SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Human Papillomavirus Virus Type 16 and Type 18 DNA Detection Kit.

Device Trade Name: Cervista™ HPV 16/18

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: P080015

Date of FDA Notice of Approval: March 12, 2009

Expedited: Not Applicable

II. INDICATIONS FOR USE

Cervista™ HPV 16/18 Indications For Use:

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

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

The Cervista™ HPV 16/18 test is indicated:

1) In women 30 years and older the Cervista™ HPV 16/18 test can be used adjunctively with the Cervista™ HPV HR test in combination with cervical cytology to screen to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

2) To be used adjunctively with the Cervista™ HPV HR test in patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results, to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology

history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy.

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

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

III. CONTRAINDICATIONS

There are no known contraindications for use.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Cervista™ HPV 16/18 labeling.

V. DEVICE DESCRIPTION

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

The probes are present in large molar excess and cycle rapidly on and off the target sequence so that many cleaved 5' flaps are generated per target sequence. The cleaved flaps then bind to a universal hairpin FRET oligonucleotide creating another invasive structure that the Cleavase® enzyme recognizes as a substrate. The enzyme cleaves the FRET oligonucleotides between the fluorophore and quencher molecule and produces fluorescence signal as the cleaved flaps cycle on and off. The flap sequences and FRET oligonucleotides are universal since they are not complementary to the targeted sequence.

The reagents for this test are provided as two oligonucleotide mixtures, which detect HPV16 and HPV18. Oligonucleotides that bind to the human histone 2 gene (H2be, HIST2H2BE) are also present in these two oligonucleotide mixtures. HIST2H2BE serves as an internal control producing a signal from cellular DNA present in the sample. The format of the Cervista™ HPV 16/18 test allows simultaneous detection of HPV DNA sequences and HIST2H2BE in a single well by utilizing two different 5'-flap sequences on the probes as

well as two different FRET oligonucleotides, each with a spectrally distinct fluorophore (FAM and Red). By design, the released 5'-flaps bind only to their respective FRET oligonucleotides to generate target-specific signal.

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

Interpretation of Results

A signal to noise value (sample signal measured against signal from a No Target Control reaction well) is referred to as FOZ (Fold-Over-Zero). FOZ values are generated for both the HPV 16 and HPV 18 reactions. A final positive, negative or indeterminate result for any particular sample is generated based on the analysis of two separate reaction wells. When the HPV16 FOZ value and/or HPV18 FOZ value is greater than 2.13, the sample is positive for HPV 16 and/or HPV 18.

An indeterminate call is generated in three different scenarios 1) when the % difference between the gDNA FOZ values is ≥25.0% (High % difference), 2) when both HPV FOZ values are < 0.7 (Low HPV FOZ) and 3) when the 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.

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

%Difference gDNA FOZ: The absolute value of the difference between the HPV16 and HPV18 genomic DNA FOZ values divided by the average genomic DNA FOZ value of the two HPV Oligo Mixes.

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

Table 1: Interpretation of Cervista™ HPV 16/18 Test Results when High-risk (HR) HPV Results are Positive[b].

Cervista™ HPV16/18 Test	Result Report	Interpretation for patients with NILM cytology who are ≥30	Interpretation for patients with

Result[c]		years old[a]	ASC-US cytology
POS:HPV 16	HPV type 16 detected	Low but increased likelihood that underlying high-grade CIN will be detected at colposcopy. Medical literature suggests that progression to high-grade disease is possible.[1,2,3,4]	Increased likelihood that underlying high-grade CIN will be detected at colposcopy.
POS:HPV 18	HPV type 18 detected		
POS: HPV16 & HPV18	HPV types 16 and 18 detected		
NEG[d]	HPV types 16 and/or 18 not detected	Low likelihood of underlying CIN2-3 or cancer; results are not intended to prevent women from further cytology or HPV retesting.[1,2,3,4]	Likelihood of underlying CIN2-3 or cancer is lower, but infection with other non-16/18 high-risk HPV types still confers risk. Results are not intended to prevent women from proceeding to colposcopy.
IND: High % CV	Indeterminate	HPV 16/18 status unknown	
IND: Low gDNA			

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

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

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

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

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 16/18 test should only be used in patients who also have a Cervista HPV HR results, and the two Cervista tests should be interpreted in conjunction with the patient's other clinical information (as mentioned above) in accordance with appropriate patient management procedures.

The Cervista™ HPV 16/18 test is a first-of-a-kind assay for the detection of HPV type 16 and/or 18 DNA. At the time of this approval there are no alternative FDA approved devices that detect other HPV 16 and/or 18 targets (such as HPV RNA or protein).

VII. MARKETING HISTORY

Cervista™ HPV 16/18 is not marketed in any country.

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

Nine HPV-negative characterized DNA samples isolated from cervical specimens were tested in replicates of eight (9 samples x 8 replicates/sample = 72 data points) to determine the Limit of Blank (LoB). The LoB values (FAM FOZ) were 1.18 and 1.21 from HPV 16 and HPV 18 respectively.

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

The LoB and LoD were evaluated according to the CLSI document EP17-A.[5]

The Limit of Detection for each HPV type is referenced in Table 2. Limits are described in terms of the FAM FOZ and as a copy number range. Copy number per reaction LoD values were reported as the copy number range in which 95% of the observed FAM FOZ values were above the LoB.

Table 2: Cervista™ HPV 16/18 Test Analytical Sensitivity Summary

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

In addition to the analytical sensitivity study described above, cell line dilutions were prepared to evaluate the performance of the HPV 16/18 assay using two HPV positive cell lines (HeLa and SiHa) diluted with a HPV negative cell line (Jurkat) to a final concentration of 100,000 cells/ml in PreservCyt media. DNA was isolated from the cell line samples using the Genfind™ DNA Extraction Kit. Using a clinical HPV 16 and

HPV18 FOZ cut-off of 2.13, concentrations of approximately 2,500 cells/ml for both SiHa and HeLa cells were above the clinical cutoff 95% of the time.

2. Clinical Cutoff of the Cervista™ HPV 16/18 test

The clinical cut-off was evaluated based on HPV16/18 test results targeting a 5% positive rate in the NILM ≥30 population from a multi-center clinical study. The 95th percentile of the maximum HPV16 and HPV18 FOZ values was determined for NILM ≥30 subjects and based on this analysis, a FOZ value of ≥2.13 was selected as the positive cutoff value for the Cervista™ HPV 16/18 test. For more details, see reference[6] for unbiased estimates of the performance when the study was also used for determination of the cutoff.
The estimate of the positive percent agreement (PPA) of the Cervista™ HPV 16/18 test and PCR/Sequencing was 85.7% (18/21) with 95% CI: 65.4% to 95.0%; and the 95% CI for the PPA taking into account an increased uncertainty was 66.7% to 100% (the increase in uncertainty was 1.1 times; 33.3%/29.7%). The estimate of the negative percent agreement (NPA) of the Cervista™ HPV 16/18 test and PCR/Sequencing was 95.9% (1784/1860) with 95% CI: 94.9% to 96.7%; and the 95% CI for the NPA taking into account an increased uncertainty was 94.6% to 97.2% (the increase in uncertainty was 1.4 times; 2.6%/1.8%).

3. Within-Laboratory Precision

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

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

The repeatability and within-laboratory precision values were calculated for each target at each concentration. The precision values for HPV16 FOZ are shown in Table 3 and the HPV18 FOZ values are shown in Table 4. A summary of positive HPV16 and positive HPV18 results are shown in Tables 5 and 6 respectively.

