Cervista™ HPV HR

REF **92-011, PRD-01560**

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

Intended Use

The Cervista HPV HR test is intended for two uses:

1. In combination with cervical cytology screening for women age 30 and above to guide patient management.
2. To triage patients with atypical squamous cells of undetermined significance (ASC-US) Pap test results to determine the need for referral to colposcopy.

92-011- Σ 96

PRD-01560- Σ 384

-30°C **-15°C**

CE EC REP

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Do not store in a frost-free freezer.

Protect from light.

Table of Contents

Abbreviations Used

ASC-US:	Atypical squamous cells of undetermined significance
CIN:	Cervical intra-epithelial neoplasia
DNA:	Deoxyribonucleic acid
FAM:	Carboxyfluorescein 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
Max:	Maximum
Min:	Minimum
MTA	Medium Throughput Automation
NTC:	No Target Control
Oligo:	Oligonucleotide
Pap:	Papanicolau cervical cytology test
Red:	Redmond red dye
RFU:	Relative Fluorescence Unit

Summary and Explanation of the Test

Approximately 11,000 new U.S. cases of invasive cervical cancer and over 3,500 deaths are projected annually.[1] For the earliest stage of cervical cancer, the 5-year relative survival rate is 92%, and for all stages of cervical cancer, the 5-year survival rate is about 72%.[1] Cervical cancer is caused by persistent infection with human papillomavirus (HPV).[2] 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.

Over 100 HPV types have been documented in the literature, approximately 40 of which infect the anogenital area and are transmitted sexually. 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), referred to as high-risk (HR) types, are now recognized as the cause of almost all cervical cancers.[1,2] 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 (CIN 2 or CIN 3).[4,6,7] CIN 3, while occurring in only approximately 5% of ASC-US cases,[5] is an immediate precursor to cervical cancer and consequently its detection is very important for patient management.[2] Therefore, the identification of those women with ASC-US cytology in conjunction with a high-risk HPV infection is a useful aid for clinicians to decide who should be monitored or treated more aggressively.[2,4,8,9]

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 Pap test sample, and other factors.[6,10,11] 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 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 over age 20 with ASC-US.[3,11] 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.[6,8,11]

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 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.[12] The flap sequences and FRET oligonucleotides are universal since they are not complementary to the targeted sequence.

The reagents for this assay 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. 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 semi-quantitative 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.

