Cervista™ HPV HR

REF 95-438 REF PRD-00804

An *in vitro* diagnostic test
for the detection of DNA from14 high-risk Human Papillomavirus (HPV) types
(16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in Cervical Specimens.

95-438: Σ96 -15°C -30°C

PRD-00804: Σ768

Do NOT store in frost-free freezer.
Protect from light.

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General Information

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.

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

The Cervista HPV HR test may be performed either manually or using the automated Cervista High Throughput Automation (HTA) system.

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 HTA system is only to be used for Cervista HPV HR testing and cannot be utilized for testing with the Cervista HPV 16/18 test.
- 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
HTA	High Throughput Automation
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

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.[1] 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 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).[2,3] Still, the vast majority of high-risk HPV infections are cleared.[4] Very few high-risk HPV DNA positive women develop cytologic high-grade SIL (HSIL) indicating underlying CIN2-3 or cancer.[5] 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).[6,7,8] CIN3, while occurring in only approximately 5% of ASC-US cases,[9] is an immediate precursor to cervical cancer and consequently its detection is very important for patient management.**[3]** 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.[3,6,10,11]

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.[4,12,13,14] 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.[15,16]

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.[7,17,15] 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.[15,18] 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.[7,10,18]

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. 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.[19] 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). The Cervista oligonucleotide mixtures contain at least two probes for each HPV type and every type has a probe that targets the E6 or E7 HPV viral oncogenes. 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.

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.

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.

Table 1: Cervista HPV HR Contents

Reagent	Vial Label Abbreviation	Vial Quantity & Reagent Volume for 96 tests (REF 95-438)	Vial Quantity & Reagent Volume for 768 tests (REF PRD-00804)	Component Description
HPV Oligo Mix 1	O1 (Blue cap and blue stripe)	1 x 1400 µL	8 x 1400 µL	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 µL	8 x 1400 µL	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 µL	8 x 1400 µL	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 µL	8 x 1100 µL	Cleavase Enzyme suspended in 140 mM $MgCl_2$, 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 350 µL	8 x 350 µL	1000 copies/µL cloned HPV type 51 DNA and 3000 copies/µL 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 350 µL	8 x 350 µL	1000 copies/µL cloned HPV type 18 DNA and 3000 copies/µL 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 350 µL	8 x 350 µL	1000 copies/µL cloned HPV type 16 DNA and 3000 copies/µL 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 µL	8 x 350 µL	Yeast tRNA and 10 mM Tris, 0.1 mM EDTA Buffer

* PRD-00804 is intended to be used for one run of 96 on the Cervista HTA system.

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.
- Hologic 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 Hologic to order the Genfind DNA Extraction Kit (REF 95-449).

Materials Required, But Not Provided, for Manual Testing

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 appropriate reaction temperatures.

Materials Required, But Not Provided, for Automated Testing

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

- Cervista HTA System
- Pipettes
- Vortex

Specimen Collection 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 HR 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**)**.

Automated Test Procedure for the Cervista HTA System

Refer to the Cervista HTA Operator's Manual (P/N MAN-01522-001) for the use of the automated system to perform the Cervista HPV HR test.

Manual Test Procedure for Cervista HPV HR

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.

Reaction Procedure

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

	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 1: Example Cervista HPV HR 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 three HPV Oligo Mixes prior to each use. Prepared reaction mixture should be used within 30 minutes.

Table 2: Reaction Mix Preparation Instructions

Reagent	µL/Reaction	No. Of Reactions (Samples & Controls (*k*))	Total Volume
HPV Oligo Mix 1, 2, or 3	8 µL	*k*	=8*k*(1.25) µL
Cleavase Enzyme Solution	2 µL	*k*	=2*k*(1.25) µL
Total Mix Volume	**10 µL**	***k***	**=10*k*(1.25) µL**

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 1), taking care to pipette below the mineral oil.
8. Incubate the plate at 63°C setting for 4 hours.

Data Collection

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).

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.

Define the plate type to set up the coordinates and probe height for the specific type of plate. Save the settings.

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).

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. 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 1 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.

* Procedural Notes 7, 10 and 11 do not apply to the Cervista HTA system.

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 or equal to 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 ≥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 2.

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.

