SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: cobas HPV for use on the cobas 6800/8800 Systems

Device Procode: MAQ

Applicant's Name and Address: Roche Molecular Systems, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190028

Date of FDA Notice of Approval: April 3, 2020

II. INDICATIONS FOR USE

cobas HPV for use on the cobas 6800/8800 Systems (cobas HPV) is a qualitative *in vitro* test for the detection of Human Papillomavirus in clinician-collected cervical specimens using an endocervical brush/spatula or broom and placed in the ThinPrep Pap Test PreservCyt Solution. This test detects the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

cobas HPV is indicated for use for routine cervical cancer screening as per professional medical guidelines, including triage of ASC-US cytology, co-testing (or adjunctive screen) with cytology, and HPV primary screening of women to assess the risk for cervical precancer and cancer. Patients should be followed-up in accordance with professional medical guidelines, results from prior screening, medical history, and other risk factors.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the cobas HPV labeling.

V. DEVICE DESCRIPTION

cobas HPV is a qualitative real-time PCR test that detects 14 high-risk HPV genotypes. Of 14 HPV genotypes, 13 HPV genotypes are classified as carcinogenic or high-risk (HR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and an additional genotype, 66, that is classified as "possibly carcinogenic" based on its relatively low prevalence in invasive cervical carcinoma. cobas HPV uses primers to define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the Master Mix is designed to amplify HPV DNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The test includes a primer pair that amplifies the human β-globin gene as an internal control to monitor the entire sample preparation and PCR amplification process (330 base pair amplicon). Fluorescent oligonucleotide probes bind to polymorphic regions within the sequence defined by these primers. In addition, the test utilizes a low titer positive and a negative control.

cobas HPV consists of:

- cobas 6800/8800 Systems
- cobas HPV assay specific analysis package (ASAP) software
- cobas HPV reagents in cassettes
- cobas HPV Positive Control Kit
- cobas Buffer Negative Control Kit
- Specimen preparation reagents (cobas omni Reagents)

cobas HPV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas 6800/8800 software, which assigns test results for all tests as positive, negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Principle of Procedure

1. Sample Preparation (Nucleic Acid Extraction and Purification)

   Nucleic acid from a patient sample is released upon addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris, and potential PCR inhibitors are removed with subsequent wash steps, and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each cobas HPV run.

2. Nucleic Acid Amplification

   A thermostable DNA polymerase enzyme is used for PCR amplification. The HPV and β-globin sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

3. Nucleic Acid Detection

   The cobas HPV master mix contains detection probes specific for twelve High Risk HPV target sequences, one detection probe specific for the HPV16 target sequence, one detection probe specific for the HPV18 target sequence and one for β-globin. The amplified signal from twelve high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) is detected using the same fluorescent dye while HPV16, HPV18, and β-globin signals are each detected with their own dedicated fluorescent dye. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the HPV targets and β-globin, respectively.

Instrumentation and Software

1. cobas 6800/8800 System Platform Overview

   The cobas 6800/8800 System is a platform that allows users to perform multiple PCR-based *in vitro* nucleic acid amplification tests. The platform consists of two separate instruments, the cobas 6800 System and the cobas 8800 System, both of which provide automated sample preparation, nucleic acid extraction, and target amplification and detection.

2. cobas 6800/8800 Systems Software Overview

   The cobas 6800/8800 Systems Software is the primary interface for operators to access, control, and manage the cobas 6800/8800 Systems. The cobas 6800/8800 Systems Software includes off the shelf software components as well as software tools that are used for diagnosis and maintenance of the system.

   The main system functionality is provided by two software components;
   i) the cobas 6800/8800 System Software and ii) Assay Specific Analysis Package

(ASAP) software. The cobas 6800/8800 System software provides basic functionality, such as a Graphical User Interface (GUI), instrument management, database functionality, report engines, and LIS interfaces. These basic functions do not change when a new ASAP is added onto the system. The ASAPs are built using a common software framework and provide the assay test run conditions (sample preparation and PCR parameters), result analysis functionality (result calculation and algorithms), and result report formatting.

3. cobas 6800/8800 Systems Workflow

A workflow defines how the system processes the samples designated for a specific test, including any required user interactions. The sample gets loaded by the user and then processed automatically without any further user interaction until results are generated. More than one test can be ordered for the same sample. The system identifies the orders, and manages the process automatically.

The workflow on the cobas 6800/8800 Systems is centered on the batch process and linked to the design of the Process plate and Amplification plate:

- The Process Plate has 48 wells
- Both the cobas 6800 and cobas 8800 Systems have two 48 channel process heads per process cell which can process 96 test orders in parallel, using two Process plates
- The Amplification Plate has 96 wells

Both systems are capable of processing up to 96 samples in one run batch. As a result, there is only one common sample workflow on both systems. This common workflow is used for parallel processing of 96 samples in two Process plate and one Amplification plate on the cobas 6800/8800 Systems. If 48 or fewer test orders have to be processed, then only one Process plate will be used on both systems.