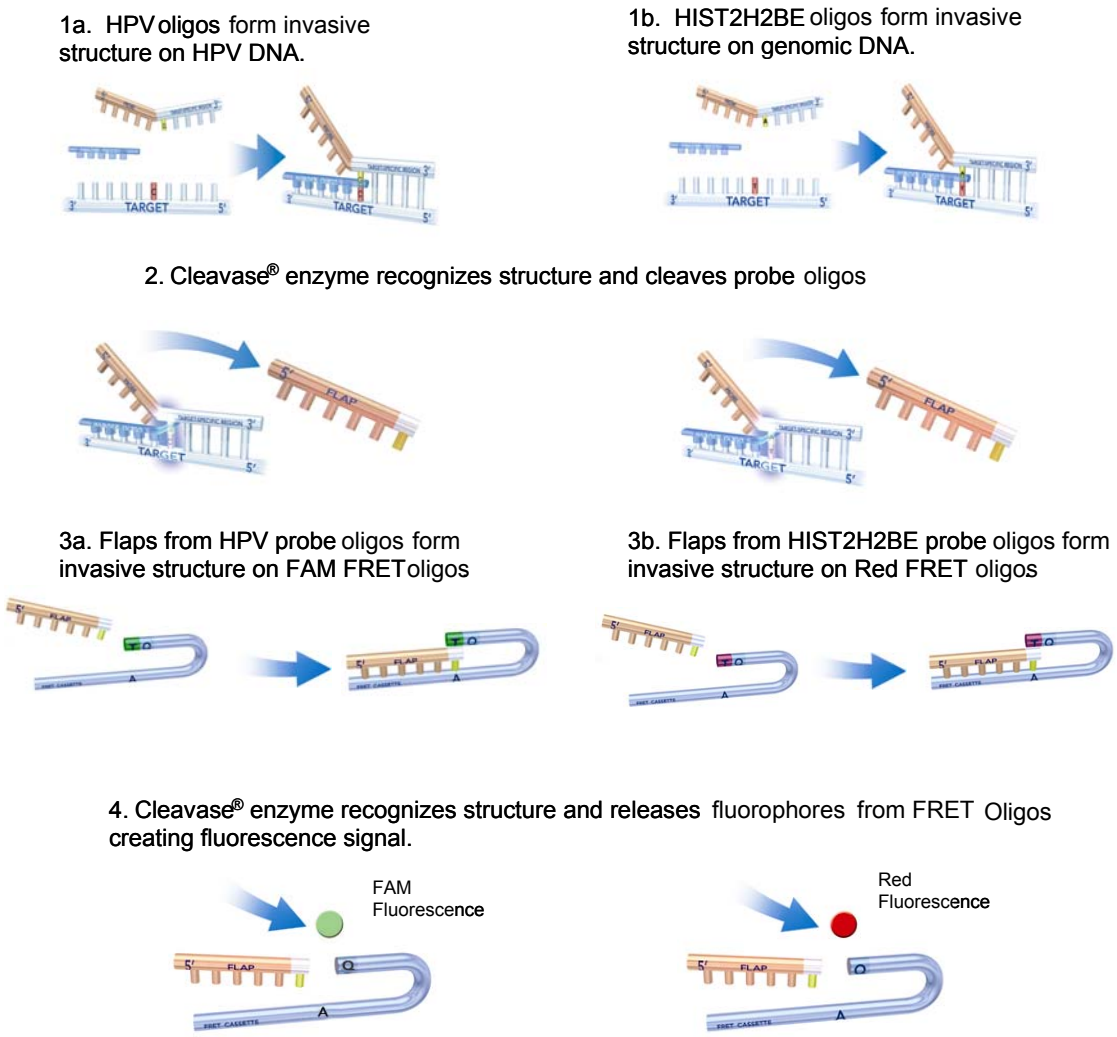

Figure 1: A graphic representation of the Invader chemistry in Cervista HPV HR

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 (REF 92-011)	Vial Quantity & Reagent Volume (REF PRD-01560)	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, and 66 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, and 68 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, and 58 suspended in water and MOPS buffer (pH 7.5)
Cleavase Enzyme Solution	E (Purple cap and purple stripe)	1 x 1100 µL	8 x 970 µL	Cleavase Enzyme suspended in 140 mM MgCl2, 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

Warnings and Precautions

1. For *in vitro* diagnostic use.
2. Universal safety precautions should be used when handling any human tissues or fluids. Specimens should be disposed according to local requirements.
3. Do not pool reagents from different lots or from different vials of the same lot.
4. Do not use reagents after their expiration date.
5. 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.

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.
- Prepare reaction mixes prior to each use. Prepared reaction mixture should be used within 30 minutes.

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. Contact your local representative if additional copies are required.

The Genfind® DNA Extraction Kit is an accessory of the Cervista HPV HR test. Contact your local representative 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

- Cervista MTA System for automation users
- Pipettes
- Vortex
- Tecan® Infinite™ F200, Tecan GENios™, or BioTek® FLx800™ fluorescence plate reader
- Desktop PC with Microsoft® Windows® XP or Windows 7 operating system and Microsoft Excel and Adobe® Reader® software.
- Thermal cycler or oven capable of maintaining appropriate reaction temperatures

Specimen Collection, DNA Extraction, and Storage for Analysis

Cervical specimens that may be tested with the Cervista HPV HR test include the following

- Specimens collected in PreservCyt™ Solution, the ThinPrep™ Pap test preservation system, using an approved collection device.
- Specimens collected in SurePath™ Preservative Fluid using an approved collection device.