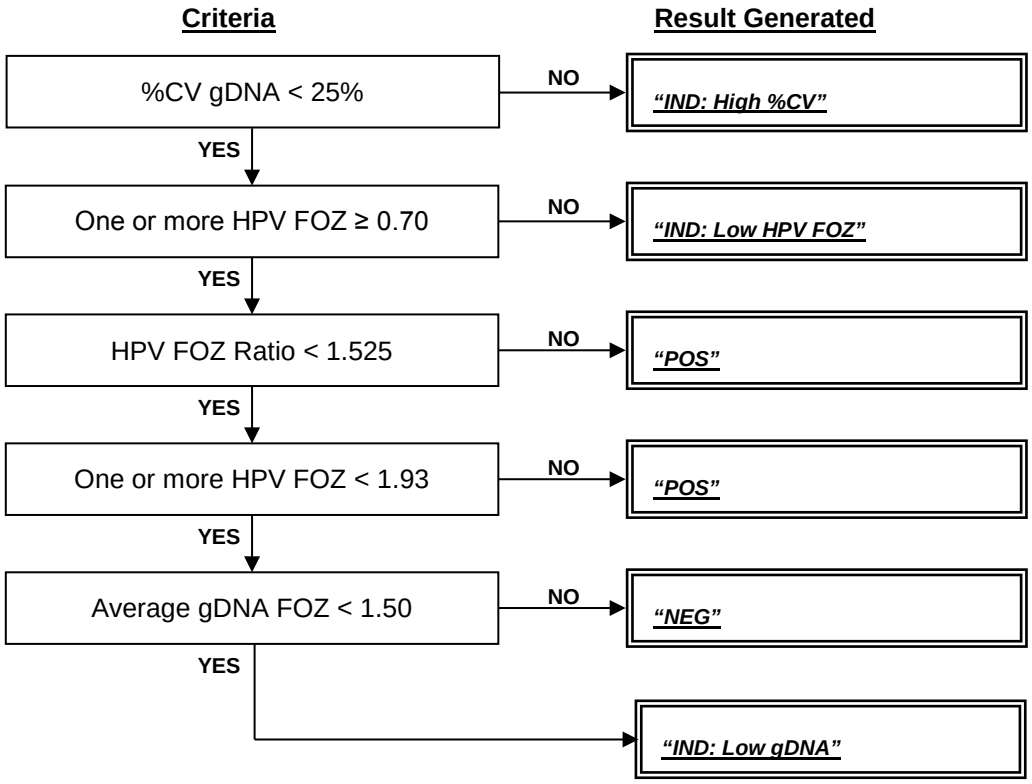

Figure 2: Sample Call Criteria Ordered Top to Bottom

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[a]
POS	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. [2,3,7,18]	Low likelihood of underlying high-grade CIN; HPV infection may be transient, resolving or persistent.
NEG	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
IND: High % CV[b]	Indeterminate	High risk HPV status unknown	
IND: Low gDNA [b]			

[a] According to the 2006 consensus guidelines[18], 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.

[b] 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 (≥ 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

Result	Min. HPV Signal	Min. gDNA Signal	Max. % CV (HPV and gDNA)
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 (≥ 1.525) for only HPV Oligo Mix 1, HPV Control 2 should yield a positive HPV FOZ value (≥ 1.525) for only HPV Oligo Mix 2, and HPV Control 3 should yield a positive HPV FOZ value (≥ 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 (≥ 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. Refer to the Troubleshooting section of the Cervista HTA Operator's Manual (P/N MAN-01522-001) for Cervista HTA systems.
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.[18]
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%.[20] 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

	ASC-US Population		NILM Population	
Center	**Subjects Tested**	**HPV HR Positive Rate**	**Subjects Tested**	**HPV HR Positive Rate**
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[21]	2001	3,863	18 - 40	26.7%
U.S. Civilian Population[22]	2007	1,921	14 - 59	15.2%
Oregon[23]	2003	20,156	16 - 94	16.3%
Arizona[24]	2001	988	15 - 79	13.9%

Performance Characteristics

The following performance characteristics of the Cervista HPV HR test have been established using the manual procedure.

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 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 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 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.

* This study was conducted prior to implementation of guidelines[18] that recommend limiting ASC-US HPV testing to women 21 years or older.

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
Total	382	714	182	47	22	1347

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

Age: 18 to <21	Central Histology ≥ CIN2		
Cervista HPV HR	Negative	Positive	Total
Positive	96	9	105
Negative	28	0	28
Total	124	9	133
Disease Prevalence:	6.8% (9/133)	95% CI	
Sensitivity:	100% (9/9)	70.1%	100.0%
Specificity:	22.6% (28/124)	16.1%	30.7%
PPV:	8.6% (9/105)	4.0%	15.70%
NPV:	100% (28/28)	87.7%	100.0%
Age: 21 to <30	Central Histology ≥ CIN2		
Cervista HPV HR	Negative	Positive	Total
Positive	321	31	352
Negative	136	0	136
Total	457	31	488
Disease Prevalence:	6.4% (31/488)	95% CI	
Sensitivity:	100% (31/31)	89.0%	100.0%
Specificity:	29.8% (136/457)	25.8%	34.1%
PPV:	8.8% (31/352)	6.1%	12.27%
NPV:	100% (136/136)	97.3%	100.0%
Age: 30 to <39	Central Histology ≥ CIN2		
Cervista HPV HR	Negative	Positive	Total
Positive	157	10	167
Negative	126	3	129
Total	283	13	296
Disease Prevalence:	4.4% (13/296)	95% CI	
Sensitivity:	76.9% (10/13)	49.7%	91.8%
Specificity:	44.5% (126/283)	38.8%	50.3%
PPV:	6.0% (10/167)	2.9%	10.74%
NPV:	97.7% (126/129)	93.4%	99.5%
Age: 39 or older	Central Histology ≥ CIN2		
Cervista HPV HR	Negative	Positive	Total
Positive	131	14	145
Negative	268	2	270
Total	399	16	415
Disease Prevalence:	3.9% (16/415)	95% CI	
Sensitivity:	87.5% (14/16)	64.0%	96.5%
Specificity:	67.2% (268/399)	62.4%	71.6%
PPV:	9.7% (14/145)	5.4%	15.67%
NPV:	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 Hologic clinical trial are similar to those seen under the controlled trial conditions described in the ASC-US/LSIL Triage Study (ALTS).[6,8,28] A comparison of the study design, disease prevalence and clinical performance characteristics for the Hologic study and ALTS is shown in Table 14. The difference in ≥ CIN2 rates observed between the two studies may reflect population differences as well as disease ascertainment differences.