Table 3: HPV 16 Precision Values for Each Target and Concentration

Target	Copies/Reaction[a] or Cells/mL[b]	N	Mean HPV 16 FOZ	Within-Run (repeatability)		Between-Run		Between- Day		Between-Operator		Total (Within-lab precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV 16	5,000[a]	84	3.708	0.196	5%	0.238	6%	0.348	9%	0.364	10%	0.411	11%
	20,000[a]	84	7.397	0.697	9%	0.460	6%	0.390	5%	0.331	4%	0.708	10%
HPV 18	5,000[a]	84	1.021	0.028	3%	0.042	4%	0.031	3%	0.027	3%	0.047	5%
	20,000[a]	84	1.024	0.041	4%	0.069	7%	0.045	4%	0.048	5%	0.073	7%

SiHa/Jurkat	5000 SiHa / 95,000 Jurkat[b]	84	2.430	0.160	7%	0.115	5%	0.138	6%	0.135	6%	0.196	8%
	20,000 SiHa / 80,000 Jurkat[b]	84	5.465	0.220	4%	0.360	7%	0.384	7%	0.324	6%	0.486	9%
Hela/Jurkat	2500 HeLa / 97,500 Jurkat[b]	84	0.784	0.029	4%	0.047	6%	0.049	6%	0.048	6%	0.063	8%
	10,000 HeLa / 90,000 Jurkat[b]	84	0.893	0.037	4%	0.037	4%	0.039	4%	0.036	4%	0.053	6%
Jurkat	10,000[b]	84	0.886	0.111	12%	0.074	8%	0.064	7%	0.029	3%	0.114	13%
	20,000[b]	84	0.870	0.029	3%	0.035	4%	0.030	3%	0.023	3%	0.044	5%
	100,000[b]	84	0.917	0.066	7%	0.042	5%	0.042	5%	0.039	4%	0.070	8%

[a] HPV16 or HPV18 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: HPV 18 Precision Values for Each Target and Concentration

Target	Copies/Reaction or Cells/mL[b]	N	Mean HPV 18 FOZ	Within-Run (repeatability)		Between-Run		Between- Day		Between-Operator		Total (Within-lab precision)	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HPV 16	5,000[a]	84	0.978	0.041	4%	0.055	6%	0.059	6%	0.050	5%	0.076	8%
	20,000[a]	84	0.990	0.055	6%	0.068	7%	0.062	6%	0.043	4%	0.087	9%
HPV 18	5,000[a]	84	3.620	0.243	7%	0.255	7%	0.265	7%	0.230	6%	0.363	10%
	20,000[a]	84	8.483	0.396	5%	0.613	7%	0.595	7%	0.378	4%	0.787	9%
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat[b]	84	0.874	0.051	6%	0.035	4%	0.045	5%	0.043	5%	0.062	7%
	20,000 SiHa / 80,000 Jurkat[b]	84	0.858	0.023	3%	0.052	6%	0.043	5%	0.044	5%	0.059	7%
Hela/Jurkat	2500 HeLa / 97,500 Jurkat[b]	84	2.988	0.163	5%	0.174	6%	0.175	6%	0.064	2%	0.243	8%
	10,000 HeLa / 90,000 Jurkat[b]	84	7.918	0.427	5%	1.466	19%	1.757	22%	0.463	6%	2.062	26%
Jurkat	10,000[b]	84	0.927	0.055	6%	0.054	6%	0.055	6%	0.043	5%	0.077	8%
	20,000[b]	84	0.920	0.035	4%	0.038	4%	0.035	4%	0.027	3%	0.051	6%
	100,000[b]	84	0.951	0.054	6%	0.042	4%	0.036	4%	0.031	3%	0.060	6%

[a] HPV16 or HPV18 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 5. Summary of Positive HPV16 Results for Precision Study.

Target	Copies/Reaction[a] or Cells/mL[b]	N	Mean HPV 16 FOZ	HPV 16 Positive % (n)			
				Operator 1	Operator 2	Operator 3	Total
HPV 16	5,000[a]	84	3.708	100%	100%	100%	100%

				(28)	(28)	(28)	(84)
	20,000[a]	84	7.397	100% (28)	100% (28)	100% (28)	100% (84)
HPV 18	5,000[a]	84	1.021	0% (0)	0% (0)	0% (0)	0% (0)
	20,000[a]	84	1.024	0% (0)	0% (0)	0% (0)	0% (0)
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat[b]	84	2.430	82% (23)	100% (28)	100% (28)	94% (79)
	20,000 SiHa / 80,000 Jurkat[b]	84	5.465	100% (28)	100% (28)	100% (28)	100% (84)
Hela/Jurkat	2500 HeLa / 97,500 Jurkat[b]	84	0.784	0% (0)	0% (0)	0% (0)	0% (0)
	10,000 HeLa / 90,000 Jurkat[b]	84	0.893	0% (0)	0% (0)	0% (0)	0% (0)
Jurkat	10,000[b]	84	0.886	0% (0)	0% (0)	0% (0)	0% (0)
	20,000[b]	84	0.870	0% (0)	0% (0)	0% (0)	0% (0)
	100,000[b]	84	0.917	0% (0)	0% (0)	0% (0)	0% (0)

[a] HPV16 or HPV18 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 6. Summary of Positive HPV18 Results for Precision Study.

Target	Copies/Reaction[a] or Cells/mL[b]	N	Mean HPV 18 FOZ	HPV 18 Positive % (n)			
				Operator 1	Operator 2	Operator 3	Total
HPV 16	5,000[a]	84	0.978	0% (0)	0% (0)	0% (0)	0% (0)
	20,000[a]	84	0.990	0% (0)	0% (0)	0% (0)	0% (0)
HPV 18	5,000[a]	84	3.620	100% (28)	100% (28)	100% (28)	100% (84)
	20,000[a]	84	8.483	100% (28)	100% (28)	100% (28)	100% (84)
SiHa/Jurkat	5000 SiHa / 95,000 Jurkat[b]	84	0.874	0% (0)	0% (0)	0% (0)	0% (0)
	20,000 SiHa / 80,000 Jurkat[b]	84	0.858	0% (0)	0% (0)	0% (0)	0% (0)
Hela/Jurkat	2500 HeLa / 97,500 Jurkat[b]	84	2.988	100% (28)	100% (28)	100% (28)	100% (84)
	10,000 HeLa / 90,000 Jurkat[b]	84	7.918	100% (28)	100% (28)	86% (24)	95% (80)
Jurkat	10,000[b]	84	0.927	0% (0)	0% (0)	0% (0)	0% (0)
	20,000[b]	84	0.920	0% (0)	0% (0)	0% (0)	0% (0)
	100,000[b]	84	0.951	0% (0)	0% (0)	0% (0)	0% (0)

[a] HPV16 or HPV18 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).

4. Reproducibility

Reproducibility of the Cervista™ HPV 16/18 test was assessed at three external sites using a panel of HPV positive and negative cultured cells and HPV positive and negative cervical specimens. DNA was extracted from 2 mL of cervical specimen or cultured cells suspended in PreservCyt® Solution. The DNA was extracted using the Genfind™ DNA Extraction Kit. Sixteen samples were extracted for DNA and tested with Cervista™ HPV 16/18 at three locations on five non-consecutive days within a two-week time period. Two lots of Cervista™ HPV 16/18 kits and three lots of Genfind™ DNA Extraction Kits were used across the 3 sites for the study. The total number of measurements for each sample was 15 = (3 sites x 5 days x 1 run per day). A summary of the percent agreement between the expected and observed results combined for all sites is shown in Table 7. A summary of individual sample results across sites with a cumulative mean and standard deviation for the HPV16 and HPV18 FOZ values are presented in Table 8 and Table 9.

Table 7. Data Summary for a Multi-Center Reproducibility Study of the Cervista™ HPV 16/18 Test.