Cervical specimens in PreservCyt Solution can be stored at room temperature (20 - 30°C) for up to 24 weeks prior to performing the test.

Cervical specimens in SurePath Preservative Fluid can be stored at room temperature (20 - 30°C) for up to 6 weeks prior to performing the test.

The Genfind DNA Extraction Kit (REF 95-449) has been validated for use with the Cervista HPV HR test. The recommended procedure for DNA extraction from cervical specimens in PreservCyt Solution or SurePath Preservative Fluid is included in the Genfind DNA Extraction Kit instructions for use.

Laboratories performing the Cervista HPV HR test with any extraction method other than that provided in the validated Genfind DNA Extraction Kit are responsible for their own validation of that method.

DNA samples can be stored at 2 to 8°C for up to four weeks. For storage greater than four weeks, place the samples in a freezer between -30°C and -15°C.

Test Procedure for Cervista MTA System

Refer to the Cervista MTA Operator's Manual (Part Number: MAN-02378-002) for the use of the automated system to perform the Cervista HPV HR test.

Manual Test Procedure for Cervista HPV HR

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 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 HPV HR test plate layout

2. Overlay each well with 20 µL of mineral oil and 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.

Table 2. Reaction Mix Preparation Instructions

Component	µL/Well	Number of Reactions Samples & Controls (*k*)	25% Overage	Total Volume
HPV Oligo Mix 1, 2, or 3	8 µL	*K*	1.25	=8*k*(1.25)
Cleavase Enzyme Solution	2 µL	*K*	1.25	=2*k*(1.25)
Total Mix Volume	10 µL	K	1.25	=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 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 the 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 = 530 nm) and Red (Excitation = 560 nm, Emission = 612 nm). To detect the HPV signal, the instrument should be set to detect the FAM dye first. To detect the sample genomic DNA, the instrument should be set to detect the Red dye.

5. Adjust the gain of the fluorescence plate reader to be in the linear dynamic range of the reader according to the manufacturer's instructions. The gain should be set so that the No Target Control (NTC) yields values that are in the background range of the reader, with a minimum RFU of 600. The NTC values do not have to be identical for the FAM and Red reads.

Procedural Notes and Precautions

1. Laboratories should use good laboratory practices and comply with all applicable federal, state and local regulatory requirements.
2. Mix the samples, reagents, and reaction mixes thoroughly and consistently.
3. Use nuclease-free, sterile disposable aerosol barrier pipette tips for each addition and transfer to avoid cross-contamination.
4. Use nuclease-free, disposable polypropylene tubes for preparing the reaction mixes.
5. 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.*
6. Use only calibrated equipment.
7. 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.
8. Use fresh mineral oil for each reaction setup (do not transfer these reagents back to the original container once they have been dispensed).
9. Refer to the test plate layout to ensure that the correct mix is added to the appropriate column.*
10. 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.*

    *Procedural Notes 5, 9 and 10 do not apply to the Cervista MTA 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 cutoff 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).

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.