Table 14: Comparison of Hologic Clinical Trial and ALTS6,8

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

[a] Immediate colposcopy arm of ALTS
[b] Number of subjects with known disease status and Cervista HPV HR results
[c] Referral rate for women 30 years of age and older was 43%

In Women 30 Years and Older with NILM Cytology, Screening Performance of the Cervista HPV HR Test as an Adjunct to Cervical Cytology to Help Guide Patient Management

The primary objective of the 3-year follow-up study was to demonstrate that subjects 30 years of age or older with negative for intraepithelial lesion or malignancy (NILM) cytology results at the time of enrollment (baseline) with Cervista HPV positive results have a higher risk of progression to ≥CIN2 over a 3-year follow-up period than subjects with Cervista HPV negative results. Subjects undergoing routine cervical cancer screening who had NILM cytology results were enrolled from 26 clinical centers throughout the United States into a 3-year follow-up study with annual cytology and, if needed, colposcopy/ biopsy visits. The residual ThinPrep cytology specimen collected at screening was tested with the Cervista HPV HR test. During follow-up annual visits, subjects whose cytology results were ASC-US or greater were referred to a colposcopic examination regardless of HPV status at baseline. Cervical biopsies were collected during colposcopy as directed by local standard of care guidelines and were sent to a central pathologist review panel for histological interpretation where cervical disease status was based on agreement of at least two expert pathologists. Cervical disease status in the study was considered “negative” based on NILM cytology or, for women with abnormal cytology test results, based on negative colposcopy or No CIN or CIN1 central histology results if biopsy was performed. Women diagnosed with ≥CIN2 were included in the analysis up to the time of the diagnosis. Clinical performance of the Cervista HPV HR test was evaluated for detection of ≥CIN2 disease.

Of the 2026 subjects enrolled, 67 subjects did not have Cervista HPV HR results. Among 1959 subjects, there were 362 subjects with Cervista HPV HR positive results at baseline, 79% (286/362), 75% (270/359) and 72% (257/358) had annual visits at year 1, 2, and 3 correspondingly. There were 1,597 subjects with Cervista HPV HR negative results at baseline, 78% (1250/1597), 77% (1227/1596) and 71% (1134/1595) had annual visits at year 1, 2, and 3 correspondingly. At the end of the 3-year follow-up period, there were 1384 subjects with known Cervista HPV HR results and disease status that completed the 3 year follow-up study. 73 of the 1384 subjects developed abnormal cytology results and 6 subjects had ≥CIN2 results and 1 subject had ≥CIN3. The prevalence of ≥CIN2 was 0.43% (6/1384). A summary of these results are presented in Table 15.

Table 15: Summary of Cytology and Histology Results at the End of the 3-Year Follow-Up Study

Cervista HPV HR Results	NILM Cytology	Abnormal Cytology					
		Neg. Colp., No Histology	Negative Histology	CIN1	CIN2	CIN3	Total
Positive	233	4	16	1	3	1	258
Negative	1078	15	27	4	2	0	1126
Total	1311	19	43	5	5	1	1384

Absolute and Relative Risk Estimates in NILM (≥30) Population from the 3-year Follow-up Study

The cumulative risks in the follow-up intervals based on Kaplan-Meier estimation (life table analysis) are shown in Table 16. The risk of ≥CIN2 was calculated for each interval by dividing the number of subjects diagnosed with ≥CIN2 in that interval by the number of subjects screened during that interval.

Table 16: Absolute Risk of ≥CIN2 by Study Year and Cervista HPV HR Status at Enrollment

	Cervista HPV HR Positive				Cervista HPV HR Negative			
Interval	Total at Risk	≥CIN2	Risk in Interval	Cumulative Risk	Total at Risk	≥CIN2	Risk in Interval	Cumulative Risk
1	286	3	1.05%	1.05%	1250	1	0.08%	0.08%
2	270	1	0.37%	1.42%	1227	1	0.08%	0.16%
3	257	0	0.00%	1.42%	1134	0	0.00%	0.16%

Probability of ≥CIN2 (regardless of HPV status at the baseline) was 0.40%

The cumulative risk of detection of (≥CIN2) over three years for subjects with Cervista HPV HR positive results at the baseline was 1.42% with 95% CI: 0.04, 2.79 and for subjects with Cervista HPV HR negative results at the baseline was 0.16% with 95% CI: 0.00, 0.38. The relative cumulative risk of detection of (≥CIN2) in the follow-up intervals was evaluated for subjects with Cervista HPV HR positive results at the baseline versus Cervista HPV HR negative results. After 3-years of follow-up, subjects with Cervista HPV HR positive results at the baseline were 8.8 times more likely (95% CI: 1.6, 47.6) to be diagnosed with ≥CIN2 disease compared to subjects with Cervista HPV HR negative results (Table 17).

