw cycles for all Cervista™ HPV HR 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 HR test and, afterwards, when incremental updates to the software are released.

The Genfind™ DNA Extraction Kit is an accessory of the Cervista™ HPV HR test. Contact Third Wave Technologies 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 operating system with Microsoft® Excel® and Adobe® Reader® software.
- Thermal cycler or oven capable of maintaining appropriate reaction temperatures.

## **SPECIMEN COLLECTION AND STORAGE FOR ANALYSIS**

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

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

DNA should be extracted from PreservCyt<sup>®</sup> specimens using the Genfind<sup>™</sup> DNA Extraction Kit ([REF](#) 95-449).

## **TEST PROCEDURE**

Note: Perform DNA extraction from cervical specimens collected in PreservCyt<sup>®</sup> Solution using the Genfind<sup>™</sup> DNA Extraction Kit ([REF](#) 95-449) prior to beginning the reaction procedure.

### **Reaction Procedure**

1. Add 10  $\mu\text{L}$  of each control and sample DNA to three wells of a 96-well plate as indicated in the test plate layout (see Figure 2).

	Mix 1	Mix 2	Mix 3	Mix 1	Mix 2	Mix 3	Mix 1	Mix 2	Mix 3	Mix 1	Mix 2	Mix 3
	1	2	3	4	5	6	7	8	9	10	11	12
A	C1	C1	C1	S5	S5	S5	S13	S13	S13	S21	S21	S21
B	C2	C2	C2	S6	S6	S6	S14	S14	S14	S22	S22	S22
C	C3	C3	C3	S7	S7	S7	S15	S15	S15	S23	S23	S23
D	NTC	NTC	NTC	S8	S8	S8	S16	S16	S16	S24	S24	S24
E	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
F	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26
G	S3	S3	S3	S11	S11	S11	S19	S19	S19	S27	S27	S27
H	S4	S4	S4	S12	S12	S12	S20	S20	S20	S28	S28	S28

**Figure 2:** Cervista<sup>™</sup> HPV HR test plate layout

2. Overlay each well with 20  $\mu\text{L}$  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<sup>™</sup> software) or according to the calculations in Table 2. Prepare one reaction mix for each of the three HPV Oligo Mixes prior to each use. Prepared reaction mixture should be used within 30 minutes.

**Table 2:** Reaction Mix Preparation Instructions

Reagent	$\mu\text{L}/\text{Reaction}$	No. Of Reactions (Samples & Controls ( <i>k</i> ))	Total Volume
HPV Oligo Mix 1, 2, or 3	8 $\mu\text{L}$	<i>k</i>	=8 <i>k</i> (1.25) $\mu\text{L}$
Cleavase <sup>®</sup> Enzyme Solution	2 $\mu\text{L}$	<i>k</i>	=2 <i>k</i> (1.25) $\mu\text{L}$
<b>Total Mix Volume</b>	<b>10 <math>\mu\text{L}</math></b>	<b><i>k</i></b>	<b>=10<i>k</i>(1.25) <math>\mu\text{L}</math></b>

6. Decrease thermal cycler temperature setting to 63°C.
7. Add 10  $\mu$ L 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 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 (See Table 3).
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:	20 $\mu$ s	20 $\mu$ s

### PROCEDURAL NOTES AND PRECAUTIONS

1. Laboratories should use good laboratory practices and comply with all applicable federal, state and local regulatory requirements.

2. These components have been tested as a unit. Do not interchange components from other sources or from different lots. Do not pool reagents from different lots or from different vials of the same lot.
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 HR 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 generated for each of the three reactions. This signal to noise value is referred to as FOZ (Fold-Over-Zero). A final positive or negative or indeterminate result for any particular sample is generated based on the analysis of three separate reaction wells.

The ratio between HPV FOZ values generated by the three reaction mixtures determines whether a sample is positive. The HPV FOZ ratio is calculated by dividing the highest HPV FOZ value from any one of the three reaction mixtures by the lowest HPV FOZ value of the three. When any FOZ value is less than 1, it is rounded up to 1 for the ratio calculation. If the HPV FOZ Ratio is greater than or equal to 1.525, then the sample is positive for HPV.

However, in a subset of mixed infections, all three reaction wells may generate a signal much higher than background. In some cases, these mixed infections may generate positive signals of similar intensity in all three reaction wells and therefore a HPV FOZ Ratio of less than 1.525. In order to avoid the chance of a false negative due to the triple positive scenario described above, a second calculation is applied as follows: when the FOZ ratio is less than 1.525, but all three individual reaction FOZ values are greater than a second cut-off value of 1.93, the sample is positive for HPV.

An indeterminate call is generated in three different scenarios 1) when the % CV between the gDNA FOZ values is  $\geq 25.0\%$  (High % CV), 2) when all three 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.