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

Table 8. Summary of Cervista™ HPV16 Results from a Multi-Center Reproducibility Study

Sample	Sample Type and Concentration (cells/ml)	N	HPV 16 FOZ		HPV 16 Positive % (n)			
			Mean	SD	Site 1	Site 2	Site 3	To (r
1 Neg	100,000 Jurkat	15	0.899	0.048	0 (0)	0 (0)	0 (0)	0
2 Pos:HPV18	10,000 HeLa 90,000 Jurkat	15	0.883	0.076	0 (0)	0 (0)	0 (0)	0
3 Pos:HPV18	5,000 HeLa 95,000 Jurkat	15	0.847	0.083	0 (0)	0 (0)	0 (0)	0
4 Pos:HPV18	2,500 HeLa 97,500 Jurkat	15	0.833	0.073	0 (0)	0 (0)	0 (0)	0
5 Pos:HPV16	20,000 SiHa	15	6.345	0.553	100 (5)	100 (5)	100 (5)	1

	80,000 Jurkat							
6 Pos:HPV16	10,000 SiHa 90,000 Jurkat	15	4.933	0.598	100 (5)	100 (5)	100 (5)	1
7 Pos:HPV16	5,000 SiHa 95,000 Jurkat	15	3.049	0.473	100 (5)	100 (5)	100 (5)	1
8 Pos:HPV18 and HPV16	5,000 SiHa 2,500 HeLa 12,500 Jurkat	15	3.047	0.387	100 (5)	100 (5)	100 (5)	1
9 Neg	Cervical Pool	15	0.905	0.078	0 (0)	0 (0)	0 (0)	0
10 Neg	Cervical Pool	15	0.888	0.097	0 (0)	0 (0)	0 (0)	0
11 Pos:HPV18	Cervical Pool	15	0.958	0.154	0 (0)	0 (0)	0 (0)	0
12 Neg	Cervical Pool	15	0.865	0.127	0 (0)	0 (0)	0 (0)	0
13 HPV16	Cervical Pool	15	9.769	0.658	100 (5)	100 (5)	100 (5)	1
14 Neg	Cervical Pool	15	0.919	0.093	0 (0)	0 (0)	0 (0)	0
15 HPV16	Cervical Pool	15	2.782	0.611	100 (5)	100 (5)	100 (5)	1
16 Neg	Cervical Pool	15	1.049	0.130	0 (0)	0 (0)	0 (0)	0

Table 9. Summary of Cervista™ HPV18 Results from a Multi-Center Reproducibility Study

Sample	Sample Type and Concentration (cells/ml)	N	HPV 18 FOZ		HPV18 Positive % (n)			
			Mean	SD	Site 1	Site 2	Site 3	To
1 Neg	100,000 Jurkat	15	0.927	0.042	0 (0)	0 (0)	0 (0)	0
2 Pos:HPV18	10,000 HeLa 90,000 Jurkat	15	9.322	0.831	100 (5)	100 (5)	100 (5)	1
3 Pos:HPV18	5,000 HeLa 95,000 Jurkat	15	6.121	1.105	100 (5)	100 (5)	100 (5)	1
4 Pos:HPV18	2,500 HeLa 97,500 Jurkat	15	3.645	0.455	100 (5)	100 (5)	100 (5)	1
5 Pos:HPV16	20,000 SiHa 80,000 Jurkat	15	0.831	0.043	0 (0)	0 (0)	0 (0)	0
6 Pos:HPV16	10,000 SiHa 90,000 Jurkat	15	0.963	0.043	0 (0)	0 (0)	0 (0)	0
7 Pos:HPV16	5,000 SiHa 95,000 Jurkat	15	0.927	0.031	0 (0)	0 (0)	0 (0)	0
8 Pos:HPV18 and HPV16	5,000 SiHa 2,500 HeLa 12,500 Jurkat	15	3.815	0.435	100 (5)	100 (5)	100 (5)	1
9 Neg	Cervical Pool	15	0.896	0.049	0 (0)	0 (0)	0 (0)	0
10 Neg	Cervical Pool	15	0.892	0.053	0 (0)	0 (0)	0 (0)	0
11 Pos:HPV18	Cervical Pool	15	10.413	1.945	100 (5)	100 (5)	100 (5)	1
12 Neg	Cervical Pool	15	1.146	0.121	0 (0)	0 (0)	0 (0)	0

13 HPV16	Cervical Pool	15	0.861	0.053	0 (0)	0 (0)	0 (0)	0
14 Neg	Cervical Pool	15	0.927	0.029	0 (0)	0 (0)	0 (0)	0
15 HPV16	Cervical Pool	15	0.921	0.035	0 (0)	0 (0)	0 (0)	0
16 Neg	Cervical Pool	15	0.921	0.050	0 (0)	0 (0)	0 (0)	0

5. Interfering Substances

Three cell-line samples (one HPV negative, one HPV16 positive, one HPV18 positive) described in Table 10 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 11). Concentration levels were chosen to represent extreme conditions that could potentially occur during specimen collection if the cervix was not cleared prior to obtaining the specimen. DNA was isolated from pure and impure samples using the Genfind™ DNA Extraction Kit and was tested with the Cervista™ HPV 16/18 test to assess interference caused by the introduced substances.

Table 10: Interfering Substances Sample Descriptions

Sample	Description
Jurkat	Cell line sample stored in PreservCyt solution containing 100,000 cells/mL Jurkat (HPV Negative) cells
SiHa/Jurkat	Cell line sample stored in PreservCyt solution containing 7,500 cells/mL SiHa cells (HPV 16 Positive) and 92,500 cells/mL Jurkat cells
HeLa/Jurkat	Cell line sample stored in PreservCyt solution containing 2,500 cells/mL HeLa cells (HPV 18 Positive) and 97,500 cells/mL Jurkat cells

Table 11: Interference 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%	No
	Magnetic Beads	5%, 10%	No

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

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

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

6. Cross-Reactivity

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

Table 12: The organisms listed below were added to PreservCyt® Solution at concentrations of approximately 1 x10^5 cfu/mL and 1x10^7 cfu/mL. DNA from these organisms and a negative cell line (Jurkat, 1x10^5 cells/mL) was extracted using the Genfind™ DNA Extraction Kit. All samples yielded negative results with the Cervista™ HPV 16/18 test.

Candida albicans	*Proteus vulgaris*
Corynebacterium pseudodiptheriticum	Staphylococcus aureus
Enterococcus faecalis	*Staphylococcus epideridis*
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 16/18 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 meningitides*
	Mycoplasma hominis

Table 14: Cloned DNA or PCR amplicons for the following samples were tested at concentrations of 1x10^5 copies/reaction and 1x10^7 copies/reaction unless noted, using the Cervista™ HPV 16/18 test. All samples yielded negative results.

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

[a] Human papillomavirus type 31 yielded positive HPV16 results with the Cervista™ HPV 16/18 test at 1X10^7 copies/reaction. Upon further titration of the HPV 31 sample, negative results were obtained with the Cervista™ HPV 16/18 test at ≤ 1x10^6 copies/reaction.

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 16/18 test does not cross-react with DNA isolated from PreservCyt® samples containing up to containing up to $1.0x10^7$ cfu/ml of *Neisseria meningitides and Mycoplasma hominis,* $5x10^6$ cfu/ml of *Neisseria gonorrhoeae* and $1.0x10^6$ cfu/ml *Chlamydia trachomatis.*

7. Sample Handling and Collection

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

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 16/18 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 16/18 test was evaluated by subjecting the test components (HPV kit Controls, HPV Oligo Mixes, and Enzyme) to one (standard condition), five or ten freeze-thaw cycles. Performance was evaluated by testing a set of samples that included 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 10 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 TP-2000, 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™ HPV16/18 test.

All 200 negative samples that were not processed on the TP-2000 generated HPV negative results with the Cervista™ HPV 16/18 test. The percent of negative samples above the clinical cut-off (HPV FOZ values ≥2.13) was 0% (0/200) with 95% CI: 0, 1.9%). The 200 negative samples processed on the TP-2000 along with alternating positive samples generated HPV negative results with the Cervista™ HPV 16/18 test. The percent of these negative samples above the clinical cut-off (HPV FOZ values≥2.13) was 0% (0/200) with 95% CI: 0, 1.9%). The percent of samples where HPV16 and or HPV18 was detectable (HPV FOZ ≥ 1.18 for HPV16 or HPV FOZ ≥ 1.21 for HPV18) in the negative sample sets was 0% (0/200) with 95% CI: 0, 1.9%). All 200 HPV16 positive samples processed on the TP-2000 generated positive HPV16 results and negative HPV18 results with the Cervista HPV 16/18 test.
The difference in the false positive rates was 0% with 95% CI: -2.0% to 2.0% for the HPV negative samples processed on the TP-2000 in the presence and in the absence of the positive samples indicating there was no sample carryover contamination from ThinPrep 2000 processer observed with the Cervista™ HPV 16/18 test.