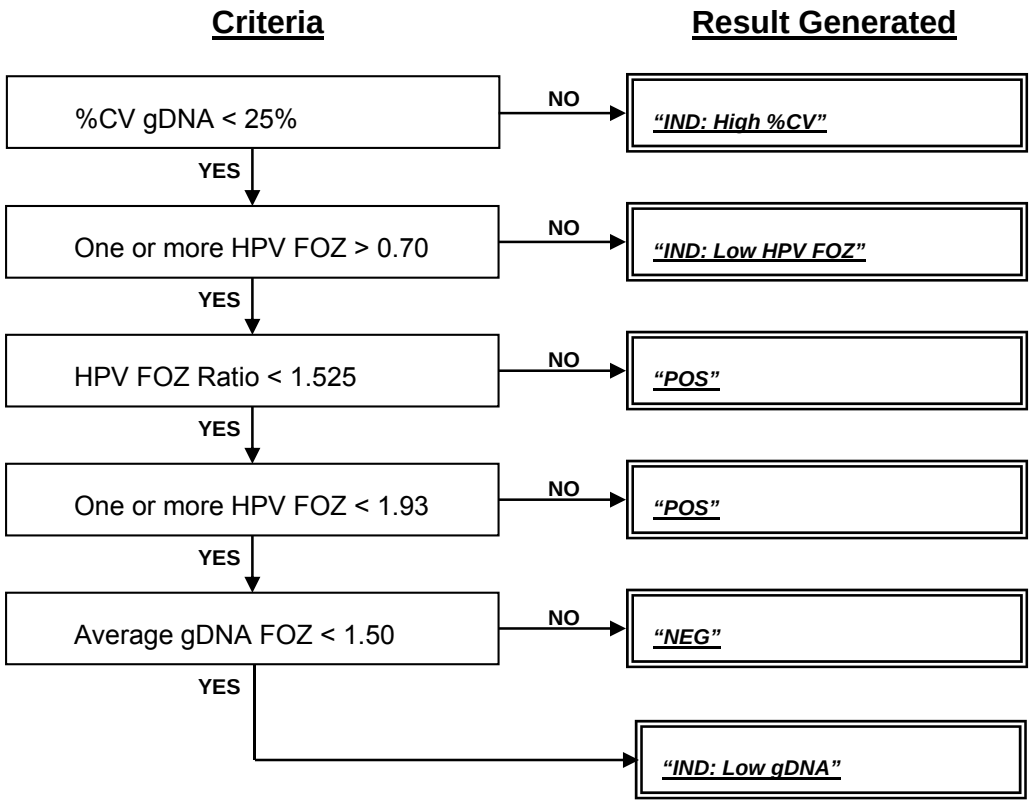

Figure 3: Sample Call Criteria Ordered Top to Bottom

Quality Control

Negative Control

1. The No Target Control must yield the appropriate results 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 3).
2. The minimum signal for each of the three mixes must be greater than or equal to 600 RFU (≥ 600).
3. The %CV of the average HPV signal from all three mixes must be less than 25.0% (<25.0%), or the samples and controls on that plate are invalid and must be repeated (See Table 3).
4. The %CV of the average gDNA signal from all three mixes must be less than 25.0% (<25.0%).

Table 3: No Target Control Criteria

Result	Min. HPV Signal	Min. gDNA Signal	Max. % CV (HPV and gDNA)
Valid	600	600	24.9%

HPV Controls

1. HPV controls (HPV Controls 1-3) must yield the appropriate results for the test to be valid. If controls do not meet these criteria, the samples on that plate are also invalid and must be repeated (see Table 4).
2. 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.
3. 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.
4. The %CV of the Mean gDNA FOZ from all three mixes should be less than 25.0% (<25.0%).

Table 4: HPV Control and Sample 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%

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 positive control(s) is invalid, all sample results on that plate are invalid and must be repeated. Refer to the Troubleshooting sections located in the instructions for use and in the User Manual for Invader Call Reporter Software. Refer to the Troubleshooting section of the Cervista MTA Operators Manual (Part Number MAN-02378-002) for the Cervista MTA system.
2. All quality control requirements should be performed in conformance with applicable national, international, and regional regulations.

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).
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 or SurePath Preservative Fluid.
5. The performance of the Cervista HPV HR test was established using DNA extracted with the Genfind DNA Extraction Kit.
6. Interference was observed in cervical specimens collected into PreservCyt Solution 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.
7. Interference was observed in cervical specimens collected into SurePath Preservative Fluid contaminated with contraceptive jelly and/or anti-fungal creams at levels of 0.5% and the personal lubricant ASTROGLIDE® at levels of 0.5% when DNA was isolated with the Genfind DNA Extraction Kit. Under these conditions, false-negative results may be obtained. The potential interference of the personal lubricant ASTROGLIDE® was not tested in cervical specimens collected into PreservCyt Solution.