Table 17: Relative Cumulative Risk of ≥CIN2 for Subjects with Cervista HPV HR Positive vs Negative Results

Follow-up Interval	Relative Cumulative Risk	95% CI
Year 1	13.1	(1.4, 125.6)
Year 2	8.8	(1.6, 47.9)
Year 3	8.8	(1.6, 47.6)

Analysis for evaluation of the impact of the missing data was performed and showed the robustness of the study conclusions.

Cervista HPV HR Test Clinical Performance for NILM (≥30) at the End of the 3-Year Follow-Up Study

The clinical performance of the Cervista HPV HR test in subjects completing the study is presented in Table 18 including sensitivity, specificity, positive likelihood ratio (PLR=sensitivity/(1-specificity)) and negative likelihood ratio (NLR=(1-sensitivity)/specificity)), positive predictive value (PPV) and negative predictive value (NPV) for detection of ≥CIN2.

Table 18: Cervista HPV HR Clinical Performance at the End of the 3-Year Follow-up Study

Performance	Estimate	95%CI
Sensitivity	66.7% (4/6)	(30.0%, 90.3%)
Specificity	81.6% (1124/1378)	(79.4%, 83.5%)
PLR	3.62	(1.60, 5.05)
NLR	0.409	(0.080, 0.874)
PPV	1.6% (4/258)	(0.6%, 2.2%)
NPV	99.8% (1124/1126)	(99.6%, 100%)
≥CIN2 Prevalence	0.43% (6/1384)	

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 19 and 20 below.

Table 19: 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 20: NILM ≥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 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.[26]

The Limit of Detection for each HPV type is referenced in Table 21. 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 21: Cervista HPV HR Test Analytical Sensitivity Summary

HPV DNA Type	LoD (Copy Number/Reaction)	LoD (FAM FOZ Ratio)	SD
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
Mean		**1.324**	**0.074**

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 [27] 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 HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 or HPV 68 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 22: Statistical Summary for 21 Day Precision Study

Target	Copies/Reaction[a] or Cells/mL[b]	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[a]	84	2.827	1.052	37%	0.694	25%	0.529	19%	0.074	3%	1.048	37%
	10,000[a]	84	3.976	0.136	3%	0.263	7%	0.276	7%	0.281	7%	0.316	8%
HPV 18	5,000[a]	84	2.236	0.280	13%	0.230	10%	0.176	8%	0.089	4%	0.304	14%
	10,000[a]	84	3.182	0.105	3%	0.153	5%	0.092	3%	0.054	2%	0.160	5%
HPV 31	5,000[a]	84	2.199	0.098	4%	0.142	6%	0.119	5%	0.079	4%	0.174	8%
	10,000[a]	84	3.032	0.123	4%	0.178	6%	0.159	5%	0.082	3%	0.219	7%
HPV 33	5,000[a]	84	1.840	0.319	17%	0.261	14%	0.214	12%	0.121	7%	0.362	20%
	10,000[a]	84	2.622	0.100	4%	0.236	9%	0.164	6%	0.102	4%	0.230	9%
HPV 35	5,000[a]	84	1.640	0.226	14%	0.249	15%	0.157	10%	0.052	3%	0.280	17%
	10,000[a]	84	2.339	0.077	3%	0.149	6%	0.084	4%	0.070	3%	0.142	6%
HPV 39	5,000[a]	84	2.078	0.061	3%	0.124	6%	0.081	4%	0.050	2%	0.116	6%
	10,000[a]	84	2.986	0.100	3%	0.259	9%	0.139	5%	0.055	2%	0.239	8%
HPV 45	5,000[a]	84	2.514	0.092	4%	0.127	5%	0.124	5%	0.117	5%	0.158	6%
	10,000[a]	84	3.606	0.172	5%	0.263	7%	0.277	8%	0.306	8%	0.325	9%
HPV 51	5,000[a]	84	2.301	0.150	7%	0.198	9%	0.171	7%	0.122	5%	0.230	10%
	10,000[a]	84	3.329	0.156	5%	0.323	10%	0.272	8%	0.274	8%	0.343	10%
HPV 52	5,000[a]	84	1.961	0.364	19%	0.275	14%	0.222	11%	0.122	6%	0.389	20%
	10,000[a]	84	2.756	0.095	3%	0.233	8%	0.150	5%	0.104	4%	0.239	9%
HPV 56	5,000[a]	84	2.280	0.123	5%	0.169	7%	0.147	6%	0.142	6%	0.209	9%
	10,000[a]	84	3.310	0.160	5%	0.266	8%	0.167	5%	0.131	4%	0.274	8%
HPV 58	5,000[a]	84	2.255	0.102	5%	0.113	5%	0.071	3%	0.040	2%	0.130	6%
	10,000[a]	84	3.121	0.158	5%	0.273	9%	0.172	6%	0.137	4%	0.276	9%
HPV 59	5,000[a]	84	1.822	0.070	4%	0.165	9%	0.144	8%	0.153	8%	0.182	10%
	10,000[a]	84	2.663	0.079	3%	0.186	7%	0.154	6%	0.174	7%	0.218	8%
HPV 66	5,000[a]	84	2.126	0.087	4%	0.150	7%	0.159	7%	0.157	7%	0.194	9%
	10,000[a]	84	2.968	0.132	4%	0.247	8%	0.290	10%	0.312	11%	0.336	11%
HPV 68	5,000[a]	84	2.015	0.058	3%	0.129	6%	0.054	3%	0.045	2%	0.119	6%
	10,000[a]	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[b]	84	3.303	0.148	4%	0.156	5%	0.107	3%	0.059	2%	0.185	6%
HeLa/ Jurkat	2500 HeLa / 97,500 Jurkat[b]	84	2.495	0.121	5%	0.209	8%	0.098	4%	0.061	2%	0.206	8%
	10,000 HeLa / 90,000 Jurkat[b]	84	6.130	0.183	3%	0.299	5%	0.214	3%	0.088	1%	0.333	5%
Jurkat	10,000[b]	84	1.030	0.161	16%	0.114	11%	0.089	9%	0.029	3%	0.159	15%
	20,000[b]	84	1.003	0.026	3%	0.018	2%	0.013	1%	0.005	0%	0.026	3%
	100,000[b]	84	1.005	0.038	4%	0.027	3%	0.019	2%	0.008	1%	0.038	4%