B. Animal Studies

Not applicable

C. Additional Studies

Not applicable

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

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 no intraepithelial lesion or malignancy (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 OF CERVISTA HPV 16/18 AMONG WOMEN WITH ASC-US CERVICAL CYTOLOGY RESULTS
A multi-center prospective clinical study was conducted to evaluate the performance of the Cervista™ HPV 16/18 test among patients with ASC-US cytology results to determine the need for referral to colposcopy. All clinical performance characteristics were established using ThinPrep liquid cytology specimens. Initial 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 16/18 testing. All patients who consented to the study underwent colposcopic examination. Investigators and patients remained blinded to the patient's HPV status until after completion of the colposcopic procedures, to avoid bias. Colposcopically directed histological specimens were examined by pathologists who were also blinded to the patient's HPV status. 1,514 women age 18 and over with ASC-US results were ultimately enrolled in the study from 89 clinical sites across the United States.

The clinical performance of the Cervista™ HPV 16/18 test was measured against colposcopy and histology results. Biopsy samples were collected from the women with ASC-US cytology as warranted by standard of care guidelines at each participating clinical site. Consensus histology results provided by a central pathologist review panel served as the 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.

IN WOMEN 30 YEARS AND OLDER WITH NILM CYTOLOGY, PERFORMANCE OF THE CERVISTA HPV 16/18 TEST AS A REFLEX HPV TEST TO HELP GUIDE PATIENT MANAGEMENT

A longitudinal 3 year post-approval study has been initiated to support the use of the Cervista HPV 16/18 test as a reflex test in women 30 years of age and older with normal cytology and positive Cervista™ HPV HR test results. The study design is described below, along with 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 and Cervista™ HPV 16/18 testing. This was used for evaluation of agreement of the Cervista HPV16/18 test with DNA sequencing as a comparator for HPV detection in the ASC-US and NILM ≥30 populations. Approval for this indication is being given prior to completion of the longitudinal studies in light of the comparator study results. Additionally, consistent data obtained from multiple cross-sectional and prospective cohort studies conducted with a variety of cell sampling methods and utilizing a variety of HPV DNA testing methods (both FDA approved, and research grade) provide strong evidence that a negative HPV DNA test implies very low risk of prevalent or incipient CIN 2-3 or cancer when cervical cytology are normal.[7,8,9,10] Furthermore, the absence of HPV 16 and 18 in this population of women further reduces the risk of developing cervical disease and conversely the presence of HPV 16 or HPV 18 augments the relative risk of cervical disease among women ≥30 years of age regardless of NILM cytological findings.[2,3,11,12]

Description of NILM ≥30 clinical study

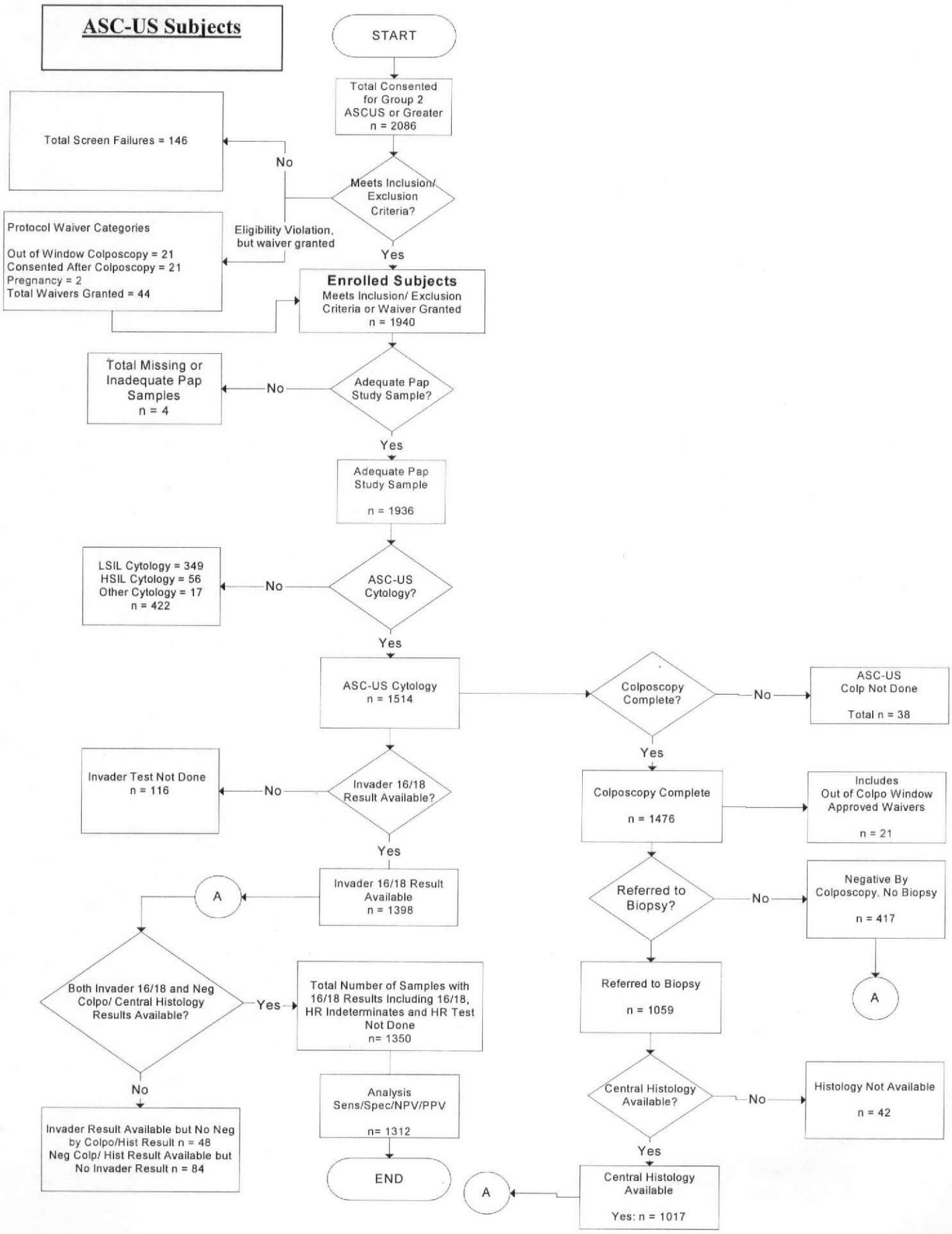
Approximately 2,000 qualified subjects with normal Pap test results (NILM) have been enrolled from 26 active clinical centers throughout the United States. At baseline T_0, initial residual NILM ThinPrep specimens were obtained for Cervista™ HPV HR and Cervista™ HPV 16/18 testing. It is anticipated that not less than 1,000 subjects will have 3-year follow-up data. The subject retention rate at the end of the first year of follow-up has been nearly 80%. Subjects will be followed for 3 years and have annual study visits.

At each follow-up visit, a cervical cytology test is performed. Women who have ASC-US or higher grade cytology results will have a colposcopy performed, and subsequently a biopsy if needed. Analysis of these data will focus on the three-year risk of cervical disease associated with NILM subjects positive for Cervista™ HPV 16/18 as compared to those negative for the test at the time of enrollment (T_0) and also the three-year risk of cervical disease associated with NILM subjects positive for Cervista™ HPV 16/18 as compared to those negative for any HPV high-risk type at the time of enrollment (T_0). The presence or absence of HPV at T_0, will be compared against the presence or absence of (a) ≥ CIN2 and (b) ≥ CIN3 throughout the study. The presence of CIN2, CIN3 or cervical cancer will be ascertained by central histology. Negative results will be defined by colposcopy unless central histology results are available to supersede an initial positive colposcopic indication. All histological interpretation will be conducted by a central pathology review panel.