Performance Characteristics

Clinical Trial Performance

A multi-center, cross-sectional and prospective clinical study was conducted to evaluate the performance of the Cervista HPV HR test for the detection of human papillomavirus and cervical intraepithelial neoplasia grade 2 or higher (CIN2+) in liquid cytology samples. Residual ThinPrep cytology specimens were collected from 3,540 women undergoing routine cervical cancer screening. This study included 2,026 women age 30 and over with normal cytology results (WNL) and 1,514 women age 18 and over with ASC-US results. Cytology samples were collected from 89 clinical sites across the United States. DNA was extracted from residual ThinPrep cervical samples that remained after routine cervical cancer screening procedures were completed. The DNA was subsequently tested using the Cervista HPV HR test.

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

A comparison of the Cervista HPV HR test with the PCR/Sequencing method among both ASC-US and WNL subjects resulted in an overall 86.1% agreement between the two methods (95% CI = 84.9 - 87.3%). The positive percent agreement between the two methods was 91.8 (89.7 – 93.6%) and the negative percent agreement was 84.2% (95% CI = 82.7 – 85.7).

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 review panel served as the "gold standard" for determining the presence or absence of disease. In the absence of histology data, the lack of colposcopically visible cervical lesions and no biopsy equated to the absence of disease.

There were 1347 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 Tables 5 and 6.

Table 5: Cervista HPV HR versus Colposcopy/Consensus Histology results (CIN2+) among women with ASC-US cytology

Cervista HPV HR	Colposcopy/ Histology		
	Positive [b]	Negative [c]	Total
Positive	64	705	769
Negative[a]	5	573	578
Total	69	1278	1347

[a] Includes indeterminate results
[b] CIN2+ Histology
[c] No CIN or CIN1 by Central Histology or Colposcopy without Central Histology

Table 6: Cervista HPV HR versus Colposcopy/Consensus Histology results (CIN3+) among women with ASC-US cytology

Cervista HPV HR	Colposcopy/ Histology		
	Positive[b]	Negative[c]	Total
Positive	22	705	727
Negative[a]	0	573	573
Total	22	1278	1300

[a] Includes indeterminate results
[b] CIN3+ including one adenocarcinoma in situ
[c] No CIN, CIN1 or CIN2 by Central Histology or Colposcopy without Central Histology

Among women with ASC-US cytology the clinical sensitivity of the test for CIN2+ was 92.8% (95% CI = 83.9% - 97.6%) and the negative predictive value was 99.1% (95% CI = 98.0 - 99.7). The clinical sensitivity and the negative predictive values of the test for CIN 3 are both 100% (95% CI = 84.6% -100% and 99.4% - 100%).

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 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).[7,4] A comparison of the study design, disease prevalence and clinical performance characteristics for the Hologic study and ALTS is shown in Table 7. The difference in CIN2+ rates observed between the two studies may reflect population differences as well as disease ascertainment differences.

Table 7: Comparison of Hologic Clinical Trial and ALTS[7,4]

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%

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. 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) as a function of a Limit of Blank (LoB) measurement and a population variance measurement (SD) from multiple concentrations of the specific HPV target (CLSI/NCCLS guideline EP17-A Vol. 24 No. 34). Nine HPV-negative characterized DNA samples isolated from cervical specimens were used to determine the LoB value (FAM FOZ Ratio = 1.20). 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 and negative samples were tested in replicates of eight.

The Limit of Detection for each HPV type is referenced in Table 8. Limits are described in terms of the FAM FOZ Ratio and as a copy number range.

Table 8: 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

Accuracy and Specificity Compared to a PCR / DNA Sequencing Method

A study designed to evaluate the ability of the Cervista HPV HR test to detect High-Risk HPV DNA from clinical specimens was performed. The specimens were characterized using a research-use HPV genotyping method that utilized degenerate PCR amplification followed by HPV type-specific sequencing. The PCR/sequencing method was used as the sole determinant for the presence of HPV DNA.

