

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: High-risk Human Papillomavirus Virus DNA Detection Kit.

Device Trade Name: Cervista™ HPV HR
Genfind DNA Extraction Kit

Applicant's Name and Address: Third Wave Technologies, Inc.
502 South Rosa Road
Madison, WI 53719

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P080014

Date of FDA Notice of Approval: March 12, 2009

Expedited: Not Applicable

II. INDICATIONS FOR USE

Cervista™ HPV HR Indications For 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

Genfind™ DNA Extraction Kit Indication For Use:

The Genfind™ DNA Extraction Kit is intended for use in the extraction of DNA from cervical specimens collected in ThinPrep® Pap Test™ PreservCyt® Solution for testing by the Cervista™ HPV HR and Cervista™ HPV 16/18 tests.

III. **CONTRAINDICATIONS**

There are no known contraindications for use.

IV. **WARNINGS AND PRECAUTIONS**

The warnings and precautions can be found in the Cervista™ HPV HR labeling.

V. **DEVICE DESCRIPTION**

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 fluorescence signal as the cleaved flaps cycle on and off. 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. 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 confirm that adequate sample DNA is present 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.

Interpretation of Results

A signal to noise value (sample signal measured against signal from a No Target Control reaction well) is generated for each of the three reactions. This signal to noise value is referred to as FOZ (Fold-Over-Zero). A final positive or negative or indeterminate result for any particular sample is generated based on the analysis of three separate reaction wells.

The ratio between HPV FOZ values generated by the three reaction mixtures determines whether a sample is positive. The HPV FOZ ratio is calculated by dividing the highest HPV FOZ value from any one of the three reaction mixtures by the lowest HPV FOZ value of the three. When any FOZ value is less than 1, it is rounded up to 1 for the ratio calculation. If the HPV FOZ Ratio is greater than or equal to 1.525, then the sample is positive for HPV. However, in a subset of mixed infections, all three reaction wells may generate a signal much higher than background. In some cases, these mixed infections may generate positive signals of similar intensity in all three reaction wells and therefore a HPV FOZ Ratio of less than 1.525. In order to avoid the chance of a false negative due to the triple positive scenario described above, a second calculation is applied as follows: when the FOZ ratio is less than 1.525, but all three individual reaction FOZ values are greater than a second 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 $\geq 25.0\%$ (High % CV), 2) when all three HPV FOZ values are < 0.7 (Low HPV FOZ) and 3) when average gDNA FOZ of a negative sample is < 1.5 (low gDNA). An indeterminate call is indicative of insufficient mixing, a pipetting error or inadequate gDNA in the sample).

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.

Table 1: 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*
POS	HPV types 16, 18, 31, 33, 35, 39, 45,	Low but increased likelihood that underlying high-grade CIN will be	Low likelihood of underlying high-grade CIN; HPV infection may be

	51, 52, 56, 58, 59, 66, or 68 detected	detected at colposcopy. Medical literature suggests that progression to high-grade disease is possible. ^{1,2,3}	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**	Indeterminate	High risk HPV status unknown	
IND: Low gDNA**			

* According to the 2006 consensus guidelines², women 30 years and older with greater than ASC-US cytology (including ASC-H, LSIL or above) should proceed to colposcopy regardless of their HPV test results.

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

The patient's age, medical history and thorough physical examination, including cytology, will provide further information on a patient's risk of cervical disease, as well as the need for referral to colposcopy. The Cervista™ HPV HR test should only be used in conjunction with this clinical information in accordance with appropriate patient management procedures.

One alternative for the detection of high-risk HPV DNA is currently approved in the United States. At the time of this approval there are no alternative FDA approved devices that detect other HPV targets (such as HPV RNA or protein). Each DNA detection method has its own advantages and disadvantages.

A patient should fully discuss these alternatives with his/her physician to select the screening method(s) that best meets expectations and lifestyle.