[a] HPV plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

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

Table 23: Summary of Positive Results for 21 Day Precision Study

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

[a] HPV plasmid DNA at the indicated concentration (copies/reaction) mixed with 100ng/reaction of HPV negative genomic DNA (Jurkat).

[b] 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 23).

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 24. Individual sample results across sites along with a cumulative mean and standard deviation for the HPV FOZ ratio are presented in Table 25.

Table 24. 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 25: 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 26 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 27). 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 26: 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 27: 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[a]
	Anti-fungal Cream containing 2% clotrimizole	0.5%, 2%	Yes[a]
	Anti-fungal Cream containing 4% miconazole	0.5%, 2%	Yes[a]
Genfind DNA Extraction Kit Sample Processing	PreservCyt Solution	0.5%, 2%	No
	70% Ethanol	5%, 10%	Yes[b]
	Magnetic Beads	5%, 10%	Yes[b]

[a]The levels of interferent required to cause testing failures (2%) are unusually high and should not be encountered in actual clinical specimens.
[b]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 28-30).

Table 28: The organisms listed below were added to PreservCyt Solution at concentrations of approximately 1 x10^5 cfu/mL and 1x10^7 cfu/mL. DNA from these organisms and a negative cell line (Jurkat, 1x10^5 cells/mL) was extracted using the Genfind DNA Extraction Kit. All samples yielded negative results with the Cervista HPV HR test.

Candida albicans	*Proteus vulgaris*
Corynebacterium pseudodiptheriticum	*Staphylococcus aureus*
Enterococcus faecalis	*Staphylococcus epidermidis*
Escherichia coli	*Streptococcus mitis*
Lactobacillus acidophilus	*Streptococcus pyogenes*

Table 29: Purified DNA obtained from the organisms listed below was tested at concentrations of 1x10^5 copies/reaction and 1x10^7 copies/reaction using the Cervista HPV HR test. All samples yielded negative results.

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

Table 30: Cloned DNA or PCR amplicons for the following samples were tested at concentrations of 1x10^5 copies/reaction and 1x10^7 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 1x10^5 and 1x10^7 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 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 HR test does not cross-react with DNA isolated from PreservCyt® samples containing up to 1.0 x 10^7 cfu/mL of *Neisseria gonorrhoeae* and N*eisseria meningitidis,* 5x10^6 cfu/mL of *Mycoplasma hominis* and 1.0 x 10^6 cfu/mL *Chlamydia trachomatis*.

Test Performance Characteristics Using the Cervista HTA System

Testing was conducted to evaluate that results from the Cervista HPV HR test performed on the Cervista HTA system are in agreement with results obtained using the manual procedure. Precision and reproducibility testing were performed using the appropriate sample panels and the results were compared between the manual procedure and the HTA system for the Cervista HPV HR test. Additionally, a comparison panel study using clinical specimens was conducted to evaluate the percent agreement of the results between the automated and the manual procedure.

The following sections describe the testing in more detail.

Precision

The precision study was performed for the Cervista HTA system using HPV plasmid DNA samples and HPV positive cell lines. The tests were conducted with two operators over the course of 12 days with 2 runs per day and 2 replicates per sample in each run for a total of 48 measurements of each sample. In one study (Table 31), HPV plasmid DNA samples consisting of 14 HPV HR types at 3 concentrations of each plasmid in a background of human genomic DNA were tested. The data are summarized in the following table.