The study involved 192 specimens stored in PreservCyt Solution, of which 189 had clear sequencing results. Of these 189 samples, two samples were indeterminate by the Cervista HPV HR test. Indeterminate results were not included in the comparative analysis of the Cervista HPV HR test and PCR/sequencing methods.

The proportion of PCR/sequencing negative results that were positive by the Cervista HPV HR test was 5/187. Conversely, the proportion of PCR/sequencing positive results that were negative by the Cervista HPV HR test was 11/187 (see Table 9).

When analyzed in this manner, an overall 91.4% agreement (171/187; 95% CI = 86.5-95.0) was observed between the methods, with a positive and negative agreement of 89.8% and 93.7%, respectively (95% CI = 82.5-94.8 and 85.8-97.9).

Table 9: Detection of HPV DNA Comparing Cervista HPV HR Test to PCR with Type-Specific Sequencing

		PCR/Sequencing		
		Negative	Positive	Total
Cervista HPV HR test	Negative	74	11	85
	Positive	5	97	102
	Total	79	108	187

Reproducibility

In this investigational study, overall reproducibility of the Cervista HPV HR test was assessed at three sites using a panel of HPV positive and negative cultured cells and HPV positive and negative cervical specimens. DNA was extracted from 2 mL of cervical specimen or cultured cells suspended in PreservCyt Solution. The DNA was extracted using the Genfind DNA Extraction Kit. Sixteen samples were tested at three locations 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 for the study.

Within day/site agreement was assessed by calculating the between run percent agreement for the three possible pairings within each of the days/sites. The average percent agreement and one-sided exact 95% confidence interval is presented first for each site (intra-site reproducibility), then across all three sites (inter-site reproducibility).

Between day/within site agreement was assessed by calculating the between run % agreement for any two runs done on two separate days within a site for all possible pairings. The average percent agreement and one-sided exact 95% confidence interval is presented first for each site (intra-site, inter-run reproducibility), then across all three sites (inter-site, inter-run reproducibility).

Between site agreement was assessed by calculating the between run percent agreement for any two runs done by two different sites for all possible pairing [n=3 (sites 1 and 2, sites 1 and 3, sites 2 and 3)]. The average percent agreement and one-sided 95% confidence interval are presented in Tables 10 and 11.

Table 10: HPV HR Molecular Assay Between-Day (Within-Site) Percent Agreement

Site	Number of Comparisons	Number of Agreements	Percent Agreement	1-sided 95% Confidence Lower Limit
Site 1	200	200	100.0%	96.3%
Site 2	200	193	96.5%	90.8%
Site 3	200	200	100.0%	96.3%
Across All 3 Sites	600	593	98.8%	96.9%

Table 11: HPV HR Molecular Assay Between-Site Percent Agreement

Sites	Number of Comparisons	Number of Agreements	Percent Agreement	1-sided 95% Confidence Lower Limit
Site 1 vs. Site 2	500	490	98.0%	96.6%
Site 1 vs. Site 3	500	500	100.0%	99.4%
Site 2 vs. Site 3	500	490	98.0%	96.6%
All Site Pairs	1500	1480	98.7%	97.9%

Interfering Substances

Four cervical specimens (one HPV negative, three HPV positive) and three cell line samples (one HPV negative, two HPV positive) were tested with added substances that potentially could be present in the cervical specimen. The substances that were added to the specimens included PreservCyt Solution, two types of vaginal douches, contraceptive jelly, two types of anti-fungal creams, and negative clinical specimens that visually contained blood and mucous. The PreservCyt Solution, douche, contraceptive jelly and anti-fungal creams were added at two levels, 0.5% and 2%. These levels were chosen in order to represent extreme situations 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.

Contraceptive jelly and the anti-fungal creams containing either clotrimazole or miconazole at a 2% sample concentration resulted in indeterminate and false negative results. During DNA extraction, the contraceptive jelly interfered with the magnetic bead separation in the 10 mM Tris buffer, causing low DNA recovery and insufficient DNA sample for testing. This interference was visually detectable.