VII. MARKETING HISTORY

Cervista™ HPV HR is CE marked for use in the EU and approved for use in Canada by Health Canada. It has not been withdrawn from these markets for any reason.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As with any *in vitro* diagnostic test, the potential risks are associated with incorrect test results or result interpretations. Failure of this device to perform as expected or failure to correctly interpret results may lead to incorrect HPV test results and subsequently, improper patient management decisions in cervical cancer screening and treatment. False negative results may lead to delays in the timely diagnosis of cervical cancer and treatment, allowing an undetected condition to worsen and potentially increasing morbidity and mortality. False positive results could lead many women to unnecessarily undergo more frequent screening and potentially invasive procedures such as colposcopy and biopsy.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

1. 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 cutoff 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 eight 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.⁴

The Limit of Detection for each HPV type is referenced in Table 2. 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 2: Cervista™ HPV HR Test Analytical Sensitivity Summary

HPV DNA Type	LoD (Copy Number/Reaction)	LoD (FAM FOZ Ratio)	SD _s
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 cutoff 95% of the time were approximately 2,500 cells/ml for SiHa cells and 1,000 cells/ml for HeLa cells.

2. Clinical Cutoff of the Cervista™ HPV HR Test

The clinical cut-off was assessed based on the approach described in a reference paper⁵ for unbiased estimates of sensitivity and specificity. Briefly, the cutoff values were evaluated based on pre-specified clinical sensitivity level for the detection of \geq CIN2 histology. The clinical cutoff was selected as an unbiased estimate of the 93th percentile using the 69 ASC-US subjects with \geq CIN2 histology results. Based on this criterion, an HPV FOZ ratio cutoff of \geq 1.525 or a minimum HPV FOZ value of \geq 1.93 for all three reaction mixes were selected as the cutoff values for the test. The estimate of sensitivity was 92.8% (64/69); and the 95% CI for sensitivity taking into account an increased uncertainty was 82.6% to 97.1%. The estimate of specificity was 44.2% (558/1263); and the 95% CI for specificity taking into account an increased uncertainty was 39.6% to 53.8%. The 95% CI were calculated using bootstrap.

3. Precision

Repeatability and within-laboratory precision of the Cervista™ HPV HR test was demonstrated in a 21-day study with three alternating operators, each performing two runs per day on individually-assigned sets of equipment. Each run consisted of four plates. Different plate layouts were used for the runs within a day. The samples tested within each run included genomic DNA samples isolated from two HPV positive cell lines (SiHa - Type 16 and HeLa - type 18), an HPV negative cell line (Jurkat) and contrived samples containing HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 or HPV68 plasmid DNA and Jurkat DNA. Each sample was tested in duplicate at three concentrations.

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

Table 3: 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	%C V	SD	%C V	SD	%C V	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%

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	%C V	SD	%C V	SD	%C V	SD	%CV
	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%	0.299	5%	0.107	3%	0.059	2%	0.333	5%
Hela/ Jurkat	2500 HeLa / 97,500 Jurkat ^b	84	2.495	0.121	5%	0.018	2%	0.098	4%	0.061	2%	0.026	3%
	10,000 HeLa / 90,000 Jurkat ^b	84	6.130	0.183	3%	0.027	3%	0.214	3%	0.088	1%	0.038	4%
Jurkat	10,000 ^b	84	1.030	0.161	16%	0.059	6%	0.089	9%	0.029	3%	0.067	6%
	20,000 ^b	84	1.003	0.026	3%	0.112	8%	0.013	1%	0.005	0%	0.116	8%
	100,000 ^b	84	1.005	0.038	4%	0.156	5%	0.019	2%	0.008	1%	0.185	6%

^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 4: 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 4).

4. 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™ 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 5. Individual sample results across sites along with a cumulative mean and standard deviation for the HPV FOZ ratio are presented in Table 6.

Table 5. 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 6: Summary of Cervista™ 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

5. 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 7 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 8). 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 7: 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 8: 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

^aThe levels of interferent required to cause testing failures (2%) are unusually high and should not be encountered in actual clinical specimens.

^bThe 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.

6. 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 9-11).

Table 9: The organisms listed below were added to PreservCyt® Solution at concentrations of approximately 1 x10⁵ cfu/mL and 1x10⁷ cfu/mL. DNA from these organisms and a negative cell line (Jurkat, 1x10⁵ cells/mL) was extracted using the Genfind™ DNA Extraction Kit. All samples yielded negative results with the Cervista™ HPV HR test.

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

Table 10: Purified DNA obtained from the organisms listed below was tested at concentrations of 1x10⁵ copies/reaction and 1x10⁷ copies/reaction using the Cervista™ HPV HR test. All samples yielded negative results.