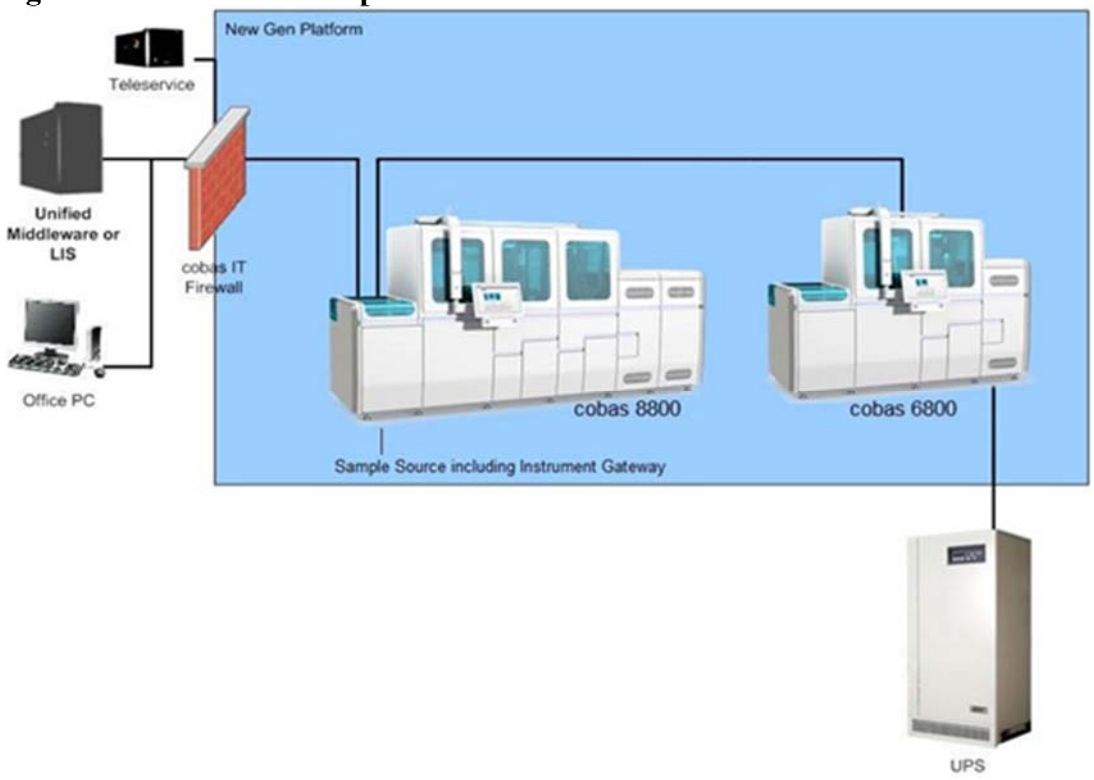
The cobas 6800/8800 Systems use the same hardware, software, and workflow for processing the samples. As a result, samples run on either system will have identical processing. The Sample Transfer process depends on the number of samples and orders and volumes being transferred to the Process plate. The timing for the Sample Preparation process is independent of the number of samples being processed, since all process steps are controlled by a time box. The timing of the amplification and detection process is independent of the number of samples, since all samples in the Amplification Plate undergo PCR in parallel.

The cobas 6800/8800 Systems utilize a core set of common reagents, the cobas omni reagents, which are designed to be used with all assays that are run on the systems. Identification, validity described by the remaining number of determinations, onboard time and expiry date of all reagents are tracked by RFID.

In addition, the omni (common) reagents and consumables, such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used by any of

the cobas 6800/8800 System assays, and on either the cobas 6800 or the cobas 8800 instrument. Figure 1 below, depicts the cobas 6800/8800 platform.

Figure 1: cobas 6800/8800 platform



Additional details can be found in the operator's manual of the device.

4. Interpretation of Test Results

   Results and their corresponding interpretation for detecting overall HR HPV and HPV-Genotyping are shown in Table 1 and Table 2, repsectively.

Table 1: The interpretations for the overall HR-HPV results

Target 1	Target 2	Target 3	Interpretation
HR HPV Positive	<Blank>	<Blank>	Specimen is positive for the DNA of any one of, or combination of, the following high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
HR HPV Negative			HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 DNA were undetectable or below the pre-set threshold.
Invalid			The result for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 is invalid.

Table 2: The interpretations for the HPV-genotyping results

Target 1	Target 2	Target 3	Interpretation
Other HR HPV Positive			Specimen is positive for the DNA of any one of, or combination of the following high risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
Other HR HPV Negative			HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were undetectable or below the pre-set threshold.
Invalid			The result for HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 is invalid.
	HPV 16 Positive		Specimen is positive for HPV type 16 DNA.
	HPV 16 Negative		HPV type 16 DNA was undetectable or below the pre-set threshold.
	Invalid		The result for HPV type 16 is invalid.
		HPV 18 Positive	Specimen is positive for HPV type 18 DNA.
		HPV 18 Negative	HPV type 18 DNA was undetectable or below the pre-set threshold.
		Invalid	The result for HPV type 18 is invalid.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several alternatives for the detection of cervical cancer precursors including testing by cytology alone, co-testing with HPV alongside or as a follow-up to cytology. or HPV testing as a first line screening test for cervical cancer. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

The patient's age, medical history, and thorough physical examination will provide further information on the risk of cervical disease, as well as the need for referral to colposcopy. The cobas HPV test should only be used in conjunction with this clinical information in accordance with professional clinical patient management guidelines.

VII. MARKETING HISTORY

The product is currently distributed/marketed in forty-one countries. The product has not been withdrawn to date from the market in any country for reasons related to the safety or effectiveness of the device.

The forty-one countries where the product is distributed includes:

Australia	Germany	Mexico
Austria	Greece	Norway
Belgium	Hungary	Poland
Brazil	Iceland	Portugal
Bulgaria	Ireland	Romania
Colombia	Italy	Slovakia
Croatia	Japan	Slovenia

Cyprus	Latvia	Spain
Czech Republic	Liechtenstein	Sweden
Denmark	Lithuania	Switzerland
El Salvador	Luxembourg	Turkey
Estonia	Malta	United Kingdom
Finland	Myanmar	Vietnam
France	Netherlands	

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As with any *in vitro* diagnostic test, the potential risks are associated with an incorrect test result or result interpretation. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect HPV test results and subsequently, improper patient management decisions.

For the specific adverse events that occurred in the clinical study, please see Section X below..