Table 31: Statistical Summary for Cervista HTA System Plasmid Precision Study

Sample Type	Copies /Reaction	N[a]	Mean HPV FOZ Ratio	Within-Run (repeatability)		Between-Run		Between-Day		Total (Within-Lab Precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV 16	2500	44	1.88	0.10	5.3	0.06	3.0	0.12	6.2	0.16	8.7
	5000	46	2.63	0.11	4.3	0.13	4.8	0.17	6.5	0.24	9.2
	10000	46	3.94	0.23	6.0	0.28	7.1	0.30	7.7	0.47	12.0
HPV 18	2500	46	1.93	0.10	5.0	0.08	3.9	0.04	2.2	0.13	6.7
	5000	46	2.85	0.16	5.6	0.16	5.6	0.10	3.4	0.25	8.6
	10000	46	4.22	0.18	4.3	0.29	6.9	0.21	4.9	0.40	9.4
HPV 31	2500	46	1.63	0.10	6.2	0.08	5.1	0.10	6.0	0.16	10.0
	5000	46	2.26	0.10	4.3	0.13	5.7	0.16	7.1	0.23	10.0
	10000	43	3.57	0.15	4.1	0.25	7.0	0.25	6.9	0.38	11.0
HPV 33	2500	46	1.43	0.04	2.6	0.07	4.8	0.08	5.2	0.11	7.5
	5000	46	1.85	0.08	4.2	0.11	5.8	0.09	4.8	0.16	8.6
	10000	46	2.71	0.07	2.5	0.21	7.7	0.17	6.4	0.28	10.0
HPV 35	2500	48	1.37	0.05	3.8	0.03	2.3	0.02	1.3	0.06	4.6
	5000	48	1.71	0.05	2.8	0.04	2.3	0.06	3.3	0.09	4.9
	10000	48	2.43	0.13	5.2	0.04	1.4	0.07	2.9	0.15	6.2
HPV 39	2500	48	1.54	0.10	6.3	0.00	0.0	0.05	3.4	0.11	7.2
	5000	47	2.19	0.09	4.3	0.09	4.3	0.03	1.5	0.14	6.3
	10000	48	3.13	0.18	5.8	0.11	3.5	0.10	3.0	0.23	7.5
HPV 45	2500	48	1.95	0.10	5.1	0.03	1.5	0.01	0.6	0.10	5.3
	5000	48	2.78	0.10	3.5	0.11	4.0	0.08	2.7	0.17	6.0
	10000	48	4.49	0.11	2.4	0.12	2.8	0.15	3.4	0.22	5.0
HPV 51	2500	48	1.74	0.07	3.8	0.01	0.7	0.04	2.5	0.08	4.6
	5000	48	2.4	0.26	11.0	0.00	0.0	0.17	7.1	0.31	13.0
	10000	47	3.44	0.19	5.5	0.00	0.0	0.10	3.0	0.22	6.3
HPV 52	2500	48	1.63	0.05	2.9	0.06	3.8	0.00	0.0	0.08	4.7
	5000	48	2.17	0.10	4.4	0.07	3.0	0.04	2.0	0.12	5.7
	10000	48	3.29	0.14	4.2	0.11	3.4	0.06	1.8	0.19	5.7
HPV 56	2500	48	1.86	0.06	3.1	0.02	1.1	0.04	2.0	0.07	3.9
	5000	48	2.55	0.09	3.6	0.00	0.0	0.10	3.8	0.13	5.2
	10000	48	3.83	0.19	5.0	0.00	0.0	0.12	3.0	0.22	5.8
HPV 58	2500	48	1.7	0.22	13.0	0.00	0.0	0.12	7.1	0.25	15.0
	5000	48	2.29	0.12	5.2	0.08	3.6	0.00	0.0	0.15	6.4
	10000	45	3.52	0.15	4.2	0.11	3.0	0.11	3.0	0.21	6.0
HPV 59	2500	48	1.71	0.05	2.6	0.04	2.5	0.02	1.2	0.07	3.8
	5000	48	2.41	0.18	7.6	0.12	5.1	0.07	2.7	0.23	9.6
	10000	48	3.47	0.08	2.2	0.16	4.6	0.00	0.0	0.18	5.1
HPV 66	2500	48	1.56	0.04	2.2	0.06	3.6	0.05	3.2	0.08	5.3
	5000	47	2.16	0.10	4.5	0.05	2.4	0.09	4.0	0.14	6.5
	10000	48	3.21	0.19	5.9	0.07	2.0	0.17	5.4	0.26	8.2
HPV 68	2500	48	1.69	0.09	5.5	0.05	2.8	0.00	0.0	0.10	6.1
	5000	48	2.41	0.12	5.2	0.03	1.1	0.03	1.3	0.13	5.4
	10000	48	3.56	0.15	4.3	0.11	3.0	0.17	4.7	0.25	7.1

[a] 1.7% of the results obtained in the precision study were invalid and were subsequently excluded from the analysis.