Herpes simplex virus, type 1 (HSV-1)	<i>Chlamydia trachomatis</i>
Herpes simplex virus, type 2 (HSV-2)	<i>Neisseria gonorrhoeae</i>

Human Immunodeficiency Virus type 1 (HIV-1, pol and env regions)	<i>Neisseria meningitides</i>
	<i>Mycoplasma hominis</i>

Table 11: Cloned DNA or PCR amplicons for the following samples were tested at concentrations of 1×10^5 copies/reaction and 1×10^7 copies/reaction or as noted. The Cervista™ HPV HR test did not exhibit any cross-reactivity to common low risk HPV types 6, 11, 42, 43, 44 and 53. Samples HPV type 1a and the internal control both generated negative results.

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

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

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

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

7. Sample Handling and Collection

Specimen stability studies demonstrated that 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.

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.

8. Reagent Stability Testing

Results of real-time stability studies indicate that the Cervista HPV HR test is stable for 12 months when stored at its labeled storage conditions (-30°C to -15°C).

Freeze-Thaw Stability Testing

The freeze/thaw stability of the Cervista HPV HR test was evaluated by subjecting the test components (HPV kit Controls, HPV Oligo Mixes, and Enzyme) to one, three, or six freeze-thaw cycles. Performance was evaluated by testing a set of samples that included both purified plasmid DNA samples, as well as DNA samples isolated from cultured cell lines and cervical specimens stored in PreservCyt solution. The data demonstrated that that Cervista HPV HR test components may be subjected to up to 6 freeze-thaw cycles.

9. ThinPrep Carryover Study

A study was conducted to evaluate the effects of sample carryover contamination from the ThinPrep 2000 Processor on the Cervista™ HPV tests. In this study, 200 vials of human HPV-negative cells (Jurkat) in PreservCyt medium and 200 vials of Jurkat cells spiked with a high load of the CaSki HPV positive cell line (100,000 cells/ml) also in PreservCyt medium were processed in an alternating pattern on a TP2000 instrument. After processing on the TP2000, DNA was prepared from the 200 HPV negative and 200 HPV positive samples using the Genfind™ DNA Extraction kit. In addition, DNA was prepared from 200 HPV negative samples (Jurkat) which have never come into contact with a TP-2000 instrument to establish whether there is a baseline false positive rate of the Cervista™ test. HPV testing was conducted using the Cervista™ HPV HR test.

All 200 negative samples that were not processed on the TP-2000 generated HPV negative results with the Cervista™ HPV HR test. The percent of negative samples above the clinical cut-off (HPV FOZ Ratio ≥ 1.525) was 0% (0/200) with 95% CI: 0 to 1.9%. The percent of samples where HPV was detectable (HPV FOZ Ratio ≥ 1.4) was 0% (0/200) with 95% CI: 0 to 1.9%. The 200 negative samples processed on the TP-2000 along with alternating positive samples generated HPV negative results with the Cervista™ HPV HR test. The percent of negative samples above the clinical cut-off (HPV FOZ Ratio ≥ 1.525) was 0% (0/200) with 95% CI: 0 to 1.9%. The percent of samples where HPV was detectable (HPV FOZ Ratio ≥ 1.4) was 0% (0/200) with 95% CI: 0 to 1.9%. All 200 HPV positive samples processed on the TP-2000 generated valid HPV positive results in the Cervista HPV HR test.

The difference in the false positive rates was 0% with 95% CI: -2.0% to 2.0%) for both the TP-2000 processed and un-processed HPV negative samples indicating there was no sample carryover contamination from the ThinPrep 2000 processor observed with the Cervista™ HPV HR Test.

B. Animal Studies

Not applicable

C. Additional Studies

Not applicable

X. SUMMARY OF CLINICAL STUDIES

A. Study Design

Subjects were enrolled between July 2006 and December 2007. The database for this PMA reflected data collected through July 2006 until March 2008. The study included 1,514 ASC-US subjects and 2,026 NILM subjects. There were 46 investigational sites and 43 satellite sites for a total of 89 enrolling locations.

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

A multi-center prospective clinical study was conducted to evaluate the performance of the Cervista™ HPV HR test for screening patients with ASC-US cytology results to determine the need for referral to colposcopy. All clinical performance characteristics were established using ThinPrep liquid cytology specimens. Initial Thin Prep cervical specimens were classified according to The 2001 Bethesda System Classification. All women (18 years or older) with cytology results of ASC-US during routine cervical cancer screening procedures were invited to participate in the study prior to learning their HPV status. For women who consented, their initial residual ASC-US ThinPrep specimens were subsequently obtained for Cervista™ HPV HR testing. All patients who consented to the study underwent colposcopic examination. Investigators and patients remained blinded to the patient's HPV status until after completion of the colposcopic procedures, to avoid bias. Colposcopically directed histological specimens were examined by pathologists who were also blinded to the patient's HPV status. 1,514 women age 18 and over¹ 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 evaluated 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 clinical reference standard ("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.