B. Accountability of PMA Clinical Study Subjects

ASC-US Subjects

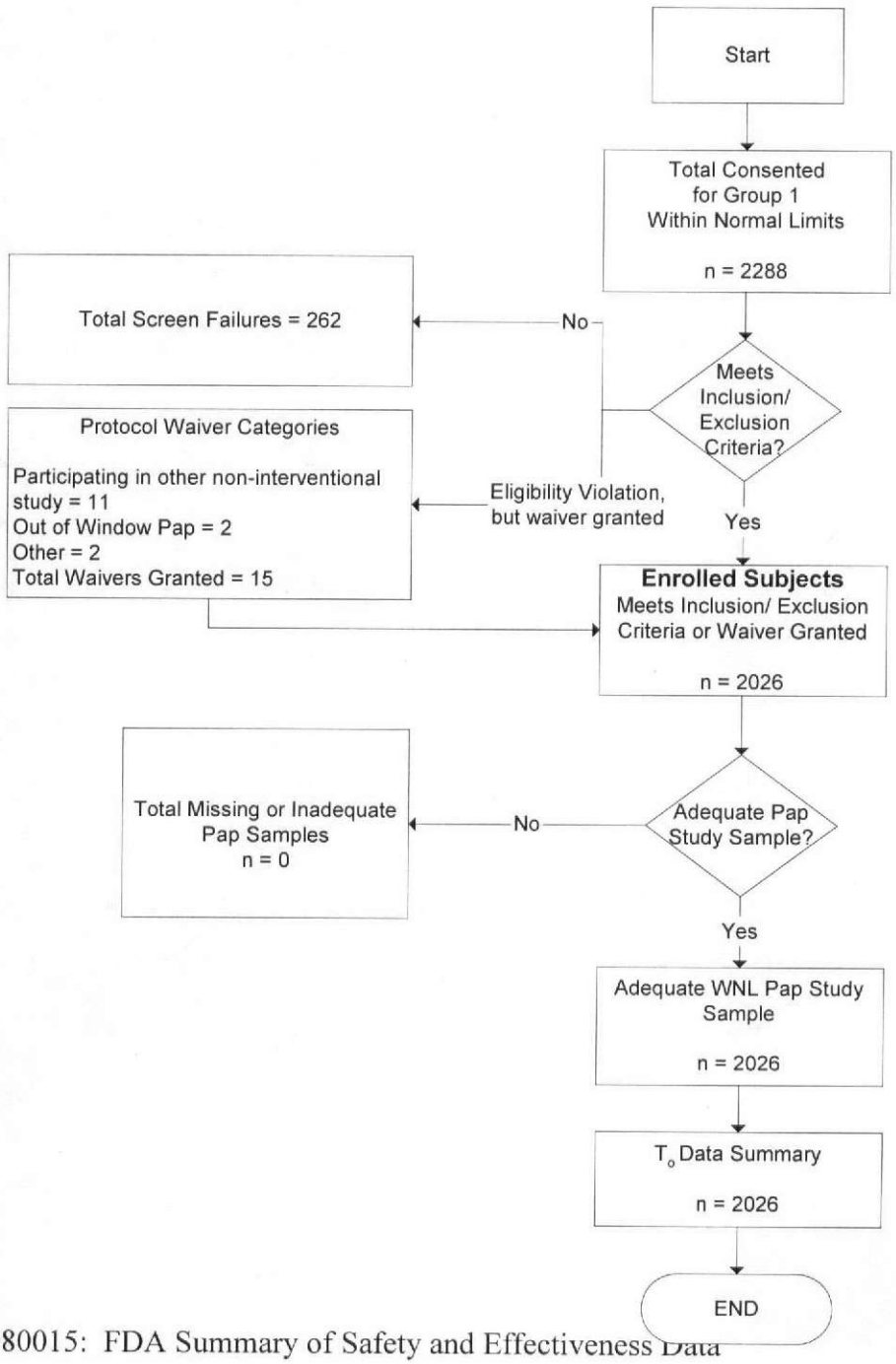
Between July 2006 and December 2007, a total of 2,086 subjects with ASC-US or greater cytology results consented to participate in the study. Out of the total number consented, 1,940 women were enrolled after it was determined that they had met the study's inclusion/exclusion criteria. A total of 1,936 (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 16/18 results were obtained from 1398 (92.3%) ASC-US subjects. Colposcopy was completed for 1,476 (97.5%) of the ASC-US subjects. A total of 1,312 subjects with known disease status (i.e. central histology or a negative colposcopy and/or no biopsy performed), Cervista™ HPV HR determinate results and Cervista™ HPV 16/18 results were available for the data analysis of Cervista™ HPV HR sensitivity, specificity, and likelihood ratios.


ASC-US Subjects
START
Total Consented for Group 2 ASCUS or Greater n = 2086
Meets Inclusion/ Exclusion Criteria?
No
Total Screen Failures = 146
Eligibility Violation, but waiver granted
Protocol Waiver Categories
Out of Window Colposcopy = 21
Consented After Colposcopy = 21
Pregnancy = 2
Total Waivers Granted = 44
Yes
Enrolled Subjects
Meets Inclusion/ Exclusion Criteria or Waiver Granted
n = 1940
Adequate Pap Study Sample?
No
Total Missing or Inadequate Pap Samples
n = 4
Yes
Adequate Pap Study Sample
n = 1936
ASC-US Cytology?
No
LSIL Cytology = 349
HSIL Cytology = 56
Other Cytology = 17
n = 422
Yes
ASC-US Cytology
n = 1514
Colposcopy Complete?
No
ASC-US Colp Not Done
Total n = 38
Yes
Colposcopy Complete
n = 1476
Includes Out of Colpo Window Approved Waivers
n = 21
Invader 16/18 Result Available?
No
Invader Test Not Done
n = 116
Yes
Invader 16/18 Result Available
n = 1398
A
Referred to Biopsy?
No
Negative By Colposcopy, No Biopsy
n = 417
A
Referred to Biopsy
n = 1059
Both Invader 16/18 and Neg Colpo/ Central Histology Results Available?
Yes
Total Number of Samples with 16/18 Results Including 16/18, HR Indeterminates and HR Test Not Done
n= 1350
No
Invader Result Available but No Neg by Colpo/Hist Result n = 48
Neg Colp/ Hist Result Available but No Invader Result n = 84
Analysis
Sens/Spec/NPV/PPV
n= 1312
END
Central Histology Available?
No
Histology Not Available
n = 42
Yes
A
Central Histology Available
Yes: n = 1017

NILM Subjects

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 determinate results and Cervista™ HPV 16/18 results were available for 1,933 (95.4%) of the subjects at their baseline T_0



C. Study Population Demographics and Baseline Parameters

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

Table 15: 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 of Cervista™ HPV 16/18 among women with ASC-US Cervical Cytology Results
   There were 1,312 ASC-US subjects with known disease status (central histology or negative colposcopy) and Cervista™ HPV HR determinate and Cervista™ HPV 16/18 results. A comparison of the Cervista™ HPV 16/18 results with Colposcopy/Consensus Histology is shown in Tables 16 – 28.

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

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

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

Table 17: Cervista™ HPV 16/18 versus Colposcopy /Consensus Histology Results (≥CIN2), among Women with ASC-US Cytology

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

In the clinical study, every woman had results of Cervista HPV HR and Cervista HPV 16/18 tests; therefore, three outcomes of these tests can be considered: (HPV HR Pos and HPV 16/18 Pos), (HPV HR Pos and HPV 16/18 Neg), and (HPV HR Neg).

Likelihood ratios are a useful method of assessing the performance of a medical test when the test has multiple (more than two) outcomes. The likelihood ratio for a test result X is a ratio of two probabilities: LR(T=X)=Pr(T=X|D+)/Pr(T=X|D-), the probability of a given test result among people with a disease divided by the probability of that test result among people without the disease. A likelihood ratio greater than 1 indicates that the test result is associated with the presence of the disease D+, whereas a likelihood ratio less than 1 indicates that the test result is associated with the absence of disease. The further likelihood ratios are from 1, the stronger the evidence for the presence or absence of disease.

The results of the Cervista HPV HR and Cervista HPV 16/18 tests for the three outcomes are presented in Table 18.