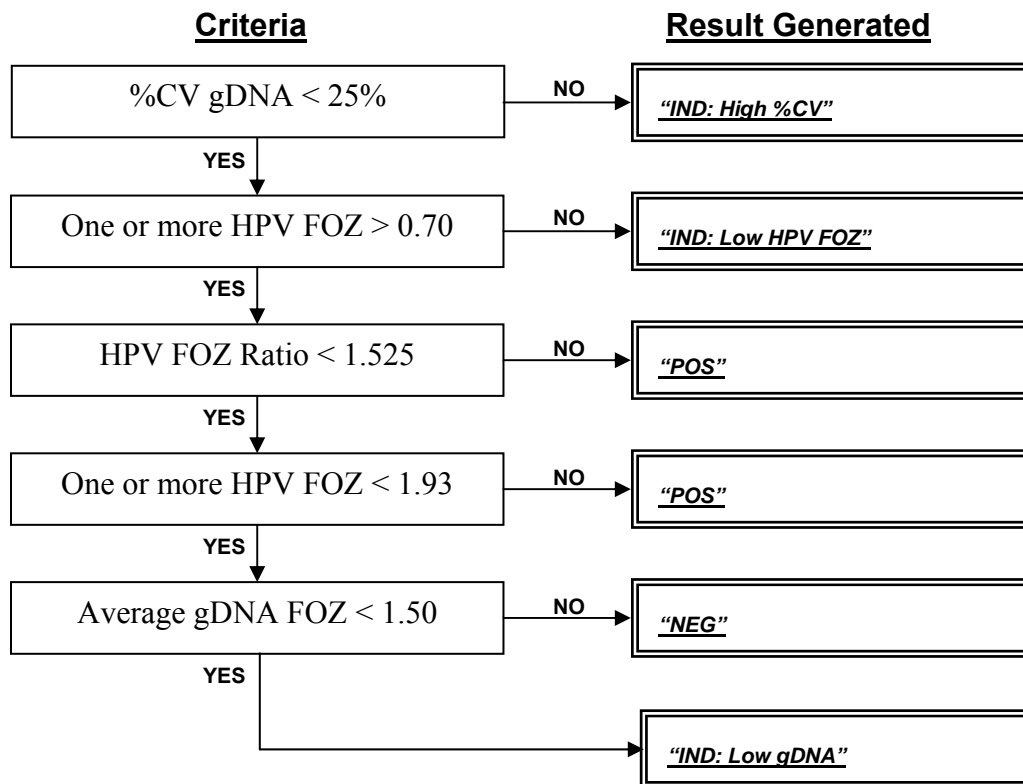
### Terminology

**HPV FOZ:** For each HPV Oligo Mix, the FAM signal of the sample divided by the FAM signal of the No Target Control.

**HPV FOZ Ratio:** The highest HPV FOZ of the three HPV Oligo Mixes divided by the lowest HPV FOZ of the three HPV Oligo Mixes (normalized to 1.0 if FOZ is lower than 1.0).

**Average gDNA FOZ:** The average value determined from the three genomic DNA FOZ values obtained from each of the three reaction mixes, calculated by dividing the Red signal of the sample by the Red signal of the No Target Control.

**%CV gDNA FOZ:** % coefficient of variation for the gDNA FOZ values generated by the three HPV Oligo Mixes.



**Figure 3:** Sample Call Criteria Ordered Top to Bottom

Note: The Cervista™ HPV HR test does not require the use of an equivocal or re-test zone.

**Table 4:** Interpretation of Cervista™ HPV HR Test Results

Cervista™ HPV HR Test Result	Result Report	Interpretation for patients with ASC-US cytology	Interpretation for patients with NILM cytology who are ≥30 years old*
<b>POS</b>	HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68 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>2,3,7,18</sup>	Low likelihood of underlying high-grade CIN; HPV infection may be transient, resolving or persistent.
<b>NEG</b>	HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 not detected	Low likelihood of underlying CIN2-3 or cancer; results are not intended to prevent women from proceeding to colposcopy.	Very low likelihood of underlying high-grade CIN or cancer; results do not preclude future HPV infection or cytologic abnormalities with underlying CIN2-3 or cancer
<b>IND: High % CV**</b> <b>IND: Low gDNA**</b>	Indeterminate	High risk HPV status unknown	

\* According to the 2006 consensus guidelines<sup>18</sup>, 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.

\*\*The Cervista™ HPV HR clinical trial demonstrated that the indeterminate rate was 0.54% (95%CI: 0.32%-0.86%) across all patient specimens tested.

## **QUALITY CONTROL**

### **Internal Control**

The Cervista™ HPV HR 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 a 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 of the three mixes must be greater than or equal to 600 RFU ( $\geq 600$ ).
2. The %CV of the average HPV signal from all three mixes must be less than 25.0% ( $<25.0\%$ ).
3. The %CV of the average gDNA signal from all three mixes must be less than 25.0% ( $<25.0\%$ ).

**Table 5:** No Target Control Criteria

<b>Result</b>	<b>Min. HPV Signal</b>	<b>Min. gDNA Signal</b>	<b>Max. % CV (HPV and gDNA)</b>
Valid	600	600	24.9%

#### **Positive Controls**

HPV controls (HPV Controls 1-3) 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. A HPV FOZ Ratio is determined by dividing the highest HPV FOZ of the three reaction mixes by the lowest HPV FOZ of the three (normalized to 1.0 if lower than 1.0). HPV Control 1 should yield a positive HPV FOZ value ( $\geq 1.525$ ) for only HPV Oligo Mix 1, HPV Control 2 should yield a positive HPV FOZ value ( $\geq 1.525$ ) for only HPV Oligo Mix 2, and HPV Control 3 should yield a positive HPV FOZ value ( $\geq 1.525$ ) for only HPV Oligo Mix 3.
2. The mean gDNA FOZ of all three mixes must be greater than or equal to 1.50 ( $\geq 1.50$ ), or the control is invalid for low gDNA.
3. The %CV of the mean gDNA FOZ from all three mixes should be less than 25.0% ( $<25.0\%$ ).

**Table 6: HPV Control Criteria**

Control	Result	HPV FOZ Ratio	Positive FOZ Mix	Average gDNA FOZ	% CV gDNA FOZ
HPV Control 1	Valid Control	≥ 1.525	Mix 1 only	≥ 1.50	< 25.0%
HPV Control 2	Valid Control	≥ 1.525	Mix 2 only	≥ 1.50	< 25.0%
HPV Control 3	Valid Control	≥ 1.525	Mix 3 only	≥ 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 both positive and negative controls yield correct results. If the No Target Control (negative control) is invalid and/or any result for the 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 Software User Manual for Invader Call Reporter™ software.
2. All quality control requirements should be performed in conformance with local, state, and federal regulations as well as accreditation requirements.

### **LIMITATIONS**

1. The Cervista™ HPV HR test detects DNA of high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This test does not detect DNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types.<sup>18</sup>
2. The Cervista™ HPV HR test exhibits cross-reactivity to two HPV types of unknown risk. An HPV positive result was observed with 5000 copies/reaction of HPV type 67 and 50,000 copies/reaction of HPV type 70.
3. A negative result does not exclude the possibility of HPV infection because very low levels of infection or sampling error may cause a false-negative result.
4. The test has been validated for use only 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 HR test was established exclusively using DNA extracted with the Genfind™ DNA Extraction Kit.
6. The performance of the Cervista™ HPV HR 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 HR 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 HR test for human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 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 High-Risk HPV 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 HR test.

## **EXPECTED RESULTS**

### **High-Risk HPV Prevalence**

The reported prevalence of HPV infection in women ranges widely, from 14% to more than 90%.<sup>20</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 HR 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. Table 7 shows the prevalence results from the four Clinical Testing Centers that performed all of the Cervista™ HPV HR testing for the trial. Samples from all enrollment sites were randomly distributed among the testing centers.

**Table 7: Prevalence of High-Risk HPV Across Clinical Trial Testing Centers**

	<b>ASC-US Population</b>		<b>NILM Population</b>	
<b>Center</b>	<b>Subjects Tested</b>	<b>HPV HR Positive Rate</b>	<b>Subjects Tested</b>	<b>HPV HR Positive Rate</b>
1	709	60.5% (429/709)	1007	18.9% (190/1007)
2	312	57.1% (178/312)	225	15.6% (35/225)
3	247	53.8% (133/247)	721	18.7% (135/721)
4	79	36.7% (29/79)	13	23.1% (3/13)
Total	1,347	57.1% (769/1347)	1,966	18.5% (363/1966)

Table 8 shows the prevalence of high-risk HPV among subjects with ASC-US cytology stratified by age.