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Clinical Cutoff Determination

The methods used for cutoff selection was chosen to achieve the maximum sensitivity for detecting ≥ CIN2 disease while maintaining a clinically acceptable level of specificity. Based on these criteria, the clinical cutoff was set at Ct of 38.5 for HPV 16, Ct of 38.0 for HPV 18 and Ct of 34.5 for all HR HPV genotypes other than 16 and 18.

2. Limit of Detection at the Clinical Cutoff

The Limit of Detection (LoD) at the clinical cutoff for HPV16 and HPV18 was assessed using SiHa and HeLa cell lines in the background of pooled HPV negative patient specimens collected in PrervCyt Solution. Cell lines were diluted to concentrations below, above, and at the expected LoD at the Clinical cutoff levels. A minimum of 24 replicates were tested for each cell line level using three reagent lots with an equal number of runs performed on the cobas 6800 and the cobas 8800 Systems. The LoD at the clinical cutoff was defined as the level of HPV DNA in the sample that has positive test results at least 95% of the time. The LoD at the clinical cutoff for SiHa and HeLa was 16 cells/mL. Table 3 and Table 4 list results from the reagent lot producing the most conservative (highest) LoD at the clinical cutoff in the analysis for HPV16 and HPV18.

Table 3: LoD at the clinical cutoff for HPV16 (SiHa cell line)

SiHa Concentration (cells/mL)	Number of Positive/Tested	% Positive	95% Confidence Interval
32	24 / 24	100%	86.2% - 100%
16	**24 / 24**	**100%**	**86.2% - 100%**
8	22 / 24	91.7%	74.1% - 97.7%

Table 4: LoD at the clinical cutoff for HPV18 (HeLa cell line)

HeLa Concentration (cells/mL)	Number of Positive/ Tested	% Positive	95% Confidence Interval
32	24 / 24	100%	86.2% - 100%
16	**24 / 24**	**100%**	**86.2% - 100%**
8	22 / 24	91.7%	74.1% - 97.7%

3. Inclusivity

Plasmids for high risk genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 were tested close to the LoD at the clinical cutoff in the background of pooled HPV negative patient specimens. All 12 of the high risk genotypes tested were detected by the assay.

4. Analytical Specificity

A panel of bacteria, fungi and viruses, including those commonly found in the female urogenital tract, as well as several human papillomavirus types classified as low or undetermined risk were tested with cobas HPV to assess analytical specificity. The organisms listed in Table 5 were spiked at concentrations of approximately 1 x 10^6 Colony Forming Units (CFU)/mL for bacteria (except for *Chlamydia trachomatis* which was quantified as Inclusion Forming Units (IFU) and *Trichomonas vaginalis* which was quantified as cells/mL) and approximately 1 x 10^5 $TCID_{50}$/mL for viruses (except Adenovirus Type 40 which was tested at 2.82 x 10^4 $TCID_{50}$/mL and Epstein Barr virus which was tested at 1 x 10^5 copies/mL) into pools of HPV negative cervical specimens in PreservCyt Solution. Testing was performed with each potential interfering organism alone as well as with each organism mixed with HPV31 plasmid, SiHa (HPV16) and HeLa (HPV18) cell lines at approximately 3x LoD at the clinical cutoff of cobas HPV. Results indicated that these organisms neither interfered with detection in the 12 Other High Risk HPV, HPV16 and HPV18 channels nor produced a false positive result in the HPV negative specimen.

Table 5: Microorganisms tested for analytical specificity

Adenovirus Type 40	Herpes Simplex Virus 2	HPV84
Bacteroides caccae	HPV6	HPV85
Bacteroides ureolyticus	HPV11	HPV89
Bifidobacterium adolescentis	HPV26	*Klebsiella oxytoca*
Bifidobacterium breve	HPV30	*Klebsiella pneumoniae*
Bifidobacterium longum	HPV34	*Lactobacillus acidophillus*
Candida albicans	HPV40	*Neisseria gonorrhoeae*
Chlamydia trachomatis	HPV42	*Peptostreptococcus anaerobius*
Clostridioides difficile	HPV53	*Peptostreptococcus asaccharolyticus*
Clostridium perfringens	HPV54	*Proteus mirabilis*
Corynebacterium genitalium	HPV55	*Proteus penneri*
Cytomegalovirus	HPV61	*Proteus vulgaris*
Enterobacter aerogenes	HPV62	*Pseudomonas aeruginosa*
Enterobacter cloacae	HPV64	*Pseudomonas fluorescens*
Enterococcus avium	HPV67	*Pseudomonas putida*
Enterococcus casseliflavus	HPV69	*Staphylococcus aureus*
Enterococcus faecalis	HPV70	*Staphylococcus epidermidis*
Enterococcus faecium	HPV71	*Streptococcus agalactiae*
Epstein Barr Virus	HPV72	*Streptococcus pyogenes*
Escherichia coli	HPV73	*Treponema pallidum*
*Finegodia magna**	HPV81	*Trichomonas vaginalis*
Fusobacterium nucleatum	HPV82	
Herpes Simplex Virus 1	HPV83	

*formerly *Peptostreptococcus magnus*

5. Interference

The effects of endogenous and exogenous substances on the performance of the cobas HPV were evaluated. HPV negative and positive specimens were tested in the presence or absence of each potential interferent that may be present in clinical cervical specimens. The concentrations of exogenous and endogenous substances tested in this study represent concentrations that could potentially occur during specimen collection. Specimens were prepared from pooled negative clinical matrix in PreservCyt Solution. All testing for interference was performed with each potential interfering substance alone as well as with the substance mixed with SiHa (HPV16) and HeLa (HPV18) cell lines at approximately 3x LoD at the clinical cutoff of cobas HPV in HPV negative samples. The study design was acceptable and the results of this study are described below.

Endogenous substances tested were cervical mucus, peripheral blood mononuclear cells and whole blood. Levels of endogenous substances tolerated by the assay are shown in Table 6. Exogenous substance testing included 18 over-the-counter (OTC)

feminine hygiene and prescription products and glacial acetic acid that are listed in Table 7.