STUDY DESIGN TO DEMONSTRATE SCREENING PERFORMANCE OF THE CERVISTA HPV HR TEST AS AN ADJUNCT TO CERVICAL CYTOLOGY IN WOMEN 30 YEARS AND OLDER

A longitudinal 3 year post-approval study has been initiated to support the use of the Cervista™ HPV HR test as an adjunct to cervical cytology, compared with cervical cytology alone. The study design is described below, along with some preliminary data obtained from the study population at enrollment. For women who consented, their initial residual NILM ThinPrep specimens were subsequently obtained for Cervista™ HPV HR testing and for HPV testing by the FDA-approved HPV test. This study was used for evaluation of agreement of the Cervista™ HPV HR test with a composite HPV comparator between the ASC-US and NILM ≥ 30 populations. Approval for this indication is being given prior to completion of the longitudinal studies in light of the results of the comparator study. Additionally, consistent data obtained from multiple cross-sectional and prospective cohort studies conducted with a variety of cell sampling methods and utilizing a variety of HPV DNA testing methods (both FDA approved, and research grade) provide strong evidence that a negative HPV DNA test implies very low risk of prevalent or incipient CIN 2-3 or cancer when cervical cytology results are normal.^{6,7,8,9}

Description of NILM ≥ 30 clinical study

Approximately 2,000 qualified subjects with normal cervical cytology results (NILM) have been enrolled from 26 active clinical centers throughout the United States. At baseline T₀, initial residual NILM ThinPrep specimens were obtained for Cervista™ HPV HR testing and for HPV testing by the FDA-approved HPV test. It is anticipated that not less than 1,000 subjects will have 3-year

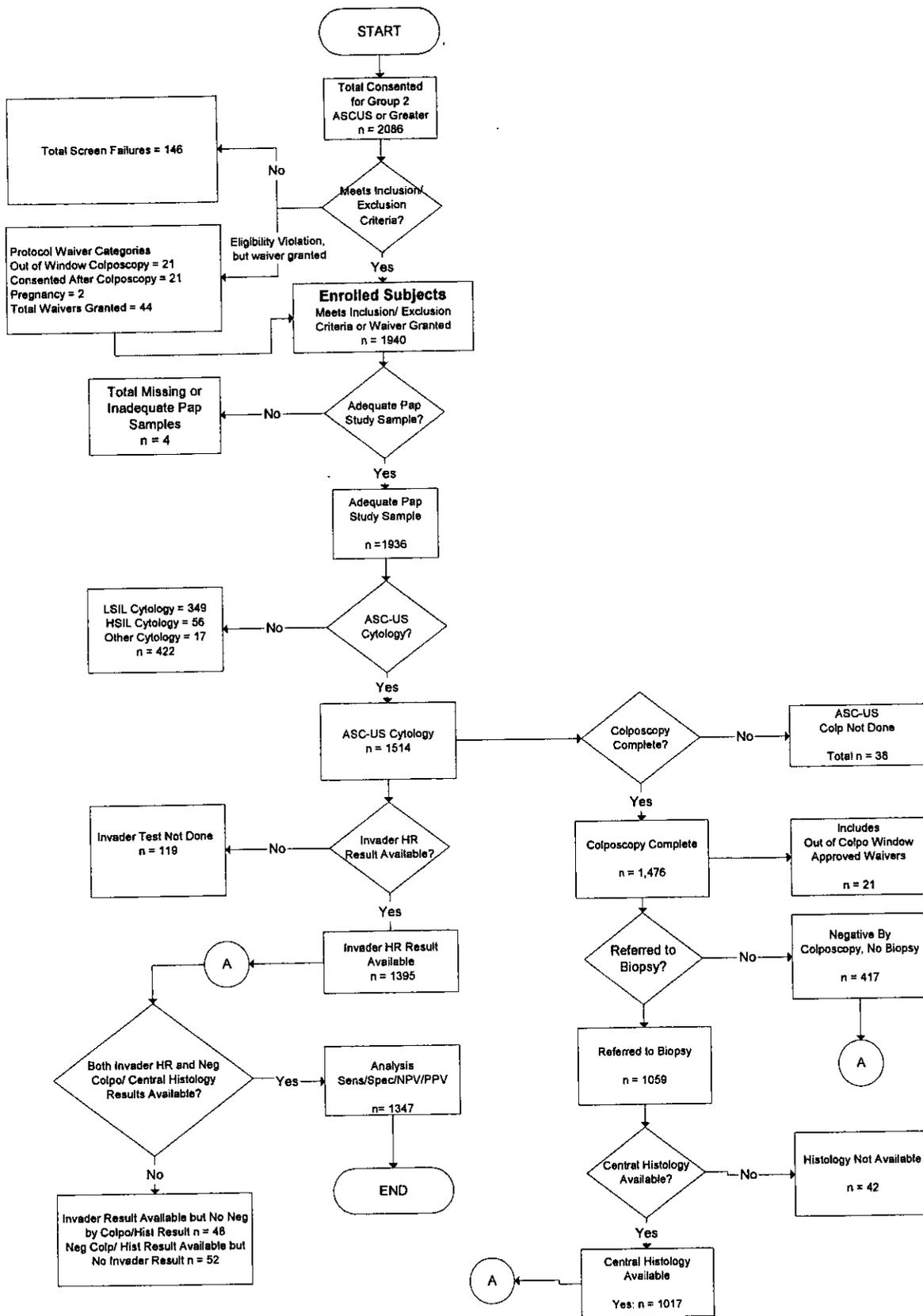
¹ This study was conducted prior to implementation of guidelines² that recommend limiting ASC-US HPV testing to woman 21 years or older.