**Table 8: Prevalence of High-Risk HPV by Age**

Age	ASC-US Population	Age	NILM Population
18 < 21	77.8% (105/135)	30 < 40	21.4% (132/618)
21 < 30	71.7% (352/491)	40 < 50	17.5% (118/674)
30 < 39	55.3% (167/302)	50 < 60	16.2% (79/489)
≥ 39	34.6% (145/419)	≥ 60	18.4% (34/185)
All	57.1% (769/1347)	All	18.5% (363/1966)

Table 9 shows the prevalence of high-risk HPV regardless of cytology as reported in various studies of women in different U.S. populations.

**Table 9: High-Risk HPV Prevalence in Various U.S. Populations**

Location	Publication Date	Total Study Size	Age Range	HPV Prevalence (%)
New Mexico <sup>21</sup>	2001	3,863	18 - 40	26.7%
U.S. Civilian Population <sup>22</sup>	2007	1,921	14 - 59	15.2%
Oregon <sup>23</sup>	2003	20,156	16 - 94	16.3%
Arizona <sup>24</sup>	2001	988	15 - 79	13.9%

## **PERFORMANCE CHARACTERISTICS**

### **CLINICAL SENSITIVITY AND SPECIFICITY FOR SCREENING PATIENTS WITH ASC-US CERVICAL CYTOLOGY RESULTS TO DETERMINE THE NEED FOR REFERRAL TO COLPOSCOPY**

A multi-center prospective clinical study was conducted to evaluate the performance of the Cervista™ HPV HR test for screening 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 Thin Prep 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 HR 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 HR test was measured against colposcopy and histology results. Biopsy samples were collected from 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

\* This study was conducted prior to implementation of guidelines<sup>18</sup> that recommend limiting ASC-US HPV testing to women 21 years or older.

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 1,347 ASC-US subjects with known disease status (central histology or negative colposcopy) and Cervista™ HPV HR results. A comparison of the Cervista™ HPV HR results with Colposcopy/Central Histology is shown in Table 10. The clinical performance of the Cervista™ HPV HR test is summarized in Tables 11, 12 and 13.

**Table 10: Cervista™ HPV HR Results as Compared to Colposcopy/Central Histology Results among Women with ASC-US Cytology**

Cervista™ HPV HR	Neg Colposcopy No Biopsy	Central Histology				Total
		No CIN	CIN1	CIN2	≥ CIN3	
HPV HR Positive	164	389	152	42	22	769
HPV HR Negative	214	314	30	5	0	563
HPV HR Indeterminate	4	11	0	0	0	15
<b>Total</b>	<b>382</b>	<b>714</b>	<b>182</b>	<b>47</b>	<b>22</b>	<b>1347</b>

**Table 11: Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN2) among Women with ASC-US Cytology**

Sensitivity	92.8% (64/69)	95% CI: (84.1% - 96.9%)
Specificity	44.2% (558/1263)	95% CI: (41.5% - 46.9%)
PPV	8.3% (64/769)	95% CI: (7.6% - 8.9%)
NPV	99.1% (558/563)	95% CI: (98.1% - 99.6%)
Disease Prevalence	5.2% (69/1332)	

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%.

**Table 12: Clinical Performance Summary of the Cervista™ HPV HR Test as Compared to Colposcopy/Central Histology Results (≥ CIN3) among Women with ASC-US Cytology**

Sensitivity	100% (22/22)	95% CI: (85.1% - 100%)
Specificity	43% (563/1310)	95% CI: (40.3% - 45.7%)
PPV	2.9% (22/769)	95% CI: (2.4% - 3.0%)
NPV	100% (563/563)	95% CI: (99.4% - 100%)
Disease Prevalence	1.7% (22/1332)	

Note: Among women with ASC-US cytology, there were 1.1% (15 out 1347) Cervista™ HPV HR indeterminate results with 95% CI: 0.7% to 1.8%.

CIN2 histology results (47) are considered negative for disease (≥ CIN3) in this table.

**Table 13:** Clinical Performance of the Cervista™ HPV HR Test Stratified by Age as Compared to Colposcopy/Central Histology Results (≥ CIN2) among Women with ASC-US Cytology

<b>Age: 18 to &lt;21</b>	Central Histology ≥ CIN2		
Cervista™ HPV HR	Negative	Positive	Total
Positive	96	9	105
Negative	28	0	28
Total	124	9	133
<b>Disease Prevalence:</b>	6.8% (9/133)	95% CI	
<b>Sensitivity:</b>	100% (9/9)	70.1%	100.0%
<b>Specificity:</b>	22.6% (28/124)	16.1%	30.7%
<b>PPV:</b>	8.6% (9/105)	4.0%	15.70%
<b>NPV:</b>	100% (28/28)	87.7%	100.0%
<b>Age: 21 to &lt;30</b>	Central Histology ≥ CIN2		
Cervista™ HPV HR	Negative	Positive	Total
Positive	321	31	352
Negative	136	0	136
Total	457	31	488
<b>Disease Prevalence:</b>	6.4% (31/488)	95% CI	
<b>Sensitivity:</b>	100% (31/31)	89.0%	100.0%
<b>Specificity:</b>	29.8% (136/457)	25.8%	34.1%
<b>PPV:</b>	8.8% (31/352)	6.1%	12.27%
<b>NPV:</b>	100% (136/136)	97.3%	100.0%
<b>Age: 30 to &lt;39</b>	Central Histology ≥ CIN2		
Cervista™ HPV HR	Negative	Positive	Total
Positive	157	10	167
Negative	126	3	129
Total	283	13	296
<b>Disease Prevalence:</b>	4.4% (13/296)	95% CI	
<b>Sensitivity:</b>	76.9% (10/13)	49.7%	91.8%
<b>Specificity:</b>	44.5% (126/283)	38.8%	50.3%
<b>PPV:</b>	6.0% (10/167)	2.9%	10.74%
<b>NPV:</b>	97.7% (126/129)	93.4%	99.5%
<b>Age: 39 or older</b>	Central Histology ≥ CIN2		
Cervista™ HPV HR	Negative	Positive	Total
Positive	131	14	145
Negative	268	2	270
Total	399	16	415
<b>Disease Prevalence:</b>	3.9% (16/415)	95% CI	
<b>Sensitivity:</b>	87.5% (14/16)	64.0%	96.5%
<b>Specificity:</b>	67.2% (268/399)	62.4%	71.6%
<b>PPV:</b>	9.7% (14/145)	5.4%	15.67%
<b>NPV:</b>	99.3% (268/270)	97.3%	99.9%

There are a number of key variables that are known to influence the performance characteristics of any HPV test in a clinical study. These include, but are not limited to, cervical sampling techniques, the quality of the cytology results, age of the population tested, disease prevalence, disease ascertainment methods and methods for histological interpretation. Given the number of variables present during routine HPV testing across multiple clinical sites, it is noteworthy that many of the results obtained from the TWT clinical trial are similar to those seen under the controlled trial conditions described in the ASC-US/LSIL Triage Study (ALTS).<sup>6,8,28</sup> A comparison of the study design, disease prevalence and clinical performance characteristics for the TWT study and ALTS is shown in Table 14. The difference in  $\geq$  CIN2 rates observed between the two studies may reflect population differences as well as disease ascertainment differences.