Of OTC feminine hygiene and prescription products tested, Metronidazole Gel, Replens, RepHresh Odor Eliminating Vaginal Gel and RepHresh Clean Balance Feminine Freshness Kit produced false negative results. An appropriate limitation has been included in the Package Insert.

Table 6: Endogenous substances tested for interference

Endogenous Substance	PreservCyt
Mucus	Presence*
Peripheral Blood Mononuclear Cells (PBMCs as cells/mL)	1.00E+06
Whole Blood (% v/v)	10%

*Presence refers to the amount of cervical mucus normally removed from the cervix prior to sampling.

Table 7: Exogenous substances tested for interference

Product Name	Concentration
Clindamycin Phosphate Vaginal Cream	1.40 mg/mL
CVS Tioconazole 1 (Equate™ tioconazole 1)	8.02 mg/mL
Equate™ Vagicaine Anti-Itch Cream	5.87 mg/mL
Estrace® Cream	4.38 mg/mL
K-Y® Ultra Gel	6.59 mg/mL
Metronidazole Vaginal Gel§	0.20 mg/mL*
Monistat® 3 Vaginal Antifungal Combination Pack	1.57 mg/mL
Monistat® Complete Care Itch Relief Cream	4.76 mg/mL
Gyne-Lotrimin® 7	3.13 mg/mL
Norforms® Suppositories	1.10 mg/mL
Premarin® Vaginal Cream	3.65 mg/mL
Replens™ Long-Lasting Vaginal Moisturizer§	0.96 mg/mL†
RepHresh™ Odor Eliminating Vaginal Gel§	‡
RepHresh™ Clean Balance™ Feminine Freshness Kit§	‡
Summer's Eve® Feminine Deodorant Spray	0.90 mg/mL
VCF® - Vaginal Contraceptive Foam	1.42 mg/mL
Yeast Gard Advanced®	3.04 mg/mL
ZOVIRAX® (acyclovir) Cream 5%	10.37 mg/mL
Glacial acetic acid	5% (v/v)

* Concentration of product that did not cause interference with test performance.
† Concentration of product that did not cause interference with test performance.
‡ Concentrations of product that did not interfere with test performance were not determined
§ Products containing carbomer(s) have been shown to cause interference

6. Competitive Inhibition

A competitive inhibition study was performed to test whether high concentrations of high risk or low risk HPV DNA could interfere with the genotyping capability of cobas HPV to detect HPV 16 and HPV18. Competitive inhibition of HPV16 and HPV18 detection was assessed by testing samples containing low concentrations of HPV16 and HPV18 along with high concentration of non-targeted low risk HPV and targeted 12 Other high risk HPV types. The HPV16 and HPV18 were spiked to concentrations close to about 1x LoD at the clinical cutoff; each of the 25 low risk and 12 Other high risk HPV tested were at a concentration 1000-fold (3log10) higher than that of HPV16 and HPV18.

Results of this study showed that cobas HPV can detect low concentrations of HPV16 and HPV18 in the presence of 1000-fold higher concentration of any of the 25 non-targeted low risk and 12 Other high risk HPV types. No competitive interference was observed from any of the competing targets.

7. Within-Laboratory Precision

Within-laboratory precision was performed using a panel composed of either HPV cell lines or HPV positive clinical samples diluted into a pool of negative cervical specimen matrix. The precision panel was designed to include members with high negative, very low (< LoD at the clinical cutoff), low (~LoD at the clinical cutoff) and moderate (~3x LoD at the clinical cutoff) concentrations of HPV as well as an HPV negative. Testing was performed with three lots of cobas HPV reagents, two instruments, and two users. There was an equal number of runs performed on the cobas 6800 and the cobas 8800 Systems over 12 days for a total of 24 runs for each panel member. The observed hit rates for each panel member are shown in Table 8. Table 9, Table 10, and Table 11 summarize the variance components analysis in positive panel members separated by 12 Other High Risk HPV, HPV16, and HPV18. The overall CV (%) ranged from 4.32% to 6.19% for 12 Other High Risk HPV, 1.09% to 4.61% for HPV16, and 1.23% to 3.76% for HPV18.

Table 8: Summary of within laboratory precision study

Panel Level	Expected Hit Rate	Target Source	HPV Concentration	Target Channel	N Tested	N Positive	Hit Rate	95% CI	
								LL	UL
Negative	0%	N/A	N/A	12 Other HR HPV	72	0	0%	0%	5%
Negative	0%	N/A		HPV16	72	0	0%	0%	5%
Negative	0%	N/A		HPV18	72	0	0%	0%	5%
High Negative	≤ 5%	Clinical sample	N/A	12 Other HR HPV	72	0	0%	0%	5%
High Negative	≤ 5%	Clinical sample		HPV16	72	0	0%	0%	5%
High Negative	≤ 5%	Clinical sample		HPV18	72	5	7%	3%	15%
< 1x LoD	< 95%	Clinical sample	N/A	12 Other HR HPV	72	30	42%	31%	53%
< 1x LoD	< 95%	Clinical sample	N/A	HPV16	71	33	47%	35%	58%
< 1x LoD	< 95%	Clinical sample	N/A	HPV18	72	49	68%	57%	78%