Table 18: Three Outcomes of the Cervista™ HPV HR and Cervista™ HPV 16/18 tests versus Colposcopy /Consensus Histology Results (≥CIN2), among Women with ASC-US Cytology

Cervista™ HPV HR and Cervista™ HPV 16/18 Result	≥ CIN2		Total
	Positive	Negative	
HPV HR Pos and HPV	44	213	257

16/18 Pos			
HPV HR Pos and HPV 16/18 Neg	20	480	500
HPV HR Neg	5	550	555
Total	69	1243	1312

The likelihood ratio for (HPV HR Pos and HPV 16/18 Pos) result is 3.72 (=(44/69)/(213/1243)); the likelihood ratio for (HPV HR Pos and HPV 16/18 Neg) result is 0.75 (=(20/69)/(480/1243)); and the likelihood ratio for (HPV HR Neg) result is 0.16 (=(5/69)/(550/1243)).

The performance of a test with multiple outcomes can also be described by risks of the disease for each test outcome. The risk of disease for the test result X depends on the corresponding likelihood ratio and prevalence of disease: $Pr(D+|T=X) = (1+(LR(T=X))^{-1}(1-\pi)/\pi)^{-1}$ where π is prevalence of the disease.

Table 19: Risks of ≥ CIN2 for Different Outcomes of Cervista™ HPV HR and Cervista™ HPV 16/18 Tests

Prevalence of ≥ CIN2: 5.3%

Cervista™ HPV HR Result	Cervista™ HPV 16/18 Result	Risk	95% CI		Likelihood Ratio	95% CI	
HPV HR Positive	HPV 16 and/or 18 Positive	17.1% (44/257)	13.0%	22.2%	3.72	2.93	4.54
	HPV 16/18 Negative	4.0% (20/500)	2.6%	6.1%	0.75	0.51	1.06
HPV HR Negative	HPV 16/18 Negative	0.9% (5/555)	0.4%	2.1%	0.16	0.07	0.36

Table 20: Performance of the Cervista™ HPV16/18 Test for Women with Cervista™ HPV HR Positive Results:

Prevalence of ≥CIN2 among Women with Cervista™ HPV Positive Results: 8.5%

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

Table 21: Cervista™ HPV 16/18 versus Colposcopy / Consensus Histology Results (≥ CIN3), among Women with ASC-US Cytology

Cervista™ HPV HR Result	Cervista™ HPV 16/18 Result	≥ CIN3		Total
		Positive	Negative	
HPV HR Positive	HPV 16 Positive	14	187	201
	HPV 18 Positive	1	42	43
	HPV 16&18 Both Positive	2	11	13

HPV HR Negative	HPV 16&18 Both Negative	5	495	500
	HPV 16 and/or 18 Positive	0	7	7
	HPV 16&18 Both Negative	0	548	548
Total		22	1290	1312

Table 22: Three Outcomes of the Cervista™ HPV HR and Cervista™ HPV 16/18 tests versus Colposcopy /Consensus Histology Results (≥CIN3), among Women with ASC-US Cytology

Cervista™ HPV HR and Cervista™ HPV 16/18 Result	≥ CIN3		Total
	Positive	Negative	
HPV HR Pos and HPV 16/18 Pos	17	240	257
HPV HR Pos and HPV 16/18 Neg	5	495	500
HPV HR Neg	0	555	555
Total	22	1290	1312

Table 23: Risks of ≥CIN3 for Different Outcomes of Cervista™ HPV HR and Cervista™ HPV16/18 Tests

Prevalence of ≥CIN3: 1.7%

Cervista™ HPV HR Result	Cervista™ HPV 16/18 Result	Risk	95% CI		Likelihood Ratio	95% CI	
HPV HR Positive	HPV 16 and/or 18 Positive	6.6% (17/257)	4.2%	10.3%	4.15	2.99	5.08
	HPV 16/18 Negative	1.0% (5/500)	0.4%	2.3%	0.59	0.26	1.14
HPV HR Negative	HPV 16/18 Negative	0.0% (0/555)	0.0%	0.7%	0.00	0.00	0.37

Table 24: Performance of the Cervista™ HPV16/18 Test for Women with Cervista™ HPV HR Positive Results:

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

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

Table 25: Clinical Performance of the Cervista™ HPV 16/18 Test Stratified by Age for Women with Cervista™ HPV HR Positive Results

		Central Histology ≥ CIN 2		
Age: 18 to <21		**Positive**	**Negative**	**Total**
HPV HR Positive	HPV 16 and/or 18 Positive	7	39	46
	HPV 16 & 18 Negative	2	54	56
HPV HR Negative		0	23	23
	Total	9	116	125
Disease Prevalence*:	8.8% (9/102)	95% CI		
Sensitivity:	77.8% (7/9)	40.0% to 97.2%		
Specificity:	58.1% (54/93)	47.4% to 68.22%		
Age: 21 to <30		**Positive**	**Negative**	**Total**
HPV HR Positive	HPV 16 and/or 18 Positive	21	117	138
	HPV 16 & 18 Negative	9	197	206
HPV HR Negative		0	138	138
	Total	30	452	482
Disease Prevalence*:	8.7% (30/344)	95% CI		
Sensitivity:	70.0% (21/30)	50.6% to 85.3%		
Specificity:	62.7% (197/314)	57.1% to 68.1%		
Age: 30 to <39		**Positive**	**Negative**	**Total**
HPV HR Positive	HPV 16 and/or 18 Positive	7	30	37
	HPV 16 & 18 Negative	3	126	129
HPV HR Negative		3	125	128
	Total	13	281	294
Disease Prevalence*:	6.0% (10/166)	95% CI		
Sensitivity:	70.0% (7/10)	34.8% to 93.3%		
Specificity:	80.8% (126/156)	73.7% to 86.6%		
Age: 39 or older		**Positive**	**Negative**	**Total**
HPV HR Positive	HPV 16 and/or 18 Positive	9	27	36
	HPV 16 & 18 Negative	6	103	109
HPV HR Negative		2	264	266
	Total	17	394	411
Disease Prevalence*:	10.3% (15/145)	95% CI		
Sensitivity:	60.0% (9/15)	32.3% to 83.7%		
Specificity:	79.2% (103/130)	71.2% to 85.8%		

* Prevalence of ≥ CIN2 among women with Cervista™ HPV HR Positive Results

Additional Subgroup Analysis

Tables 26 and 27 present clinical performance of the Cervista™ HPV 16/18 test for the collection devices tested in the clinical study.

Table 26. Clinical Performance of the Cervista HPV HR/1618 Reflex Stratified by Sample Collection Device* as Compared to Colposcopy/central Histology Results Among Women with ASC-US Cytology

Collection Device=Rovers Cervex Brush						
	Negative Colposcopy with No Biopsy	**Central Histology**				**Total**
		No CIN	**CIN 1**	**CIN 2**	**CIN 3**	

HPV HR Positive 16 and/or 18 Positive	35	38	19	10	9	111
HPV HR Positive Both 16 and 18 Negative	63	111	36	4	2	216
HPV HR Positive 16/18 Indeterminate	2	5	0	0	0	7
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						
	Negative Colposcopy with No Biopsy	**Central Histology**				**Total**
		No CIN	**CIN 1**	**CIN 2**	**CIN 3**	
HPV HR Positive 16 and/or 18 Positive	10	32	19	12	4	77
HPV HR Positive Both 16 and 18 Negative	29	95	37	6	1	168
HPV HR Positive 16/18 Indeterminate	1	2	0	0	0	3
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						
	Negative Colposcopy with No Biopsy	**Central Histology**				**Total**
		No CIN	**CIN 1**	**CIN 2**	**CIN 3**	
HPV HR Positive 16 and/or 18 Positive	6	38	16	5	4	69
HPV HR Positive Both 16 and 18 Negative	17	67	25	5	2	116
HPV HR Positive 16/18 Indeterminate	1	1	0	0	0	2
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

*The Rovers Cervex Brush group includes one case with collection device reported as Rovers Cervex Brush and Endocervical brush/spatula, and 6 cases reported simply as brush.