follow-up data. The subject retention rate at the end of the first year of follow-up has been nearly 80%. Subjects will be followed for 3 years and have annual study visits. At each follow-up visit, a cervical cytology test is performed. Women who have ASC-US or higher grade cytology results will have a colposcopy performed, and subsequently a biopsy if needed. Analysis of these data will focus on the three-year risk of cervical disease associated with NILM subjects positive for Cervista™ HPV HR as compared to those negative for the test at the time of enrollment (T₀). The positive and negative Cervista™ HPV HR results at T₀, will be compared against the presence or absence of (a) \geq CIN2 and (b) \geq CIN3 throughout the study. The presence of CIN2, CIN3 or cervical cancer will be ascertained by central histology. Negative results will be defined by colposcopy unless central histology results are available to supersede an initial positive colposcopic indication. All histological interpretation will be conducted by a central pathology review panel.

B. Accountability of PMA Clinica Study Subjects

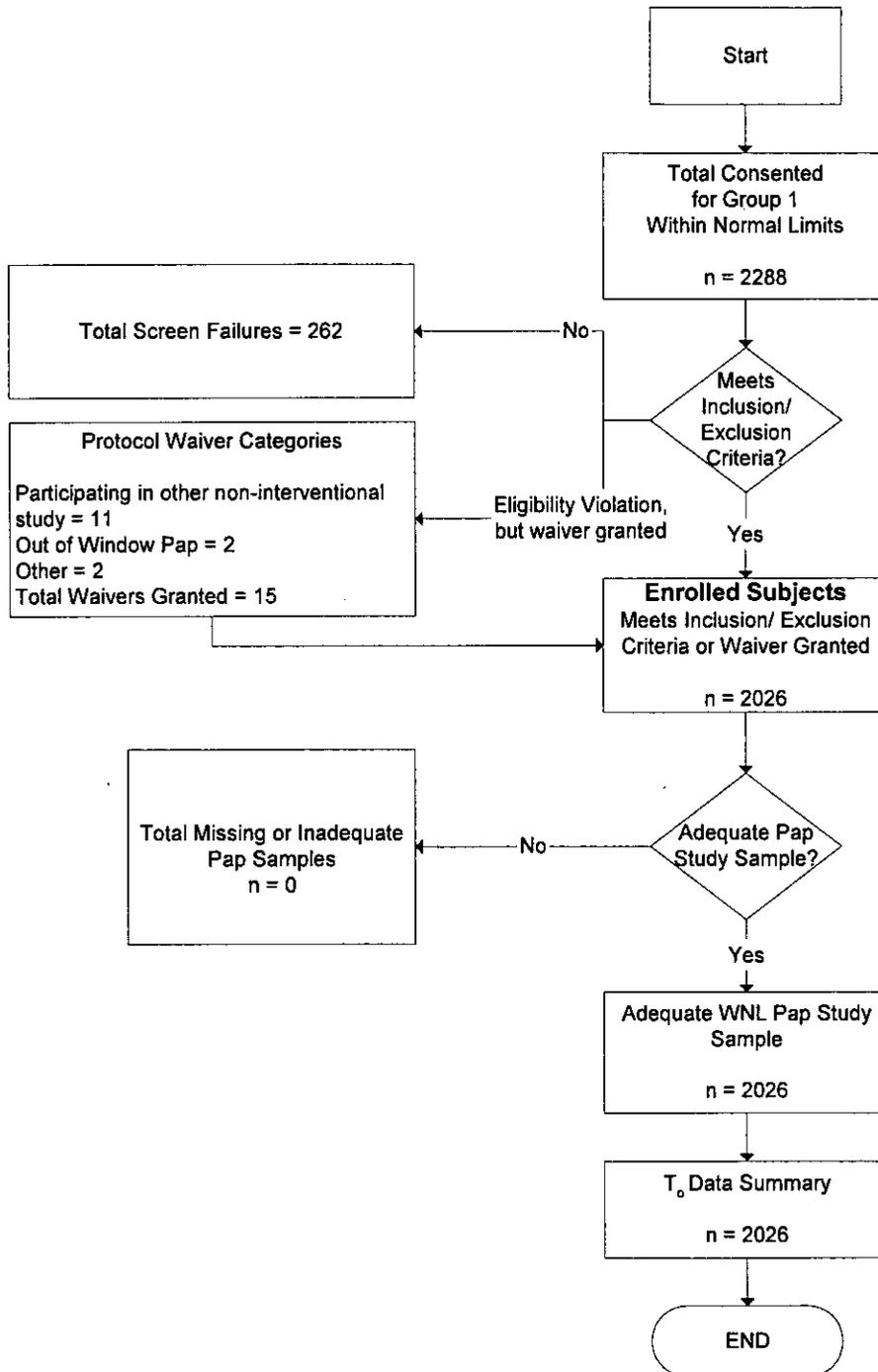
ASC-US Subjects

Between July 2006 and December 2007, a total of 2,08 subjects with ASC-US or greater cytology results consented to participate in the study. Out of the total number consented, 1,940 women were enrolled after it was determined that they had met the study's inclusion/exclusion criteria. A total of 1,936 (99.8%) subjects had adequate Pap test samples; 1,514 of these had ASC-US cytology results and the remaining 422 had LSIL, HSIL or other cytology results. Cervista™ HPV HR results were obtained from 1395 (92.1%) ASC-US subjects (119 were not reported due to insufficient volume for