Another precision study (Table 32) utilized 3 types of HPV positive cell line samples (51G – HPV 51, HeLa – HPV 18 and SiHa – HPV 16) prepared at 3 concentrations by adding HPV positive cultured cells to pooled negative cervical specimens. The data are summarized in the following table.

Table 32: Statistical Summary for Cervista HTA System Cell-Line Precision Study

Sample Type	Signal Level (cells/mL)	N	Mean HPV FOZ Ratio	Within-Run (repeatability)		Between-Run		Between-Day		Total (Within-Lab Precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
51G	High Negative (1,346)	48	1.36	0.06	4.1	0.02	1.3	0.05	3.6	0.08	5.6
	Low Positive (3,400)	48	1.76	0.10	5.5	0.05	2.9	0.08	4.5	0.14	7.7
	Moderate Positive (12,000)	48	3.22	0.21	6.6	0.03	0.8	0.22	6.7	0.30	9.4
HeLa	High Negative (278)	48	1.42	0.06	4.0	0.07	5.0	0.05	3.8	0.11	7.4
	Low Positive (798)	48	1.90	0.16	8.2	0.04	2.1	0.10	5.4	0.19	10.0
	Moderate Positive (2,500)	48	3.57	0.28	7.8	0.00	0.0	0.24	6.6	0.37	10.2
SiHa	High Negative (1,783)	48	1.42	0.07	5.1	0.03	2.4	0.04	3.1	0.09	6.4
	Low Positive (4,555)	47[a]	1.85	0.12	6.2	0.11	6.1	0.00	0.0	0.16	8.7
	Moderate Positive (12,000)	48	3.37	0.23	6.9	0.20	5.9	0.19	5.6	0.36	10.6

[a] One sample had an invalid test result and was not included in the analysis.

System Reproducibility

The reproducibility of the Cervista HTA system was evaluated by testing a panel of clinical cervical specimens and HPV positive cell lines. In one study (Table 33), 6 clinical cervical specimens that were HPV positive for Mix 1, Mix 2 or Mix 3 were diluted with pooled negative cervical specimens to produce low positive and moderate positive samples. Each panel member was tested at 2 sites over 6 days. Two operators at each site performed the testing that consisted of 2 runs per day with 2 replicates each sample per run. The data are summarized in Table 33.

Table 33: Summary of Cervista HTA System Results from Reproducibility Study with Clinical Specimens

Sample Type	Signal Level	N[a]	HPV FOZ Ratio		HPV Positive % (n)		
			Mean	SD	Site 1	Site 2	Total (n)
Mix 1	Low Positive	46	1.97	0.94	70 (16)	52 (12)	28
	Moderate Positive	46	3.32	1.58	83 (20)	96 (21)	41
Mix 2	Low Positive	48	2.28	1.00	79 (19)	58 (14)	33
	Moderate Positive	46	4.55	1.76	100 (24)	100 (22)	46
Mix 3	Low Positive	48	2.13	0.95	52 (13)	75 (18)	31
	Moderate Positive	46	4.59	1.20	100 (24)	100 (22)	46

[a] Results which were not obtained due to assay run failure (invalid results), or which were determined to be statistical outliers, were excluded. The values for SD and %CV may be underestimated.

In another study (Table 34), 9 panel members consisting of 3 types of HPV positive cell line samples (51G – HPV 51, HeLa – HPV 18 and SiHa – HPV 16) were mixed with pooled negative cervical specimens and tested for reproducibility. Each sample was evaluated by testing at 3 sites (two external sites, one in-house; 2 operators per site) over 6 days with 2 separate runs per day and 4 replicates of each panel member for a total of 144 measurements of each sample. The data are summarized in Table 34.

Table 34: Summary of Cervista HTA System Results from Reproducibility Study with Cell-Line Samples

Sample Type	Signal Level (cells/mL)	N[a]	HPV FOZ Ratio		HPV Positive % (n)			
			Mean	SD	Site 1	Site 2	Site 3	Total (n)
51G	High Negative (1,100)	141	1.28	0.12	0 (0)	2 (1)	2 (1)	2
	Low Positive (2,950)	141	1.80	0.15	94 (44)	100 (48)	98 (45)	137
	Moderate Positive (12,000)	141	3.43	0.34	100 (47)	100 (48)	100 (46)	141
HeLa	High Negative (590)	141	1.29	0.12	0 (0)	4 (2)	0 (0)	2
	Low Positive (1,325)	141	1.75	0.16	92 (43)	98 (47)	89 (41)	131
	Moderate Positive (5,000)	141	3.39	0.28	100 (47)	100 (48)	100 (46)	141
SiHa	High Negative (1,875)	141	1.32	0.13	2 (1)	2 (1)	4 (2)	4
	Low Positive (5,000)	141	1.83	0.16	92 (43)	100 (48)	98 (45)	136
	Moderate Positive (18,000)	141	3.46	0.27	100 (47)	100 (48)	100 (46)	141

[a] Three results per sample were excluded due to assay run failure.