**Table 14:** Comparison of TWT Clinical Trial and ALTS<sup>6,8</sup>

Criterion	ALTS	TWT
Number of Enrollment Sites / States	4 / 4	89 / 22
Mean Age of Subjects	29	33
Subjects with colposcopy completed	1149 <sup>a</sup>	1347 <sup>b</sup>
Subjects with no lesion; no biopsy performed (%)	25%	28%
Subjects with no pathologic lesion on biopsy (%)	49%	53%
Subjects with $\geq$ CIN1 (%)	15%	14%
Subjects with $\geq$ CIN2 (%)	11%	5%
Detection rate for $\geq$ CIN2	96%	93%
Detection rate for $\geq$ CIN3	96%	100%
Negative Predictive Value for $\geq$ CIN2	98.9%	99.1%
Negative Predictive Value for $\geq$ CIN3	99.5%	100.0%
Referral rate to colposcopy	57%	57% <sup>c</sup>
PCR concordance	82.7%	86.1%

<sup>a</sup> Immediate colposcopy arm of ALTS

<sup>b</sup> Number of subjects with known disease status and Cervista™ HPV HR results

<sup>c</sup> Referral rate for women 30 years of age and older was 43%

**IN WOMEN 30 YEARS AND OLDER, SCREENING PERFORMANCE OF THE CERVISTA™ HPV HR TEST AS AN ADJUNCT TO CERVICAL CYTOLOGY TO HELP GUIDE PATIENT MANAGEMENT**

A longitudinal 3 year post-approval study has been initiated to support the use of the Cervista™ HPV HR test as an adjunct to cervical cytology, compared with cervical cytology alone. The study design is described below, along with some preliminary analytical data obtained from the study population at enrollment. This analytical study was used for evaluation of agreement of the Cervista™ HPV HR test with a composite HPV comparator between the ASC-US and NILM  $\geq$ 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 results are normal.<sup>5,16,23,25</sup>

### Description of NILM≥30 clinical study

Approximately 2,000 qualified subjects with normal cervical cytology 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 HR as compared to those negative for the test at the time of enrollment (T<sub>0</sub>). The positive and negative Cervista™ HPV HR results at T<sub>0</sub>, 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.

### Agreement with a Composite Comparator between the ASC-US and NILM ≥30 populations

The analytical performance of the test was measured against a composite comparator of an FDA-approved HPV assay and PCR/Sequencing. The composite comparator was defined as: Positive if the FDA-Approved HPV assay and PCR/Sequencing results were positive; Negative if the FDA-Approved HPV assay and PCR/Sequencing results were negative; and Indeterminate if the FDA-Approved HPV assay and PCR/Sequencing results were discordant. A random subset of the same samples collected during the clinical study for the ASC-US populations (collected over a 17 month enrollment period) and longitudinal post-approval evaluations at the baseline for the NILM≥30 populations (collected over a 15 month enrollment period), respectively, was utilized for this analytical study.

For PCR/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. The FDA-Approved HPV assay was performed according to its approved labeling. Results of the composite analysis are shown in Tables 15 and 16 below.

**Table 15:** ASC-US Population Cervista™ HPV HR vs. Composite Comparator (PCR Sequencing and FDA-Approved HPV test)

		Cervista™ HPV HR		Total
		Positive	Negative	
Composite HR Result	Positive	536	25	561
	Negative	44	370	414
	Indeterminate	60	64	124
Total		640	459	1099

Positive percent agreement: 95.5% (536/561) with 95% CI: (93.5% - 97.1%)

Negative percent agreement: 89.4% (370/414) with 95% CI: (86.0% - 92.2%)

**Table 16:** NILM  $\geq 30$  Population Cervista™ HPV HR vs. Composite Comparator (PCR Sequencing and FDA-Approved HPV test)

		Cervista™ HPV HR		Total
		Positive	Negative	
Composite HR Result	Positive	17	1	18
	Negative	67	357	424
	Indeterminate	10	9	19
Total		94	367	461

Positive percent agreement: 94.4% (17/18) with 95% CI: (72.7% - 99.9%)

Negative percent agreement: 84.2% (357/424) with 95% CI: (80.4% - 87.5%)

### Analytical Sensitivity

Cloned HPV plasmid DNA, representing the 14 HPV types detected by the Cervista™ HPV HR 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 value (FAM FOZ Ratio) was = 1.20.

Limit of Detection (LoD) is the lowest amount of analyte in a sample that the sample has the test results “HPV detected” (FOZ >1.20) 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 the 14 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Each HPV plasmid DNA was tested at concentrations of 7500, 5000, 2500, and 1250 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  $\mu$ g 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>26</sup>

The Limit of Detection for each HPV type is referenced in Table 17. Limits are described in terms of the FAM FOZ Ratio 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 ratios were above the LoB.

**Table 17: Cervista™ HPV HR Test Analytical Sensitivity Summary**

HPV DNA Type	LoD (Copy Number/Reaction)	LoD (FAM FOZ Ratio)	SD <sub>s</sub>
16	1250-2500	1.34	0.08
18	1250-2500	1.34	0.08
31	1250-2500	1.30	0.06
33	2500-5000	1.31	0.07
35	5000-7500	1.34	0.09
39	2500-5000	1.30	0.06
45	1250-2500	1.31	0.06
51	2500-5000	1.35	0.09
52	1250-2500	1.28	0.04
56	1250-2500	1.37	0.10
58	2500-5000	1.35	0.09
59	2500-5000	1.35	0.09
66	2500-5000	1.30	0.06
68	2500-5000	1.30	0.06
<b>Mean</b>		<b>1.324</b>	<b>0.074</b>

In addition to the analytical sensitivity study described above, cell line dilutions were prepared to evaluate the performance of the Cervista™ HPV HR test 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. Using the clinical FAM FOZ Ratio cut-off of 1.525, the concentrations which were above the clinical cut-off 95% of the time were approximately 2,500 cells/mL for SiHa cells and 1,000 cells/mL for HeLa cells.