Table 27: Clinical Performance of the Cervista™ HPV 16/18 Test Stratified by Collection Device as Compared to Colposcopy/Central Histology Results (≥CIN2) for the Cervista™ HPV HR Positive Results

Collection Device	**Sensitivity**	**Specificity**	**Prevalence of ≥ CIN2**	**Percent of Subjects with Positive Results by Cervista HPV 16/18**	**Percent of Subjects with Indeterminate Results by Cervista HPV 16/18**

Rovers Cervex Brush	76.0% (19/25)	69.5% (210/302)	7.6% (25/327)	33.9% (111/327)	3.0% (7/234)
Wallach Papette	69.6% (16/23)	72.5% (161/222)	9.4% (23/245)	31.4% (77/245)	1.2% (3/248)
Endocervical Brush/Spatula	56.3% (9/16)	64.5% (109/169)	8.6% (16/185)	37.3% (69/185)	1.1% (2/187)

Table 28 presents the clinical performance of the Cervista™ HPV 16/18 test by molecular testing site.

Table 28: Clinical Performance (≥CIN2) of the Cervista™ HPV 16/18 Test for the Cervista™ HPV Positive Results by Molecular Testing Center

Molecular Testing Site	Sensitivity	Specificity	Prevalence of ≥ CIN2	Percent of Subjects with Positive Results by Cervista HPV 16/18	Percent of Subjects with Indeterminate Results by Cervista HPV 16/18
1	78.6% (11/14)	72.2% (114/158)	8.1% (14/172)	32.0% (55/172)	3.4% (6//178)
2	0.0% (0/1)	65.4% (17/26)	3.7% (1/27)	33.3% (9/27)	6.9% (2/29)
3	70.3% (26/37)	68.1% (267/392)	8.6% (37/429)	35.2% (151/429)	0.0% (0/429)
4	58.3% (7/12)	70.1% (82/117)	9.3% (12/129)	32.6% (42/129)	3.0% (4/133)
Total	68.8% (44/64)	69.3% (480/693)	8.5% (64/757)	33.9% (257/757)	1.6% (12/769)

Comparison of DNA Sequencing and Cervista HPV16/18 for the ASC-US and NILM ≥ 30 Populations

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

Below in Tables 29-32 is a comparison between Cervista™ HPV 16/18 and DNA sequencing for the detection of HPV 16 and 18 in both ASC-US and NILM ≥ 30 populations.

Table 29: Performance of Cervista HPV16/18 and PCR Sequencing Results, NILM ≥30

	PCR Sequencing										
Cervista Result	**HR Indeterminate**	**HR Negative**	**One HR Type**			**Two HR Types**				**Mult. HR Types**	**Tota**
			16	**18**	**Other**	**16&18**	**16& Other**	**18& Other**	**Others**	**16& Other**	
HPV HR Positive											

HPV 16 Positive	1	48	8	0	6	0	2	0	0	1	66
HPV 18 Positive	0	2	0	6	0	0	0	0	0	0	8
HPV 16&18 Both Positive	0	1	0	0	1	0	0	0	0	0	2
HPV 16/18 Negative	12	203	1	0	60	0	0	0	3	0	279
HPV HR Negative											
HPV 16 Positive	0	12	1	0	0	0	0	0	0	0	13
HPV 18 Positive	0	6	0	0	0	0	0	0	0	0	6
HPV 16/18Negative	39	1512	2	0	6	0	0	0	0	0	155
Total	52	1784	12	6	73	0	2	0	3	1	193

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

Table 30: Comparison of Cervista HPV16 and/or HPV18 Results vs PCR Sequencing Results for Women with Cervista HPV HR Positive Results, NILM ≥30

	PCR Sequencing HR Positve HPV16 and/or HPV 18		**Total**
Cervista™ HPV HR Positive:	**Positive**	**Negative**	
Cervista™ HPV 16/18 Positive	17	58	75
Cervista™ HPV 16/18 Negative	1	266	267
Total	18	324	342

Positive Percent Agreement and Negative Percent Agreement

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

Table 31: Performance of Cervista HPV16/18 and PCR Sequencing Results, ASC-US

			PCR Sequencing											
			One HR Type			**Two HR Types**				**Multiple HR Types**				**Total**
rvista™ sult	**HR IND**	**HR Negative**	**16**	**18**	**Other**	**16&18**	**16 & Other**	**18 & Other**	**Others**	**16 & 18 & Other**	**16 & Other**	**18 & Other**	**Others**	
'V HR sitive														
IPV 16 sitive	7	25	96	0	41	0	29	0	1	0	6	0	0	205
IPV 18 sitive	0	0	0	27	7	0	0	9	0	0	0	0	0	43
IPV 16&18 th Positive	0	1	2	0	1	2	1	1	0	3	0	1	1	13

IPV 16&18 th Negative	32	95	6	2	335	0	1	0	27	0	2	0	3	503
IPV 16/18 leterminate	2	1	0	0	7	0	0	0	2	0	0	0	0	12
'V HR gative														
IPV 16 sitive	1	3	2	0	0	0	0	0	0	0	0	0	0	6
IPV 16&18 th Negative	35	510	9	0	10	0	0	0	1	0	0	0	0	565
IPV 16/18 leterminate	1	5	0	0	1	0	0	0	0	0	0	0	0	7
tal	78	640	115	29	402	2	31	10	31	3	8	1	4	1354

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

Table 32: Comparison of Cervista™ HPV16 and/or HPV18 Results vs PCR Sequencing Results for Women with Cervista™ HPV HR Positive Results, ASC-US

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

Positive Percent Agreement and Negative Percent Agreement

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

Expected Results

The reported prevalence of HPV infection in women ranges widely, from 14% to more than 90%.[13] Several factors can affect the HPV prevalence among patient populations due to heterogeneity in geographic location, age, number of sexual partners, history of abnormal cervical cytology, coupled with differences in sampling techniques and testing methods and the intermittent nature of the infection. The Cervista™ HPV 16/18 multi-center prospective clinical study enrolled women from 89 clinical sites across 23 states throughout the United States which produced a demographically diverse patient population. Tables 33 and 34 show the prevalence of HPV16 and HPV 18 observed in the study stratified by age.

Table 33. Prevalence of HPV 16 and HPV 18 Among Women with ASC-US Cytology Stratified by Age

Age Group	Prevalence of HPV 16	Prevalence of HPV 18	Prevalence of HPV 16&18

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

Table 34. Prevalence of HPV 16 and HPV 18 Among Women with NILM Cytology Stratified by Age

Age Group	Prevalence of HPV 16	Prevalence of HPV 18	Prevalence of HPV 16/18 Co-Infection
30 < 40	3.4% (21/616)	0.8% (5/616)	0.2% (1/616)
40 < 50	4.0% (27/674)	0.7% (5/674)	0% (0/674)
50 < 60	5.1% (25/486)	1.0% (5/486)	0.2% (1/486)
60 < 70	3.9% (6/154)	0% (0/154)	0% (0/154)
≥70	0% (0/30)	0% (0/30)	0% (0/30)
All	4.0% (79/1960)	0.8% (15/1960)	0.1% (2/1960)

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

A panel homework assignment for 3 panel members was conducted for the Cervista HPV 16/18 test in lieu of an advisory panel meeting. The homework assignment asked a series of questions designed to help FDA determine the most appropriate intended use for this device. Notable comments from these assignments and FDA's consideration of the panelist's comments are described below:

ASC-US indication

Panelist 1 initially stated that "the data from the interim clinical trial report justify the proposed use as a follow-up test to an HR HPV DNA test (in women with ASC-US cytology)" because "the fraction of women who would be referred for colposcopy would be reduced considerably." Panelist 1 then goes on to express concern about the sensitivity of the HPV 16/18 test if the HPV 16/18 test is used without the HPV HR test, stating that "data from the 'interim clinical trial report' show that 22 of 69 (32%) CIN2+ lesions were types 16/18 negative (ie, false negatives). I believe that most clinicians will not adopt the use of a test in which nearly 1/3 of the significant lesions were missed. Therefore, I doubt that there will be fewer colposcopic examinations performed as a result of the availability of this test." This concern of missing lesions was expressed repeatedly during the homework response. Panelist 2 expressed similar concerns about false negatives if the device were to be used as a stand alone assay. However, when asked: "Would the HPV 16/18 test result be more useful if done in conjunction with a

HR test result...?" Panelist 1 responded: "Yes. That combination of test results is more information that the clinician may use to help determine a course of action. For example, a positive HR HPV test with a negative 16/18 test suggests that the patient has a low risk of progression, at least in a short timeframe, and thus may be watched without intervention for a longer period of time that one who is pos/pos."