testing or due to a deviation to the protocol). Colposcopy was completed for 1,476 (97.5%) of the ASC-US subjects. A total of 1,347 subjects with known disease status (i.e. central histology or a negative colposcopy and/or no biopsy performed) and Cervista™ HPV HR were available for the data analysis of Cervista™ HPV HR sensitivity, specificity, negative predictive value, and positive predictive value..



WNL Subjects

Between July 2006 and October 2007, a total of 2,288 subjects with normal (NILM) cytology results were consented to participate in the study and 2,026 of these subjects were enrolled after meeting the inclusion/exclusion criteria for the study. All of these subjects had adequate Pap test samples. Cervista™ HPV HR results were available for 1,966 (97.0%) of the subjects at their baseline T₀



C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for a prospective study performed in the US.

Table 12: Study Demographics

ASC-US Subject Demographics	
Age (years) at consent	
n	1514
Mean	33.7
SD	11.76
Median	31.0
Min	18
Max	79
Race	
n	1514
Asian	33 (2.2)
Black or African	282 (18.6)
Native American or Alaskan	6 (0.4)
Native Hawaiian or Pacific Islander	4 (0.3)
White	1172 (77.4)
Other	17 (1.1)
Ethnicity	
n	1514
Hispanic or Latino	132 (8.7)
Not Hispanic or Latino	1382 (91.3)
NILM Subject Demographics	
Age (years) at consent	
n	2026
Mean	45.6
SD	10.10
Median	45.0
Min	30
Max	85
Race	
n	2026

Asian	40 (2.0)
Black or African	447 (22.1)
Native American or Alaskan	2 (0.1)
Native Hawaiian or Pacific Islander	0
White	1482 (73.1)
Other	55 (2.7)
Ethnicity	
n	2026
Hispanic or Latino	108 (5.3)
Not Hispanic or Latino	1918 (94.7)

D. Safety and Effectiveness Results

1. Safety Results

Not applicable, this was an IDE-exempt study.