### Clinical Cut-off of the Cervista™ HPV HR Test

The clinical cut-off was assessed based on the approach described in a reference paper<sup>27</sup> for unbiased estimates of sensitivity and specificity. Briefly, the cut-off values were evaluated based on pre-specified clinical sensitivity targets for the detection of ≥CIN2 histology that were correspondingly near the cut-off values previously defined in analytical studies. Based on these criteria, an HPV FOZ ratio cut-off of ≥1.525 or a minimum HPV FOZ value of ≥1.93 for all three reaction mixes were selected as the cut-off values for the test.

### Precision

Repeatability and within-laboratory precision of the Cervista™ HPV HR 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 four plates. Different plate layouts were used for the runs within a day. The samples tested within each run included genomic DNA samples isolated from two HPV positive cell lines (SiHa - Type 16 and HeLa - Type 18), an HPV negative cell line (Jurkat) and contrived samples containing HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 or HPV68 plasmid DNA and Jurkat DNA. Each sample was tested in duplicate at three concentrations.

The total number of measurements per sample was 84 (21 days, 2 runs per day, 2 replicates per run).

**Table 18: Statistical Summary for 21 Day Precision Study**

Target	Copies/Reaction <sup>a</sup> or Cells/mL <sup>b</sup>	N	Mean HPV FOZ Ratio	Within-Run (repeatability)		Between- Run		Between- Day		Between- Operator		Total (Within- lab precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV 16	5,000 <sup>a</sup>	84	2.827	1.052	37%	0.694	25%	0.529	19%	0.074	3%	1.048	37%
	10,000 <sup>a</sup>	84	3.976	0.136	3%	0.263	7%	0.276	7%	0.281	7%	0.316	8%
HPV 18	5,000 <sup>a</sup>	84	2.236	0.280	13%	0.230	10%	0.176	8%	0.089	4%	0.304	14%
	10,000 <sup>a</sup>	84	3.182	0.105	3%	0.153	5%	0.092	3%	0.054	2%	0.160	5%
HPV 31	5,000 <sup>a</sup>	84	2.199	0.098	4%	0.142	6%	0.119	5%	0.079	4%	0.174	8%
	10,000 <sup>a</sup>	84	3.032	0.123	4%	0.178	6%	0.159	5%	0.082	3%	0.219	7%
HPV 33	5,000 <sup>a</sup>	84	1.840	0.319	17%	0.261	14%	0.214	12%	0.121	7%	0.362	20%
	10,000 <sup>a</sup>	84	2.622	0.100	4%	0.236	9%	0.164	6%	0.102	4%	0.230	9%
HPV 35	5,000 <sup>a</sup>	84	1.640	0.226	14%	0.249	15%	0.157	10%	0.052	3%	0.280	17%
	10,000 <sup>a</sup>	84	2.339	0.077	3%	0.149	6%	0.084	4%	0.070	3%	0.142	6%
HPV 39	5,000 <sup>a</sup>	84	2.078	0.061	3%	0.124	6%	0.081	4%	0.050	2%	0.116	6%
	10,000 <sup>a</sup>	84	2.986	0.100	3%	0.259	9%	0.139	5%	0.055	2%	0.239	8%
HPV 45	5,000 <sup>a</sup>	84	2.514	0.092	4%	0.127	5%	0.124	5%	0.117	5%	0.158	6%
	10,000 <sup>a</sup>	84	3.606	0.172	5%	0.263	7%	0.277	8%	0.306	8%	0.325	9%
HPV 51	5,000 <sup>a</sup>	84	2.301	0.150	7%	0.198	9%	0.171	7%	0.122	5%	0.230	10%
	10,000 <sup>a</sup>	84	3.329	0.156	5%	0.323	10%	0.272	8%	0.274	8%	0.343	10%
HPV 52	5,000 <sup>a</sup>	84	1.961	0.364	19%	0.275	14%	0.222	11%	0.122	6%	0.389	20%
	10,000 <sup>a</sup>	84	2.756	0.095	3%	0.233	8%	0.150	5%	0.104	4%	0.239	9%
HPV 56	5,000 <sup>a</sup>	84	2.280	0.123	5%	0.169	7%	0.147	6%	0.142	6%	0.209	9%
	10,000 <sup>a</sup>	84	3.310	0.160	5%	0.266	8%	0.167	5%	0.131	4%	0.274	8%
HPV 58	5,000 <sup>a</sup>	84	2.255	0.102	5%	0.113	5%	0.071	3%	0.040	2%	0.130	6%
	10,000 <sup>a</sup>	84	3.121	0.158	5%	0.273	9%	0.172	6%	0.137	4%	0.276	9%
HPV 59	5,000 <sup>a</sup>	84	1.822	0.070	4%	0.165	9%	0.144	8%	0.153	8%	0.182	10%
	10,000 <sup>a</sup>	84	2.663	0.079	3%	0.186	7%	0.154	6%	0.174	7%	0.218	8%
HPV 66	5,000 <sup>a</sup>	84	2.126	0.087	4%	0.150	7%	0.159	7%	0.157	7%	0.194	9%
	10,000 <sup>a</sup>	84	2.968	0.132	4%	0.247	8%	0.290	10%	0.312	11%	0.336	11%
HPV 68	5,000 <sup>a</sup>	84	2.015	0.058	3%	0.129	6%	0.054	3%	0.045	2%	0.119	6%
	10,000 <sup>a</sup>	84	2.823	0.098	3%	0.127	4%	0.103	4%	0.059	2%	0.173	6%
SiHa/ Jurkat	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	3.303	0.148	4%	0.299	5%	0.107	3%	0.059	2%	0.333	5%
Hela/ Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	2.495	0.121	5%	0.018	2%	0.098	4%	0.061	2%	0.026	3%
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	6.130	0.183	3%	0.027	3%	0.214	3%	0.088	1%	0.038	4%
Jurkat	10,000 <sup>b</sup>	84	1.030	0.161	16%	0.059	6%	0.089	9%	0.029	3%	0.067	6%
	20,000 <sup>b</sup>	84	1.003	0.026	3%	0.112	8%	0.013	1%	0.005	0%	0.116	8%
	100,000 <sup>b</sup>	84	1.005	0.038	4%	0.156	5%	0.019	2%	0.008	1%	0.185	6%

<sup>a</sup> HPV 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 19: Summary of Positive Results for 21 day Precision Study.**