The levels of the above substances that are required to cause testing failure are unusually high and should not be encountered in actual clinical specimens if the clinician follows the proper Pap sampling procedure of clearing the cervix before obtaining the cell sample for Pap analysis.

The Cervista HPV HR test was also tested with components that could potentially be transferred inadvertently during sample extraction using the Genfind DNA Extraction Kit. DNA containing three levels (0, 5% and 10%) each of 70% ethanol or Genfind magnetic beads was tested to assess interference caused by the introduced substances. Interference was observed when 10% of the DNA sample volume contained either 70% ethanol or the magnetic beads.

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 12-14).

Table 12

The organisms listed below were added to PreservCyt Solution at concentrations of approximately $1x10^5$ 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 13

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 (HIV-1, pol and env regions)	*Neisseria meningitidis*
	Mycoplasma hominis

Table 14

Purified cloned DNA or PCR amplicon samples for the following HPV types were tested at concentrations of $1x10^5$ copies/reaction and $1x10^7$ copies/reaction, unless noted, using the Cervista HPV HR test. All samples yielded 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[a]
Human papillomavirus type 42	Human papillomavirus type 70[a]
Human papillomavirus type 43	Human Internal Control gene

[a]Human papillomavirus types 67 and 70 yielded positive results with the Cervista HPV HR test at 1x105 and 1x107 copies/reaction. Upon further titration of these samples, negative results were obtained with the Cervista HPV HR test at 1x103 copies/reaction and 1x104 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.

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.

Each run consisted of 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.

At 2500 copies/reaction, the plasmid DNA samples yielded 57.4% (675/1176) positive results. At 5000 copies/reaction the plasmid DNA samples yielded 97.2% (1143/1176) positive results. At 10000 copies/reaction, the plasmid DNA samples yielded 100.0% (1176/1176) positive results (see Table 15).

Table 15: Summary of positive and negative values for each sample condition tested

	Target		N	HPV Positive n (%)	HPV Negative n (%)
Copies/Reaction	HPV 16	2,500	84	82 (98%)	2 (2%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 18	2,500	84	64 (76%)	20 (24%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 31	2,500	84	58 (69%)	26 (31%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 33	2,500	84	13 (15%)	71 (84%)
		5,000	84	81 (96%)	3 (4%)
		10,000	84	84 (100%)	0 (0%)
	HPV 35	2,500	84	1 (1%)	83 (99%)
		5,000	84	60 (71%)	24 (29%)
		10,000	84	84 (100%)	0 (0%)
	HPV 39	2,500	84	52 (62%)	32 (38%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 45	2,500	84	84 (100%)	0 (0%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 51	2,500	84	77 (92%)	7 (8%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 52	2,500	84	21 (25%)	63 (75%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 56	2,500	84	64 (76%)	20 (24%)
		5,000	84	83 (99%)	1 (1%)
		10,000	84	84 (100%)	0 (0%)
	HPV 58	2,500	84	60 (71%)	24 (29%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 59	2,500	84	16 (19%)	68 (81%)
		5,000	84	79 (94%)	5 (6%)
		10,000	84	84 (100%)	0 (0%)
	HPV 66	2,500	84	40 (48%)	44 (52%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)
	HPV 68	2,500	84	43 (51%)	41 (49%)
		5,000	84	84 (100%)	0 (0%)
		10,000	84	84 (100%)	0 (0%)

	Target		N	HPV Positive n (%)	HPV Negative n (%)
Cells/mL Extracted	SiHa/Jurkat	2500 SiHa / 97,500 Jurkat	84	0 (0%)	84 (100%)
		5000 SiHa / 95,000 Jurkat	84	15 (18%)	69 (82%)
		20,000 SiHa / 80,000 Jurkat	84	84 (100%)	0 (0%)
	HeLa/Jurkat	1,250 HeLa / 98,750 Jurkat	84	65 (77%)	19 (23%)
		2,500 HeLa / 97,500 Jurkat	84	84 (100%)	0 (0%)
		10,000 HeLa / 90,000 Jurkat	84	84 (100%)	0 (0%)
	Jurkat	10,000	84	2 (2%)	82 (98%)
		20,000	84	0 (0%)	84 (100%)
		100,000	84	0 (0%)	84 (100%)