2. Effectiveness Results

Clinical Sensitivity and Specificity for Screening Patients with ASC-US Cervical Cytology Results to Determine the Need for Referral to Colposcopy

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 13. The clinical performance of the Cervista™ HPV HR test for women with ASC-US cytology is summarized in Tables 14, 15 and 16.

Table 13: 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	CIN 1	CIN 2	≥CIN 3	
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 14: 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% to 96.9%)
Specificity	44.2% (558/1263)	95% CI: (41.5% to 46.9%)
PPV	8.3% (64/769)	95% CI: (7.6% to 8.9%)

NPV	99.1% (558/563)	95% CI: (98.1% to 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 15: 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% to 100%)
Specificity	43% (563/1310)	95% CI: (40.3% to 45.7%)
PPV	2.9% (22/769)	95% CI: (2.4% to 3.0%)
NPV	100% (563/563)	95% CI: (99.4% to 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 16: 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
	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%
Disease Prevalence:	6.8% (9/133)		
<hr/>			
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
	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%
Disease Prevalence:	6.4% (31/488)		

Age: 30 to <39			
Cervista™ HPV HR	Central Histology ≥ CIN2		
	Negative	Positive	Total
Positive	157	10	167
Negative	126	3	129
Total	283	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%
Disease Prevalence:	4.4% (13/296)		
Age: 39 or older			
Cervista™ HPV HR	Central Histology ≥ CIN2		
	Negative	Positive	Total
Positive	131	14	145
Negative	268	2	270
Total	399	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%
Disease Prevalence:	3.9% (16/415)		

Additional Subgroup Analyses

Tables 17 and 18 present clinical performance of the Cervista™ HPV HR Test for the collection devices tested in the clinical study.

Table 17: Clinical Performance of the Cervista™ HPV HR Test Stratified by Collection Device as Compared to Colposcopy/Central Histology Results (≥CIN2) among Women with ASC-US Cytology

Collection Device=Rovers Cervex Brush						
Cervista HPV HR	Central Histology					Total
	Neg Colposcopy No Biopsy	No CIN	CIN 1	CIN 2	CIN 3	
HPV HR Positive	100	154	55	14	11	334
HPV HR Negative	118	138	12	2	0	270
HPV HR Indeterminate	2	2	0	0	0	4

Total	220	294	67	16	11	608
Collection Device=Wallach Papette						
Cervista HPV HR	Central Histology					Total
	Neg Colposcopy No Biopsy	No CIN	CIN 1	CIN 2	CIN 3	
HPV HR Positive	40	129	56	18	5	248
HPV HR Negative	60	118	14	2	0	194
HPV HR Indeterminate	0	3	0	0	0	3
Total	100	250	70	20	5	445
Collection Device=Endocervical Brush/Spatula						
Cervista HPV HR	Central Histology					Total
	Neg Colposcopy No Biopsy	No CIN	CIN 1	CIN 2	CIN 3	
HPV HR Positive	24	106	41	10	6	187
HPV HR Negative	36	58	4	1	0	99
HPV HR Indeterminate	2	6	0	0	0	8
Total	62	170	45	11	6	294

Table 18: Clinical Performance of the Cervista™ HPV HR Test Stratified by Collection Device as Compared to Colposcopy/Central Histology Results (\geq CIN2)

Collection Device	Sensitivity	Specificity	Prevalence of \geq CIN2	Percent of Subjects with Positive Results by Cervista HPV HR	Percent of Subjects with Indeterminate Results by Cervista HPV HR
Rovers Cervex Brush	92.3% (25/27)	46.1% (268/581)	4.4% (27/608)	54.9% (334/608)	0.7% (4/608)
Wallach Papette	92.0% (23/25)	45.7% (192/420)	5.6% (25/445)	55.7% (248/445)	0.7% (3/445)
Endocervical Brush/Spatula	94.1% (16/17)	35.4% (98/277)	5.8% (17/294)	63.6% (187/294)	2.7% (8/294)

Table 19: Clinical Performance of the Cervista™ HPV HR Test by Molecular Testing Center

Molecular Testing Site	Sensitivity	Specificity	Prevalence of \geq CIN2	Percent of Subjects with Positive Results by Cervista HPV HR	Percent of Subjects with Indeterminate Results by Cervista HPV HR