Target	Copies/Reaction <sup>a</sup> or Cells/mL extracted <sup>b</sup>	N	Mean HPV FOZ Ratio	HPV Positive % (n)			
				Operator 1	Operator 2	Operator 3	Total
HPV 16	5,000 <sup>a</sup>	84	2.827	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.976	100% (28)	100% (28)	100% (28)	100% (84)
HPV 18	5,000 <sup>a</sup>	84	2.236	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.182	100% (28)	100% (28)	100% (28)	100% (84)
HPV 31	5,000 <sup>a</sup>	84	2.199	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.032	100% (28)	100% (28)	100% (28)	100% (84)
HPV 33	5,000 <sup>a</sup>	84	1.840	89% (25)	100% (28)	100% (28)	96% (81)
	10,000 <sup>a</sup>	84	2.622	100% (28)	100% (28)	100% (28)	100% (84)
HPV 35	5,000 <sup>a</sup>	84	1.640	61% (17)	71% (20)	82% (23)	71% (60)
	10,000 <sup>a</sup>	84	2.339	100% (28)	100% (28)	100% (28)	100% (84)
HPV 39	5,000 <sup>a</sup>	84	2.078	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	2.986	100% (28)	100% (28)	100% (28)	100% (84)
HPV 45	5,000 <sup>a</sup>	84	2.514	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.606	100% (28)	100% (28)	100% (28)	100% (84)
HPV 51	5,000 <sup>a</sup>	84	2.301	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.329	100% (28)	100% (28)	100% (28)	100% (84)
HPV 52	5,000 <sup>a</sup>	84	1.961	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	2.756	100% (28)	100% (28)	100% (28)	100% (84)
HPV 56	5,000 <sup>a</sup>	84	2.280	96% (27)	100% (28)	100% (28)	99% (83)
	10,000 <sup>a</sup>	84	3.310	100% (28)	100% (28)	100% (28)	100% (84)
HPV 58	5,000 <sup>a</sup>	84	2.255	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	3.121	100% (28)	100% (28)	100% (28)	100% (84)
HPV 59	5,000 <sup>a</sup>	84	1.822	82% (23)	100% (28)	100% (28)	94% (79)
	10,000 <sup>a</sup>	84	2.663	100% (28)	100% (28)	100% (28)	100% (84)
HPV 66	5,000 <sup>a</sup>	84	2.126	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	2.968	100% (28)	100% (28)	100% (28)	100% (84)
HPV 68	5,000 <sup>a</sup>	84	2.015	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 <sup>a</sup>	84	2.823	100% (28)	100% (28)	100% (28)	100% (84)
SiHa/Jurkat	20,000 SiHa / 80,000 Jurkat <sup>b</sup>	84	3.303	100% (28)	100% (28)	100% (28)	100% (84)
Hela/Jurkat	2500 HeLa / 97,500 Jurkat <sup>b</sup>	84	2.495	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 HeLa / 90,000 Jurkat <sup>b</sup>	84	6.130	100% (28)	100% (28)	100% (28)	100% (84)
Jurkat	10,000 <sup>b</sup>	84	1.030	4% (1)	0% (0)	4% (1)	2% (2)
	20,000 <sup>b</sup>	84	1.003	0% (0)	0% (0)	0% (0)	0% (0)
	100,000 <sup>b</sup>	84	1.005	0% (0)	0% (0)	0% (0)	0% (0)

<sup>a</sup> HPV 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).

At 5000 copies/reaction the plasmid DNA samples yielded 97.2% (1143/1176) of expected positive results. At 10,000 copies/reaction, the plasmid DNA samples yielded 100.0% (1176/1176) of the expected positive results (see Table 19).

## Reproducibility

Reproducibility of the Cervista™ HPV HR 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 specimens 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 HR at three sites on five non-consecutive days within a two-week time period. Two lots of Cervista™ HPV HR 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 expected and observed results combined for all sites is shown in Table 20. Individual sample results across sites along with a cumulative mean and standard deviation for the HPV FOZ ratio are presented in Table 21.

**Table 20.** Data Summary for a Multi-center Reproducibility Study of the Cervista™ HPV HR Test.

Expected Result	Number of Results	Results in Agreement	Percent Agreement	Lower Limit of 95% CI
Positive	210	208	99.0%	96.6%
Negative	30	30	100.0%	88.7%

**Table 21:** Summary of Cervista™ HPV HR Results for Each Sample from a Multi-Center Reproducibility Study

Sample	Sample Type and Concentration (cells/mL)	N	HPV FOZ Ratio		HPV Positive % (n)			
			Mean	SD	Site 1	Site 2	Site 3	Total (n)
1 Neg	100,000 Jurkat	15	1.021	0.044	0 (0)	0 (0)	0 (0)	0
2 Pos	10,000 HeLa 90,000 Jurkat	15	6.369	1.522	100 (5)	100 (5)	100 (5)	15
3 Pos	5,000 HeLa 95,000 Jurkat	15	5.004	1.004	100 (5)	100 (5)	100 (5)	15
4 Pos	2,500 HeLa 97,500 Jurkat	15	3.337	0.886	100 (5)	100 (5)	100 (5)	15
5 Pos	20,000 SiHa 80,000 Jurkat	15	4.803	1.087	100 (5)	100 (5)	100 (5)	15
6 Pos	10,000 SiHa 90,000 Jurkat	15	3.194	0.780	100 (5)	100 (5)	100 (5)	15
7 Pos	5,000 SiHa 95,000 Jurkat	15	2.401	0.970	100 (5)	60 (3)	100 (5)	13
8 Pos	5,000 SiHa 2,500 HeLa 12,500 Jurkat	15	3.402	0.774	100 (5)	100 (5)	100 (5)	15
9 Pos	Cervical Pool (A5/A6 Pos)	15	5.930	2.212	100 (5)	100 (5)	100 (5)	15
10 Pos	Cervical Pool (A5/A6 Pos)	15	8.359	2.532	100 (5)	100 (5)	100 (5)	15
11 Pos	Cervical Pool (A7 Pos)	15	5.793	1.493	100 (5)	100 (5)	100 (5)	15

12 Pos	Cervical Pool (A7 Pos)	15	7.127	1.762	100 (5)	100 (5)	100 (5)	15
13 Pos	Cervical Pool (A9 Pos)	15	8.008	2.313	100 (5)	100 (5)	100 (5)	15
14 Pos	Cervical Pool (A9 Pos)	15	7.735	2.318	100 (5)	100 (5)	100 (5)	15
15 Pos	Cervical Pool (Mixed Pos)	15	7.345	2.143	100 (5)	100 (5)	100 (5)	15
16 Neg	Cervical Pool (Neg)	15	1.196	0.137	0 (0)	0 (0)	0 (0)	0

### Interfering Substances

Four cervical specimens (one HPV negative, three HPV positive) and three cell line samples (one HPV negative, two HPV positive) described in Table 22 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 23). 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 HR test to assess interference caused by the introduced substances.