FDA agrees with the panelist's concerns of using an HPV 16/18 test as a stand-alone assay and subsequently has approved the assay only for use as a follow-up to, or alongside the Cervista HPV HR assay. With this dual testing, women with ASC-US cytology who are HR positive and 16/18 negative can be recognized as being lower risk than a woman who is simply HR positive. Likewise, a woman who is HR positive and 16/18 positive can be recognized as being higher risk than if only a HR positive result were obtained. This information may or may not influence a physician's decision to send a woman immediately to colposcopy. It is notable that in the submitted dataset, women with ASC-US cytology who were HR positive and 16/18 negative had a similar approximate absolute risk of ≥CIN2 as women with ASCUS cytology where HPV status is unknown (per the 2006 consensus guidelines[4], repeat cytology at 6&12 months may be considered over immediate colposcopy in women with ASC-US cytology who do not have an HPV test result). The ASC-US indication for use is worded such that the device is approved to provide additional information on risk to utilize in patient management. This general indication is directly supported by the data provided by the sponsor – since the sponsor has shown a statistically significant difference in absolute risk of ≥CIN2 in patients with ASC-US cytology who are 16/18 positive vs. 16/18 negative (also, statistically significant differences were demonstrated in absolute risks of ≥CIN2 between all groups of ASC-US patients: HR pos/HPV16/18 pos, HR pos/HPV 16/18 neg). This general indication also gives physicians the freedom to decide how the additional information on risk will influence their practice decisions.

NILM ≥30 indication

Panelists 1 and 2 expressed similar concerns regarding the NILM ≥30 population as they did for the ASC-US population about the false negative rate of the Cervista HPV 16/18 test if used as a stand-alone assay. FDA agrees with the panelist's concerns of using an HPV 16/18 test as a stand-alone assay and subsequently has approved the assay only for use as a follow-up to, or alongside the Cervista HPV HR assay in the NILM 30 and older population.

Panelist 3 had the following 2 major concerns with the submission:

1. "The proposal does not address assay reproducibility of Invader HPV16/18 in real practice, and constructed specimens are not sufficiently variable or difficult to address achievable reproducibility."

When Panelist 3 was asked about their expectations for assay reproducibility, the panelist indicated a preference to see essentially a repeatability experiment, where the entire (or majority of) clinical specimens in the study are run at multiple clinical sites to see if the same outcomes are obtained. OIVD generally recommends that clinical laboratories follow CLSI guidelines EP5-A2[14] and EP12-A2[15] for establishing precision (repeatability

and reproducibility) of their assay. The study design followed by the sponsor was consistent with these guidelines, and focused on reproducibility over time, between operators, laboratories and days, utilizing a panel of both contrived specimens with defined levels of analyte, and real clinical specimens, with a focus on including specimens that challenge the medical decision points of the assay. OIVD subsequently concluded that assay reproducibility had been adequately established.

2. "The clinical sensitivity of Invader HPV 16/18 might be excellent, but clinical specificity at the chosen threshold of assay positivity is possibly poor. The overall concern is that many women without HPV16/18 could be classified as high-risk (HPV16/18 positive) using this test with resultant excessive concern, overly-aggressive management, and over-treatment... the positivity by Third Wave HPV16/18 is approximately 12% in WNL subjects 227/1889. If this calculation is correct, the value is problematic. It is several times higher than the PCR-based meta-analytic estimates. Perhaps it is correct and these are analytically true positives, but that should be established. Moreover, even if true, the resultant positive predictive values would be much lower than those shown in the references. Ultra-sensitivity is not necessarily desirable in producing optimal clinical accuracy... I believe that there is substantial concern that the test is non-specific either due to false positivity or poor threshold choice for optimal definition of clinically meaningful positives. Accuracy is a combination of sensitivity and specificity. The test is not sufficiently accurate compared to virologic or disease reference standards... There might be a tendency to over-treat women with HPV 16/18 at first detection without a proper period of waiting to see whether there is, in fact, a persistent infection. Specificity is very important. I do not think the test should be approved at present. If it were, there should be a strong warning not to treat on the basis of this test. It can not be a weak warning or it would not work to dissuade concerned clinicians and patients... A new choice of threshold could, a posteriori, show the problem. Without knowing, and without access to proper ROC analysis, I urge rejection of the application."

To address the third Panelist's concerns about specificity and the lack of a receiver operator curve (ROC) analysis for this assay – the ROC analysis for the ASC-US subjects was in fact requested. Per this analysis, the cutoff would be optimal at 2.14 (the submitted cutoff was 1.50). The statistician for this submission was concerned that utilizing a ROC analysis on the same dataset used to establish performance would create a bias in the performance estimates when the cutoff is selected on the ROC curve such that the sum of sensitivity and specificity is maximized.[6.16] Unusually high prevalence with the original 1.50 cutoff in the adjunct screening (NILM 30 and older) population was one of the major signs of a specificity problem identified by the third panelist in subsequent phone conversations with the lead reviewer. The statistician suggested targeting the desired level of HPV 16/18 prevalence in the NILM 30 and older population to establish an unbiased cutoff for this assay. Ultimately, the clinical cut-off was evaluated based on HPV16/18 test results targeting a 5% positive rate in the NILM ≥30 population from a multi-center clinical study. The 95th percentile of the maximum HPV16 and HPV18 FOZ values was determined for NILM ≥30 subjects and based on this analysis, a FOZ value of ≥2.13 was selected as the positive cutoff value for the

Cervista™ HPV 16/18 test. The third panelist indicated that this course of action addressed their concerns about specificity.

Consideration of guidelines

The 2006 consensus guidelines[4] recommend sending cytology normal women age 30 and older directly to colposcopy if they are HPV 16 and/or 18 positive. Note that without a 16/18 result, women 30 and older with normal cytology and a HR positive result would be advised to return for cytology and HPV testing in 12 months, while a HR negative woman would be re-screened by cytology in 3 years per these guidelines. FDA has not given the sponsor an explicit indication for triage to colposcopy in woman 30 and older with normal cytology, because the sponsor's study was not designed to directly evaluate this indication. (Such a study would require that cytology normal women, both 16/18 positive and 16/18 negative be sent to colposcopy. At the time the sponsor's study was commenced, the 2006 consensus recommendations were not yet in place, and there was logical resistance to sending women with normal cytology to colposcopy). Instead, the approved NILM 30 and older ("adjunct") indication for use is worded such that the device is used to provide additional information on risk to utilize in patient management. This general indication gives physicians the freedom to decide how the additional information on risk will influence their practice decisions. The following elements were taken into consideration in approving this adjunctive screening claim: 1. This HPV 16/18 detection device demonstrated acceptable clinical performance in a population where clinical endpoints were readily accessible (an ASCUS screening population). 2. Evaluation of HPV detection using prospectively collected clinical specimens from the ASCUS and adjunctive screening (NILM ≥30) populations showed comparable performance in these two populations. The patients from the NILM ≥30 dataset will be followed longitudinally as part of a post-approval study to establish the cumulative 3-year risk of precancer/cancer in patients positive vs. negative by the Cervista HPV 16/18 test in this population. 3. Use of HPV 16/18 testing in the NILM ≥30 population for evaluating risk is supported by current clinical practice guidelines.

General Comments

In all cases, the physicians on the panel proposed additional specific intended uses that had not been directly evaluated by the sponsor (and subsequently can not be given without supporting data).

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

Based on the results of the preclinical and clinical laboratory studies, the safety of the Cervista HPV 16/18 Test, when used according to the provided directions and in conjunction with the Cervista HPV HR test, 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 16/18 test has been demonstrated for use in conjunction with the Cervista HPV HR test and cervical cytology. The test may be used in women 30 years and older to adjunctively screen to assess the presence or absence of human papillomavirus (HPV) types 16 and 18. Additionally, a reasonable determination of effectiveness of the Cervista HPV 16/18 test for use in screening patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results has been demonstrated. The results of this test, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

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 16/18 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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