Panel Level	Expected Hit Rate	Target Source	HPV Concentration	Target Channel	N Tested	N Positive	Hit Rate	95% CI	
								LL	UL
< 1x LoD	20-80%	SiHa cell line	4.8 cells/mL	HPV16	72	44	61%	50%	72%
< 1x LoD	20-80%	HeLa cell line	4.8 cells/mL	HPV18	72	49	68%	57%	78%
~ 1x LoD	≥ 95%	Clinical sample	N/A	12 Other HR HPV	72	72	100%	95%	100%
~ 1x LoD	≥ 95%	SiHa cell line	16 cells/mL	HPV16	72	72	100%	95%	100%
~ 1x LoD	≥ 95%	HeLa cell line	16 cells/mL	HPV18	72	72	100%	95%	100%
> 1x LoD	≥ 99%	Clinical sample	N/A	12 Other HR HPV	72	72	100%	95%	100%
> 1x LoD	≥ 99%	SiHa cell line	48 cells/mL	HPV16	72	72	100%	95%	100%
> 1x LoD	≥ 99%	HeLa cell line	48 cells/mL	HPV18	72	72	100%	95%	100%

CI = Confidence interval, LL = Lower limit, UL = Upper limit

Table 9: Overall mean, standard deviations and coefficients of variation (%) for cycle threshold – 12 Other High Risk HPV

			Between-Day		Between-Instrument		Between-Operator		Between-Lot		Between-Run		Within-Run		Total	
Level	Hit Rate	Mean Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
< LoD	41.7%	33.2	0	0	0	0	0	0	0	0	0.47	1.43	1.72	5.18	1.78	5.37
~ LoD	100%	32.4	0	0	0	0	0.49	1.50	0.16	0.51	0	0	1.94	5.98	2.01	6.19
> LoD	100%	30.7	0	0	0	0	0	0	0.27	0.88	0	0	1.30	4.23	1.33	4.32

Table 10: Overall mean, standard deviations and coefficients of variation (%) for cycle threshold - HPV16

			Between-Day		Between-Instrument		Between-Operator		Between-Lot		Between-Run		Within-Run		Total	
Level	Hit Rate	Mean Ct	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
< LoD	46.5 %	35.7	0.84	2.34	0.29	0.80	0.85	2.39	0	0	0	0	1.10	3.07	1.65	4.61
< LoD	61.1 %	36.1	0.44	0.67	0	0	0.16	0.45	0.21	0.57	0	0	0.49	1.36	0.61	1.68
~ LoD	100 %	35.0	0	0	0.02	0.06	0.02	0.07	0.38	1.09	0	0	0.45	1.28	0.59	1.69
> LoD	100 %	34.0	0.03	0.09	0.04	0.12	0	0	0.27	0.78	0	0	0.25	0.74	0.37	1.09

Table 11: Overall mean, standard deviations and coefficients of variation (%) for cycle threshold - HPV18

			Between-Day		Between-Instrument		Between-Operator		Between-Lot		Between-Run		Within-Run		Total	
Level	**Hit Rate**	**Mean Ct**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
< LoD	68.1%	35.9	0	0	0.55	1.52	0	0	0.18	0.51	0.17	0.49	1.21	3.37	1.35	3.76
< LoD	68.1%	35.3	0.19	0.54	0	0	0.02	0.06	0	0	0	0	0.97	2.75	0.99	2.80
~ LoD	100%	33.8	0	0	0	0	0	0	0.37	1.11	0	0	0.73	2.17	0.82	2.44
> LoD	100%	32.2	0	0	0	0	0	0	0.22	0.68	0.03	0.10	0.33	1.02	0.39	1.23

8. Lot-to-lot variability

Lot-to-lot variability was evaluated at one testing site, using three reagent lots for each of the two systems separately (cobas 6800 and cobas 8800). This study was performed along with the reproducibility study at testing site 3. Each panel member was tested over 15 days (5 days per lot) with three replicates per run on each of the two cobas systems. Two operators performed one run per day for 5 days for each reagent lot. The lot-to-lot variability study design was identical for both the cobas 6800 and the cobas 8800 Systems. A 13-member panel composed of pools made from clinical samples collected into PreservCyt Solution and from samples derived from SiHa and HeLa cell lines was tested.

Table 12 and Table 13 show results by reagent lot, operator/run, and day on the cobas 6800 System for the negative and positive panel members, respectively. All negative panel members were correctly identified as negative across reagent lot, operator/run and testing day. Analysis of variance of the Ct values from valid tests performed on positive panel members yielded total CV (%) ranging from 0.9% to 5.0% across all panel members. The CV(%) ranged from 0.9% to 2.2% for the cell line panel members and 1.7% to 5.0% for the pooled clinical panel members.

Table 12: Agreement and variability of negative panel members by lot, operator/run, and day on the cobas 6800 System

			Number Negative / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV%	ID	Negative agreement (%)	Negative/ Valid	ID*	Negative agreement (%)	Negative/ Valid	ID	Negative agreement (%)	Negative/ Valid
Negative background cell line	n/a	n/a	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Negative pooled clinical samples	n/a	n/a	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

*Note: Operators 5 and 6 were at testing site 3.
Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation; n/a=not applicable.

Table 13: Agreement and variability of positive panel members by lot, operator/run, and day on the cobas 6800 System

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV %	ID	Positive Agreement (%)	Positive/ Valid	ID*	Positive Agreement (%)	Positive/ Valid	ID	Positive Agreement (%)	Positive/ Valid
Positive Cell Line Panel Members: HPV16/18 Weak Positive (0.3x LOD)											
HPV16 Weak Positive (0.3x LOD)	0.75	2.0	1	46.7	14/30	5	57.8	26/45	1	61.1	11/18
			2	46.7	14/30	6	53.3	24/45	2	44.4	8/18
			3	73.3	22/30				3	38.9	7/18
									4	77.8	14/18
									5	55.6	10/18
HPV18 Weak Positive (0.3x LOD)	0.77	2.2	1	60.0	18/30	5	62.2	28/45	1	66.7	12/18
			2	60.0	18/30	6	66.7	30/45	2	66.7	12/18
			3	73.3	22/30				3	66.7	12/18
									4	66.7	12/18
									5	55.6	10/18