Performance of the Cervista HPV HR Test

Performance of the Cervista HPV HR test on specimens collected into SurePath Preservative Fluid compared to specimens collected into PreservCyt Solution:

A total of 418 subjects were enrolled in a co-collection study to obtain paired cervical specimens collected into SurePath Preservative Fluid and PreservCyt Solution from each subject. Each pair of specimens was tested with the Cervista HPV HR test. A total percent agreement of 92% was observed for the results obtained for specimens collected into the SurePath Preservative Fluid compared to the results obtained for specimens collected into the PreservCyt Solution.

Table 16: Summary of Cervista HPV HR results from cervical specimens co-collected into SurePathPreservative Fluid and PreservCyt Solution

	SurePath Specimen Results	PreservCyt Specimen Results
Total	418	418
% Positive	29.4%	29.2%
% Negative	69.9%	70.6%
% Indeterminate	0.7%	0.2%

Troubleshooting Manual Test Procedure for Cervista HPV HR

Problem	Potential Cause	Possible Solution
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: • 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.)" "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.

Problem	Potential Cause	Possible 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 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.

Problem	Potential Cause	Possible 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.

Problem	Potential Cause	Possible 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.
Sample displays “IND: Low gDNA” result.	Insufficient number of cells in specimen.	• Mix the specimen 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 assay.	
	DNA sample inhibition	Repeat DNA extraction from the specimen.
		Refer to Instructions for Use, Performance Characteristics (Interfering Substances) section.
	The DNA sample(s) may not have been completely denatured.	Verify that the sample was denatured at the correct temperature and for an appropriate amount of time.
Sample displays “IND: Low HPV FOZ” result.	Suspected error during DNA extraction	• Repeat DNA extraction from the specimen. • Verify that proper procedure was followed for DNA extraction. • Refer to Instructions for Use, Performance Characteristics (Interfering Substances) section.
	DNA sample inhibition	

Problem	Potential Cause	Possible Solution
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 MTA System

Refer to the Troubleshooting section of the Cervista MTA Operator's Manual (Part Number: MAN-02378-002) for Cervista MTA systems.

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LIMIT OF LIABILITY. HOLOGIC SHALL NOT BE LIABLE FOR ANY SPECIAL, INCIDENTAL, PUNITIVE, EXEMPLARY OR CONSEQUENTIAL LOSSES, DAMAGES, OR EXPENSES (INCLUDING BUT NOT LIMITED TO LOSS OF PROFITS, DATA, OR USE), DIRECTLY OR INDIRECTLY ARISING FROM THE SALE, HANDLING, SERVICE OR USE OF PRODUCT ORDERED OR FURNISHED, OR FROM ANY CAUSE RELATING THERETO UNLESS EXPRESSLY AGREED TO BY THE PARTIES IN WRITING. EXCEPT FOR PERSONAL INJURY OR DEATH TO THE EXTENT RESULTING FROM HOLOGIC’S NEGLIGENT OR INTENTIONALLY WRONGFUL ACTS OR OMISSIONS, IN NO EVENT SHALL HOLOGIC BE LIABLE UNDER ANY LEGAL THEORY OR FOR ANY CAUSE WHATSOEVER, WHETHER BASED UPON WARRANTY, CONTRACT, TORT, NEGLIGENCE, OR OTHER THEORY, EVEN IF ADVISED OF THE POSSIBILITY THEREOF, FOR ANY AMOUNT IN EXCESS OF THE PRICE, FEE OR CHARGE THEREFORE RECEIVED BY HOLOGIC.

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