Percent Agreement Between the Cervista HPV HR Manual Procedure and the Cervista HTA System

The percent agreement between the Cervista HPV HR manual procedure and the Cervista HTA system was evaluated by performing a comparison study with clinical specimens. A sample panel containing 288 residual cervical cytological ThinPrep specimens was processed at each of 3 different sites (two external sites, one in-house). The sample panel included 144 positive (60% low positive and moderate positive and 40% high positive) and 144 negative (40% high negative) samples created from pooled clinical specimens. Aliquots of each specimen were tested twice at each site; once with the manual procedure and once with the Cervista HTA system. A total of 844 paired measurements were obtained from the testing. The data are summarized in Table 35.

Table 35: Percent Agreement of Results between the Cervista HPV HR Manual Procedure and the Cervista HTA System

HTA	Number Tested[a]	Positive Agreement	95% Confidence Interval	Negative Agreement	95% Confidence Interval
Site 1[b]	281	95.4% ($\frac{125}{131}$)	[0.904, 0.979]	98.6% ($\frac{144}{146}$)	[0.951, 0.996]
Site 2[b]	286	97.8% ($\frac{131}{134}$)	[0.936, 0.992]	95.3% ($\frac{141}{148}$)	[0.906, 0.977]
Site 3[b]	277	96.3% ($\frac{129}{134}$)	[0.916, 0.984]	96.5% ($\frac{137}{142}$)	[0.92, 0.985]
Combined[c]	844	96.5%	[0.943, 0.982]	96.9%	[0.947, 0.984]

[a] Sufficient volume was not available for some samples to generate manual and Cervista HTA system results at all sites. These samples (7 from site 1, 2 from site 2, and 11 from site 3) were excluded on a per site basis.

[b] 95% confidence intervals for individual sites were calculated by the Wilson (score) method.

[c] Combined site agreement and confidence intervals calculated using a bootstrap approach.

Troubleshooting

Table 36: Manual Test Procedure for Cervista HPV HR

Troubleshooting Guide		
Observation	**Probable Cause**	**Solution**
Insufficient volume made for reaction mixes	Number of samples entered in 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: • Increase gain for scan 1 • Increase gain for scan 2 • Increase gain for both scans	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.)"	Fluorometer issues	See Troubleshooting Guide in the Invader Call Reporter Software User Manual for fluorometer issues that may contribute to this error.
"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.)"	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.

Troubleshooting Guide		
Observation	**Probable Cause**	**Solution**
No Target Control displays the following results: High %CV (HPV NTC) High %CV (gDNA NTC)	Insufficient or inconsistent mixing of reagents	• Be sure all samples, reagents and reaction mixes are mixed thoroughly. • 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. • Verify all liquid is expelled from the pipette tip during additions. • Verify the correct reagent was added to each well. • Verify the correct reagent volumes were added to each well. • Verify the calibration information on equipment is current. • Visually inspect plate for consistent volumes between wells.
	Incorrect preparation of reaction mixes	
	Inconsistent addition of the No Target Control or reaction mix to the microplate	
	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. • 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 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.

Troubleshooting Guide		
Observation	**Probable Cause**	**Solution**
Control(s) displays “Invalid Control” result.	Insufficient or inconsistent mixing of controls	• Be sure all controls and reagents are mixed thoroughly and consistently. • 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. • Make sure that all liquid is expelled from the pipette tip during additions. • Verify that the correct control was added to each well. • Verify that the correct control volume was added to each well. • Verify the calibration information on equipment. • Visually inspect plate for consistent volumes between wells.
	Inconsistent addition of reaction mix	
	Insufficient or inconsistent addition of control	
	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.

Troubleshooting Guide		
Observation	**Probable Cause**	**Solution**
Sample displays “IND: High %CV” result.	Insufficient or inconsistent mixing of samples	• Be sure all samples and reagents are mixed thoroughly. • 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. • Verify all liquid is expelled from the pipette tip during additions. • Verify the correct sample was added to each well. • Verify the correct sample volume was added to each well. • Verify the calibration information on equipment is current. • Visually inspect plate for consistent volumes between wells.
	Inconsistent addition of reaction mix	
	Inconsistent addition of sample	
	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.

Troubleshooting Guide		
Observation	**Probable Cause**	**Solution**
Sample displays “IND: Low gDNA” result.	Insufficient number of cells in specimen	• Mix the specimen well and repeat DNA extraction. • Verify the correct sample volume was added to each well. • Verify that proper procedure was followed for DNA extraction
	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	• Repeat DNA extraction from the specimen. • Verify that proper procedure was followed for DNA extraction. • Refer to the Package Insert, Performance Characteristics (Interfering Substances) section.
	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	• Repeat DNA extraction from the specimen. • Verify that proper procedure was followed for DNA extraction.
	Suspected DNA extraction reagent contamination	

Troubleshooting for Cervista HTA system

Refer to the Troubleshooting section of the Cervista HTA Operator’s Manual (Part Number MAN-01522-001) for the Cervista HTA system.

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