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV %	ID	Positive Agreement (%)	Positive/ Valid	ID*	Positive Agreement (%)	Positive/ Valid	ID	Positive Agreement (%)	Positive/ Valid
Positive Cell Line Panel Members: HPV16/18 Low Positive (1x LOD)											
HPV16 Low Positive (1x LOD)	0.50	1.4	1	100.0	30/30	5	97.8	44/45	1	94.4	17/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	96.7	29/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
HPV18 Low Positive (1x LOD)	0.67	2.0	1	96.7	29/30	5	97.8	44/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	94.4	17/18
									5	100.0	18/18
Positive Cell Line Panel Members: HPV16/18 Positive (3x LOD)											
HPV16 Positive(3x LOD)	0.31	0.9	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
HPV18 Positive(3x LOD)	0.39	1.2	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Positive Clinical Panel Members											
Pooled HPV16 Low Positive (1x LOD)	1.13	3.4	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV16 Positive (3x LOD)	1.00	3.0	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV %	ID	Positive Agreement (%)	Positive/ Valid	ID*	Positive Agreement (%)	Positive/ Valid	ID	Positive Agreement (%)	Positive/ Valid
Pooled HPV18 Low Positive (1x LOD)	0.60	1.7	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV18 Positive (3x LOD)	0.86	2.5	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV45 Low Positive (1x LOD)	1.60	5.0	1	100.0	30/30	5	97.8	44/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	96.7	29/30				3	100.0	18/18
									4	94.4	17/18
									5	100.0	18/18
Pooled HPV45 Positive (3x LOD)	1.46	4.9	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV39 Low Positive (1x LOD)	0.75	2.3	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV39 Positive (3x LOD)	0.84	2.6	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

*Note: Operators 5 and 6 were at testig site 3.
Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation.

Table 14 and Table 15 show results for the negative and positive panel member by reagent lot, operator/run, and day on the cobas 8800 System respectively. All negative panel members were correctly identified as negative across reagent lot, operator/run and testing day. Analysis of variance of the Ct values from valid tests performed on positive panel members yielded total CV (%) ranging from 1.1% to 7.4% across all panel members. The CV (%) ranged from 1.1% to 3.0% for the cell line panel members and 2.0% to 7.4% for the pooled clinical panel members.

Table 14: Agreement and variability of negative panel members by lot, operator/run, and day on the cobas 8800 System

			Number Negative / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	**Ct SD**	**Ct CV%**	**ID**	**Negative Agreement (%)**	**Negative/ Valid**	**ID***	**Negative Agreement (%)**	**Negative/ Valid**	**ID**	**Negative Agreement (%)**	**Negative/ Valid**
Negative background cell line	n/a	n/a	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Negative pooled clinical samples	n/a	n/a	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

*Note: Operators 5 and 6 were at testig site 3.
Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation; n/a=not applicable.

Table 15: Agreement and variability of positive panel members by lot, operator/run, and day on the cobas 8800 System

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	**Ct SD**	**Ct CV %**	**ID**	**Positive Agreement (%)**	**Positive / Valid**	**ID[1]**	**Positive Agreement (%)**	**Positive/ Valid**	**ID**	**Positive Agreement (%)**	**Positive / Valid**
Positive Cell Line Panel Members: HPV16/18 Weak Positive (0.3x LOD)											
HPV16 Weak Positive (0.3x LOD)	0.67	1.8	1	60.0	18/30	5	57.8	26/45	1	66.7	12/18
			2	63.3	19/30	6	68.9	31/45	2	61.1	11/18
			3	66.7	20/30				3	72.2	13/18
									4	66.7	12/18
									5	50.0	9/18
	1.07	3.0	1	70.0	21/30	5	73.3	33/45	1	77.8	14/18

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	**Ct SD**	**Ct CV %**	**ID**	**Positive Agreement (%)**	**Positive / Valid**	**ID[1]**	**Positive Agreement (%)**	**Positive/ Valid**	**ID**	**Positive Agreement (%)**	**Positive / Valid**
HPV18 Weak Positive (0.3x LOD)			2	70.0	21/30	6	64.4	29/45	2	72.2	13/18
			3	66.7	20/30				3	72.2	13/18
									4	72.2	13/18
									5	50.0	9/18
Positive Cell Line Panel Members: HPV16/18 Low Positive (1x LOD)											
HPV16 Low Positive (1x LOD)	0.44	1.2	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	96.7	29/30	6	95.6	43/45	2	100.0	18/18
			3	96.7	29/30				3	94.4	17/18
									4	100.0	18/18
									5	94.4	17/18
HPV18 Low Positive (1x LOD)	0.74	2.2	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Positive Cell Line Panel Members: HPV16/18 Positive (3x LOD)[2]											
HPV16 Positive (x LOD)	0.38	1.1	1	100.0	29/29	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	44/44	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	17/17
									5	100.0	18/18
HPV18 Positive (3x LOD)	0.41	1.2	1	100.0	29/29	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	44/44	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	17/17
									5	100.0	18/18
Positive Clinical Panel Members											
Pooled HPV16 Low Positive (1x LOD)	0.91	2.7	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
	0.88	2.7	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	**Ct SD**	**Ct CV %**	**ID**	**Positive Agreement (%)**	**Positive / Valid**	**ID[1]**	**Positive Agreement (%)**	**Positive/ Valid**	**ID**	**Positive Agreement (%)**	**Positive / Valid**
Pooled HPV16 Positive (3x LOD)			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV18 Low Positive (1x LOD)	0.70	2.0	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV18 Positive (3x LOD)	1.02	3.0	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV45 Low Positive (1x LOD)	2.32	7.4	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV45 Positive (3x LOD)	1.74	5.9	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18
Pooled HPV39 Low Positive (1x LOD)	1.06	3.2	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

			Number of Positives / Total Number Valid Results								
			Between-Lot			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV %	ID	Positive Agreement (%)	Positive / Valid	ID[1]	Positive Agreement (%)	Positive/ Valid	ID	Positive Agreement (%)	Positive / Valid
Pooled HPV39 Positive (3x LOD)	1.52	4.8	1	100.0	30/30	5	100.0	45/45	1	100.0	18/18
			2	100.0	30/30	6	100.0	45/45	2	100.0	18/18
			3	100.0	30/30				3	100.0	18/18
									4	100.0	18/18
									5	100.0	18/18

[1]Note: Operators 5 and 6 were at testing site 3.
[2]One replicate failed due to processing error and excluded from analysis.
Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation.