**Table 22: Interfering Substance Sample Descriptions**

Sample	Description
Cervical Specimen HPV Positive	Cervical specimen stored in PreservCyt® solution PCR/Sequencing result: "Positive"
Cervical Specimen HPV Negative	Cervical specimen stored in PreservCyt® solution PCR/Sequencing result: "Negative"
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 10,000 cells/mL SiHa cells (HPV positive) and 90,000 cells/mL Jurkat cells
HeLa/Jurkat	Cell line sample stored in PreservCyt® solution containing 5,000 cells/mL HeLa cells (HPV positive) and 95,000 cells/mL Jurkat cells

**Table 23: Interfering Substances Results**

Interferent		Concentrations Tested	Interference Observed?
Source	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%	Yes <sup>b</sup>
	Magnetic Beads	5%, 10%	Yes <sup>b</sup>

<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.

<sup>b</sup>The levels of interferent that may cause testing failures are unusually high and should not be encountered in purified DNA samples.

During DNA extraction, the contraceptive jelly showed visually detectable interference with the magnetic bead separation in the 10 mM Tris buffer, resulting in 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 cloned Human papillomavirus types of low or undetermined risk were tested with the Cervista™ HPV HR test to assess potential cross-reactivity (see Tables 24-26).

**Table 24:** 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 HR test.

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

**Table 25:** 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 HR 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 meningitides</i>
	<i>Mycoplasma hominis</i>

**Table 26:** 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 or as noted. The Cervista™ HPV HR test did not exhibit any cross-reactivity to common low risk HPV types 6,11,42,43,44 and 53. Samples HPV type 1a and the internal control both generated negative results.

Human papillomavirus type 1a	Human papillomavirus type 44
Human papillomavirus type 6	Human papillomavirus type 53
Human papillomavirus type 11	Human papillomavirus type 67*
Human papillomavirus type 42	Human papillomavirus type 70*
Human papillomavirus type 43	Human Internal Control gene

\*Human papillomavirus types 67 and 70 yielded positive results with the Cervista™ HPV HR test at  $1 \times 10^5$  and  $1 \times 10^7$  copies/reaction. Upon further titration of these samples, negative results were obtained with the Cervista™ HPV HR test at 1000 copies/reaction and 10,000 copies/reaction respectively.

In addition, DNA extracted from a panel of twelve cervical specimens that were stored in PreservCyt® Solution and previously confirmed to contain HPV types of low or undetermined risk (HPV types 6, 42, 43, 44, 53 or 70) by PCR/sequencing was also tested and yielded negative results with the Cervista™ HPV HR test.

An additional cross-reactivity study was conducted for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Neisseria meningitides*, 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 HR test does not cross-react with DNA isolated from PreservCyt® samples containing up to  $1.0 \times 10^7$  cfu/mL of *Neisseria gonorrhoeae* and *Neisseria meningitides*,  $5 \times 10^6$  cfu/mL of *Mycoplasma hominis* and  $1.0 \times 10^6$  cfu/mL *Chlamydia trachomatis*.

## REFERENCES

1. Wallboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189(1):12-19.
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. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM; ALTS Group. 2007. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *J Infect Dis* Jun 1;195(11):1582-1589. Epub 2007 Apr 16.
5. Castle PE, Wacholder S, Sherman ME, Lorincz AT, Glass AG, Scott DR, Rush BB, Demuth F, Schiffman M. 2002. Absolute risk of a subsequent abnormal Pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. *Cancer* Nov;95(10):2145-2151.
6. 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). *Jour Nat Can Inst* 94(2): 102-107.
7. Wright TC, Jr., Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. 2002. Consensus Guidelines for the management of women with cervical cytological abnormalities. *JAMA* 287: 2120-2129.
8. 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. *Jour Nat Can Inst* 93(4): 293-299.
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. 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.
11. 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.
12. Bosch FX, Lorincz A, Munoz N, Meijer CJLM, Shah KV. 2002. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* Apr;55(4):244-265.
13. Woodman CBJ, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP. 2001. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* Jun;357(9271):1831-1836.
14. Wallin K-L, Wiklund F, Angstrom T, Bergman F, Stendahl U, Wadell G, Hallmans G, Dillner J. 1999. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med* Nov;341(22):1633-1638.
15. Wright TC Jr, Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, Hatch K, Noller KL, Roach N, Runowicz C, Saslow D. 2004. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol* 103: 304-309.
16. Kjaer S, Hogdall E, Frederiksen K, et al. 2006. The absolute risk of cervical abnormalities in highrisk human papillomavirus-positive, cytologically normal women over a 10-year period. *Cancer Res* 66:10630-10636.

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., 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): 346-55.
19. 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.
20. 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.
21. Peyton C, Gravitt P, Hunt W, Hundley R, Zhao M, Apple R, Wheeler C. 2001. Determinants of Genital Human Papillomavirus Detection in a US Population. *J Infect Dis* 183:1554-1564.
22. Dunne E, Unger E, Sternberg M, McQuillan G, Swan D, Patel S, Markowitz L. 2007. Prevalence of HPV Infection Among Females in the United States. *JAMA* Feb;297(8): 813-819.
23. Sherman M, et al. 2003. Baseline Cytology, Human Papillomavirus Testing, and Risk for Cervical Neoplasia: A 10-Year Cohort Analysis. *Jour Nat Can Inst* 95: 46–52.
24. Giuliano AR, Peppenfuss M, Abrahamsen M, et al. 2001. Human papillomavirus infection in the United States-Mexico border: implications for cervical cancer prevention and control. *Cancer Epidemiol Biomarkers Prev* 10:1129-1136.
25. Liaw K, et al., 1999. Detection of Human Papillomavirus DNA in Cytologically Normal Women and Subsequent Cervical Squamous Intraepithelial Lesions. *J Natl Cancer Inst* 91:954–960.
26. CLSI document EP17-A. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.
27. Kondratovich M, Yousef W. (2005) Evaluation of Accuracy and 'Optimal' Cut-off of Diagnostic Devices in the Same Study. *Proceedings of the 2005 Joint Statistical Meeting, Biopharmaceutical Section*, p.2547-2551.
28. Arbyn M, et al. 2004. Virologic Versus Cytologic Triage of Women With Equivocal Pap Smears: A Meta-analysis of the Accuracy To Detect High-Grade Intraepithelial Neoplasia. *J Natl Cancer Inst*: 96:280-293.