1	100% (14/14)	43.3% (129/298)	4.5% (14/312)	57.1% (178/312)	1.6% (5/312)
2	100% (1/1)	59.0% (46/78)	1.3% (1/79)	36.7% (29/79)	5.1% (4/79)
3	94.9% (37/39)	41.0% (275/670)	5.5% (39/709)	60.5% (429/709)	0.4% (3/709)
4	80.0% (12/15)	46.6% (108/232)	6.1% (15/247)	53.8% (133/247)	1.2% (3/247)
Total	92.8% (64/69)	43.7% (558/1278)	5.1% (69/1347)	57.1% (769/1347)	1.1% (15/1347)

There are a number of key variables that are known to influence the performance characteristics of any HPV test in a clinical study. These include, but are not limited to, cervical sampling techniques, the quality of the cytology results, age of the population tested, disease prevalence, disease ascertainment methods and methods for histological interpretation. Given the number of variables present during routine HPV testing across multiple clinical sites, it is noteworthy that many of the results obtained from the TWT clinical trial are similar to those seen under the controlled trial conditions described in the ASC-US/LSIL Triage Study (ALTS).³ A comparison of the study design, disease prevalence and clinical performance characteristics for the TWT study and ALTS is shown in Table 20. The difference in CIN2+ rates observed between the two studies may reflect population differences as well as disease ascertainment differences.

Table 20: Comparison of TWT Clinical Trial and ALTS³

Criterion	ALTS	TWT
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%

Comparison of a Composite Comparator and Cervista HPV HR for 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 21 and 22 below.

Table 21: 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 22: NILM \geq 30 Population Cervista™ HPV HR vs. Composite Comparator (PCR Sequencing and FDA-Approved HPV test)

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

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

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

Expected Results

High-Risk HPV Prevalence

The reported prevalence of HPV infection in women ranges widely, from 14% to more than 90%.¹⁰ 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 23 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 23: Prevalence of High-Risk HPV Across Clinical Trial Testing Centers

Center	ASC-US Population		NILM Population	
	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 24 shows the prevalence of high-risk HPV among subjects with ASC-US or NILM cytology stratified by age.

Table 24: 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)
\geq 39	34.6% (145/419)	\geq 60	18.4% (34/185)
All	57.1% (769/1347)	All	18.5% (363/1966)

Table 25 shows the prevalence of high-risk HPV regardless of cytology as reported in various studies of women in different U.S. populations.

Table 25: High-Risk HPV Prevalence in Various U.S. Populations

Location	Publication Date	Total Study Size	Age Range	HPV Prevalence (%)
New Mexico ¹¹	2001	3,863	18 - 40	26.7%
U.S. Civilian Population ¹²	2007	1,921	14 - 59	15.2%
Oregon ⁸	2003	20,156	16 - 94	16.3%
Arizona ¹³	2001	988	15 - 79	13.9%

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to a panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

Based on the results of the preclinical and clinical studies, the safety of the Cervista HPV HR Test, when used according to the provided directions and in conjunction with cytology results and other clinical information, should be safe and pose minimal risk to the patient due to false test results.

B. Effectiveness Conclusions

The effectiveness of the Cervista HPV HR test has been demonstrated for use in conjunction with cervical cytology in women 30 years and older to adjunctively screen to assess the presence or absence of high-risk human papillomavirus (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. Additionally, a reasonable determination of effectiveness of the Cervista™ HPV HR test for use in screening patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy has been demonstrated.

C. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the nonclinical studies demonstrated acceptable analytical sensitivity, precision, and analytical specificity of the Cervista HPV HR test when used according to the instructions for use, the warnings and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application has shown that the assay is safe and effective for its approved indications when used according to the directions for use in the labeling.

XIV. CDRH DECISION

CDRH issued an approval order on March 12, 2009. The final conditions of approval cited in the approval order are described below.

The applicant's manufacturing facility was inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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⁵ Kondratovich M, Yousef WA. Evaluation of accuracy and 'optimal' cutoff of diagnostic devices in the same study. Joint Statistical Meeting. 2005. ASA Section on Statistics in Epidemiology.

⁶ Kjaer S, Hogdall E, Frederiksen K, et al. 2006. The absolute risk of cervical abnormalities in highrisk human papillomavirus-positive, cytologically normal women over a 10-year period. *Cancer Res.* 66:10630-10636.

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