Analyses were performed for between lot, between operator, between day, and within run variability for both the cobas 6800 and 8800 Systems separately as shown in Table 16 and Table 17, respectively.

Table 16: Overall mean, standard deviation, and coefficients of variation (%) for cycle threshold, estimated from positive panel members on cobas 6800 System

			Standard Deviation, Coefficient of Variation (%)				
Panel Member	N	Mean Ct	Between-Lot	Between-Operator	Between - Day	Within - Run	Total CV
Positive Cell Line Panel Members							
HPV16/18 Weak Positive (0.3x LOD)							
HPV16 Weak Positive (0.3x LOD)	52	36.5	0.07, (0.20%)	0.00, (0.00%)	0.28, (0.78%)	0.69, (1.88%)	2.0
HPV18 Weak Positive (0.3x LOD)	58	35.4	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.77, (2.19%)	2.2
HPV16/18 Low Positive (1x LOD)							
HPV16 Low Positive (1x LOD)	89	35.6	0.09, (0.25%)	0.04, (0.12%)	0.00, (0.00%)	0.49, (1.37%)	1.4
HPV18 Low Positive (1x LOD)	89	34.1	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.67, (1.97%)	2.0
HPV 16/18 Positive (3x LOD)							
HPV16 Positive (3x LOD)	90	34.6	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.31, (0.88%)	0.9
HPV18 Positive (3x LOD)	90	32.9	0.00, (0.00%)	0.00, (0.00%)	0.13, (0.41%)	0.36, (1.10%)	1.2
Positive Clinical Panel Members							
Pooled HPV16 Low Positive (1x LOD)	90	33.5	0.11, (0.31%)	0.00, (0.00%)	0.00, (0.00%)	1.12, (3.35%)	3.4

Panel Member	N	Mean Ct	Standard Deviation, Coefficient of Variation (%)				
			Between-Lot	Between-Operator	Between - Day	Within - Run	Total CV
Pooled HPV16 Positive (3x LOD)	90	33.1	0.11, (0.32%)	0.00, (0.00%)	0.00, (0.00%)	1.00, (3.01%)	3.0
Pooled HPV18 Low Positive (1x LOD)	90	35.1	0.14, (0.41%)	0.00, (0.00%)	0.00, (0.00%)	0.58, (1.67%)	1.7
Pooled HPV18 Positive (3x LOD)	90	33.7	0.00, (0.00%)	0.26, (0.76%)	0.14, (0.43%)	0.81, (2.39%)	2.5
Pooled HPV45 Low Positive (1x LOD)	90	32.0	0.00, (0.00%)	0.00, (0.00%)	0.42, (1.31%)	1.55, (4.84%)	5.0
Pooled HPV45 Positive (3x LOD)	90	29.7	0.18, (0.62%)	0.00, (0.00%)	0.00, (0.00%)	1.45, (4.89%)	4.9
Pooled HPV39 Low Positive (1x LOD)	90	33.3	0.00, (0.00%)	0.00, (0.00%)	0.23, (0.69%)	0.71, (2.14%)	2.3
Pooled HPV39 Positive (3x LOD)	90	31.6	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.84, (2.65%)	2.6

Table 17: Overall mean, standard deviation, and coefficients of variation (%) for cycle threshold, estimated from positive panel members on cobas 8800 System

Panel Member	N	Mean Ct	Standard Deviation, Coefficient of Variation (%)				
			Between-Lot	Between-Operator	Between-Day	Within - Run	Total CV
Positive Cell Line Panel Members							
HPV16/18 Weak Positive (0.3x LOD)							
HPV 16 Weak Positive (0.3 x LOD)	58	36.6	0.00, (0.00%)	0.16, (0.45%)	0.16, (0.44%)	0.63, (1.72%)	1.8
HPV 18 Weak Positive (0.3 x LOD)	63	35.5	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	1.07, (3.01%)	3.0
HPV16/18 Low Positive (1x LOD)							
HPV 16 Low Positive (1 x LOD)	88	35.6	0.00, (0.00%)	0.00, (0.00%)	0.14, (0.39%)	0.42, (1.18%)	1.2
HPV 18 Low Positive (1 x LOD)	90	34.2	0.00, (0.00%)	0.16, (0.46%)	0.30, (0.86%)	0.66, (1.94%)	2.2
HPV16/18 Positive (3x LOD)							
HPV 16 Positive (3 x LOD)	89	34.6	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.38, (1.10%)	1.1
HPV 18 Positive (3 x LOD)	89	32.7	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.41, (1.24%)	1.2
Positive Clinical Panel Members							
Pooled HPV16 Low Positive (1x LOD)	90	33.6	0.07, (0.21%)	0.00, (0.00%)	0.00, (0.00%)	0.91, (2.71%)	2.7