## **TROUBLESHOOTING GUIDE**

**Table 27:** Troubleshooting Guide

<b>Observation</b>	<b>Probable Cause</b>	<b>Solution</b>
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: "Check FAM & Red gain settings and read the whole plate again. (Partial plate reads are not allowed.)" "Check FAM gain setting and read the whole plate again. (Partial plate reads are not allowed.)" "Check Red gain setting and read the whole plate again. (Partial plate reads are not allowed.)"	Fluorometer issues	See Troubleshooting Guide in the Invader Call Reporter™ Software User Manual for fluorometer issues that may contribute to this error.
	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.

<p>No Target Control displays the following results:</p> <p>High %CV (HPV NTC)</p> <p>High %CV (gDNA NTC)</p>	<p>Insufficient or inconsistent mixing of reagents</p>	<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>
	<p>Incorrect preparation of reaction mixes</p>	<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>
	<p>Inconsistent addition of the No Target Control or reaction mix to the microplate</p>	<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>
	<p>Suspected contamination during sample addition or reaction mix preparation</p>	<ul style="list-style-type: none"> <li>• Use nuclease-free aerosol barrier tips and sterile tubes when making the reaction mixes.</li> <li>• Wear gloves when setting up the test.</li> <li>• Make sure that pipette tips touch only the solution being dispensed.</li> <li>• Do not touch pipette tips with hands.</li> <li>• Clean lab surfaces using appropriate materials.</li> </ul>
	<p>Sample evaporation</p>	<p>Verify mineral oil addition to each well.</p>
	<p>Bubbles in reaction plate wells</p>	<p>If possible, spin down plates prior to fluorescence scanning.</p>
	<p>Prepared reaction mixes were not used within recommended time period</p>	<p>Use reaction mixes within 30 minutes of preparation.</p>

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.
Improper plate orientation	When scanning the plate, orient the plate so well A-1 is in the upper left-hand corner	
Bubbles in the reaction plate 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.	

Sample displays "IND: High %CV" 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.
		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.	

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.</li> <li>• Verify the correct sample volume was added to each well.</li> <li>• Verify that proper procedure was followed for DNA extraction</li> </ul>
	Suspected error during DNA extraction	
	Insufficient amount of DNA was used in the test	
	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 prior to testing	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> <li>• Refer to the Package Insert, Performance Characteristics (Interfering Substances) section.</li> </ul>
	DNA sample inhibition	
Insufficient Sample DNA volume	Insufficient elution volume during DNA extraction	Repeat DNA extraction from the specimen.
		Verify that proper procedure was followed for DNA extraction
High number of DNA samples with positive FAM FOZ values in all three reaction mixes	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>
	Suspected DNA extraction reagent contamination	

## **CONTACT INFORMATION:**

### **Manufacturer:**

Third Wave Technologies, Inc.,  
502 S. Rosa Road,  
Madison, WI, 53719 USA  
Phone: 608.273.8933  
Website: www.twt.com

### **Technical Support:**

Third Wave Technologies, Inc.,  
Phone: 888-898-2357, option 3

## **NOTICE TO RECIPIENT ABOUT LIMITED LICENSE**

The receipt of this product from Third Wave Technologies, Inc. or its authorized distributor includes a limited, non-exclusive license under patent rights held by Third Wave Technologies, Inc. Acquisition of this product constitutes acceptance by the recipient of this limited license. Recipients unwilling to accept the limited license must return the product for a full refund. Such license is solely for the purposes of using this product to detect a specific analyte. For avoidance of doubt, the foregoing license does not include rights to use this product for agriculture or veterinary medicine applications. The foregoing license does not include a license to use the product for new product research or development, product manufacture, or any reverse-engineering purposes. The purchaser of this product is not authorized to transfer this product to any third party for any purpose without the express written consent of Third Wave Technologies, Inc. Except as expressly provided in this paragraph, no other license is granted expressly, impliedly, or by estoppel. For information concerning the availability of additional licenses to practice the patented methodologies, contact:

Legal Department, Third Wave Technologies, Inc., 502 South Rosa Rd., Madison, WI, 53719, (608) 273-8933.

The Cervista™ HPV HR test uses a proprietary Invader® chemistry and specific components covered under: U.S. Patent Nos. 5,614,402; 5,795,763; 5,846,717; 5,985,557; 5,994,069; 6,001,567; 6,090,543; 6,090,606; 6,348,314, 6,458,535; 6,555,357; 6,562,611; 6,635,463; 6,673,616; 6,759,226; 6,872,816; 6,875,572; 6,913,881; 7,060,436; 7,067,643; 7,087,381; Canadian Patent Nos. 2,163,015; 2,203,627; Australian Patent Nos. 694,736; 731,062; 737,449; 738,849; 744,369; 779,443; 781,188; Japanese Patent No. 3,665,648; European Patent No. 711,361. All U.S. patents and foreign patents where applicable that have or may hereafter issue in respect of such applications for patents; and all U.S and foreign patent applications and patents issuing thereon where applicable whose subject matter in whole or in part is entitled to the benefit of the filing date(s) of any of the foregoing patents/patent applications listed in this product insert.

## LIMITED PRODUCT WARRANTY

Third Wave Technologies, Inc. warrants that this product will meet the specifications stated on the product information sheet. If any component of this product does not conform to these specifications, Third Wave Technologies, Inc. will at its sole discretion, as its sole and exclusive liability and as the users sole and exclusive remedy, replace the product at no charge or refund the cost of the product; provided that notice of nonconformance is given to Third Wave Technologies, Inc. within sixty (60) days of receipt of the product.

This warranty limits Third Wave Technologies, Inc. liability to the replacement of this product or refund of the cost of the product. NO OTHER WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT, ARE PROVIDED BY THIRD WAVE TECHNOLOGIES, INC. Third Wave Technologies, Inc. shall have no liability for any direct, indirect, consequential or incidental damages arising out of the use, the results of use or the inability to use this product and its components.

Third Wave<sup>®</sup>, Cleavase<sup>®</sup>, and Invader<sup>®</sup> are registered trademarks of Third Wave Technologies, Inc. Cervista<sup>™</sup> and Invader Call Reporter<sup>™</sup> are trademarks of Third Wave Technologies, Inc. All other Trademarks / Registered Trademarks referenced within this product insert, are the property of each of their respective companies.

Some components of nucleic acid analysis, such as specific methods and compositions for manipulating or visualizing nucleic acids for analysis, may be covered by one or more patents owned by other parties. Similarly, nucleic acids containing specific nucleotide sequences may be patented. Making, using or selling such components or nucleic acids may require one or more licenses. Nothing in this document should be construed as an authorization or implicit license to make, use or sell any so covered component or nucleic acid under any such patents.

©2009 Third Wave Technologies, Inc.

P/N 15-3100, Revision C