			Standard Deviation, Coefficient of Variation (%)				
Panel Member	**N**	**Mean Ct**	**Between-Lot**	**Between-Operator**	**Between-Day**	**Within - Run**	**Total CV**
Pooled HPV16 Positive (3x LOD)	90	32.9	0.00, (0.00%)	0.00, (0.00%)	0.13, (0.39%)	0.87, (2.64%)	2.7
Pooled HPV18 Low Positive (1x LOD)	90	35.0	0.00, (0.00%)	0.05, (0.15%)	0.16, (0.47%)	0.68, (1.94%)	2.0
Pooled HPV18 Positive (3x LOD)	90	33.6	0.24, (0.70%)	0.25, (0.75%)	0.18, (0.54%)	0.94, (2.80%)	3.0
Pooled HPV45 Low Positive (1x LOD)	90	31.2	0.40, (1.27%)	0.00, (0.00%)	0.74, (2.37%)	2.16, (6.93%)	7.4
Pooled HPV45 Positive (3x LOD)	90	29.5	0.00, (0.00%)	0.41, (1.40%)	0.59, (2.00%)	1.59, (5.39%)	5.9
Pooled HPV39 Low Positive (1x LOD)	90	33.1	0.00, (0.00%)	0.00, (0.00%)	0.30, (0.92%)	1.02, (3.07%)	3.2
Pooled HPV39 Positive (3x LOD)	90	31.4	0.00, (0.00%)	0.00, (0.00%)	0.59, (1.88%)	1.40, (4.46%)	4.8

9. Equivalence between cobas 6800 and 8800 Systems

The lot-to-lot variability study data was also analyzed to compare the results obtained between the cobas 6800 cobas 8800 Systems to assess systems equivalency. The two systems generated comparable hit rates as shown below in Table 18.

Table18: Comparison of hit rates between cobas 6800 and cobas 8800 Systems

	cobas 6800 System			**cobas 8800 System**		
Panel Member	**No. of Valid Tests**	**No. of Correct Results N**	**Percent of Correct Results % (95% CI)**	**No. of Valid Tests**	**No. of Correct Results N**	**Percent of Correct Results % (95% CI)**
Negative background cell line	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
HPV 16/18 Weak Positive (0.3 x LoD)						
HPV 16 Weak Positive (0.3x LoD)	90	50	55.6 (44.7, 66.0)	90	57	63.3 (52.5, 73.2)
HPV 18 Weak Positive (0.3x LoD)	90	58	64.4 (53.7, 74.3)	90	62	68.9 (58.3, 78.2)
HPV 16/18 Low Positive (1 x LOD)						
HPV 16 Low Positive (1x LoD)	90	89	98.9 (94.0, 100.0)	90	88	97.8 (92.2, 99.7)
HPV 18 Low Positive (1x LoD)	90	89	98.9 (94.0, 100.0)	90	90	100.0 (96.0, 100.0)
HPV 16/18 Positive (3 x LoD)						
HPV 16 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	89	89	100.0 (95.9, 100.0)

	cobas 6800 System			cobas 8800 System		
Panel Member	**No. of Valid Tests**	**No. of Correct Results N**	**Percent of Correct Results % (95% CI)**	**No. of Valid Tests**	**No. of Correct Results N**	**Percent of Correct Results % (95% CI)**
HPV 18 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	89	89	100.0 (95.9, 100.0)
Negative pooled clinical samples	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 16 Low positive (1x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 16 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 18 Low Positive (1x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 18 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 45 Low Positive (1x LoD)	90	89	98.9 (94.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 45 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 39 Low Positive (1x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)
Pooled HPV 39 Positive (3x LoD)	90	90	100.0 (96.0, 100.0)	90	90	100.0 (96.0, 100.0)

10. Reproducibility

A site-to-site reproducibility study was performed using the same panel as described in the lot-to-lot variability study. Testing was conducted at three testing sites using one reagent lot and four cobas systems (three cobas 6800 Systems at all three testing sites and one cobas 8800 System at one of those three sites). Each panel member was tested over five days with three replicates per run on the four systems. Two operators performed one run per day for five days for each system. Overall, 41 runs were performed, with 30 runs on the cobas 6800 and 11 on the cobas 8800 (with 1 failed run).

Testing of the negative panel members by site/instrument, operator/run, and day on the three cobas 6800 Systems and one cobas 8800 System are summarized in Table 19. All negative panel members were correctly identified as negative across site/instrument, operator/run and testing day. The study demonstrated that the cobas HPV for use on the cobas 6800/8800 Systems produced results that were reproducible across reagent lots, sites/systems, operators, days, and within- and between-runs.

Table 19: Agreement and variability for negative panel member for site/instrument, operator/run, and day on the cobas 6800/8800 System

			Number of Negatives/Total Number of Valid Results								
			Between-Site/Instrument			Between-Operator			Between-Day		
Panel Member	Ct SD	Ct CV%	ID	Negative Agreement (%)	Negative/ Valid	ID[1]	Negative Agreement (%)	Negative/ Valid	ID	Negative Agreement (%)	Negative/ Valid
Negative background cell line	n/a	n/a	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Negative pooled clinical samples	n/a	n/a	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			

Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation; n/a=not applicable.
[1]Operators 1 and 2 were at testing site 1; Operators 3 and 4 were ar testing site 2; Operators 5 and 6 were at testing site 3. Operators 5 and 6 from site 3 tan both the cobas 6800 and cobas 8800 Systems.

Percent of positive results for the positive panel members are presented in Table 20. Analysis of variance of the Ct values from tests performed on the positive panel members yielded total CV(%) ranging from 1.1% to 5.6% across all panel members. The CV(%) ranged from 1.1% to 2.7% for the cell line panel members and 2.1% to 5.6% for the pooled clinical panel members. The overall mean, standard deviation, and coefficient of variation were also calculated for positive panel members as shown in Table 21.

Table 20: Agreement and variability for positive panel members for site/instrument, operator/run, and day on the cobas 6800/8800 Systems

			Number of Positive Results / Total Number of Valid Results								
			Between-Site/Instrument			Betweeb-Operator/Run			Between-Day		
Panel Member	Ct SD	Ct CV%	ID	Positive Agreement (%)	Positive/ Valid	ID[1]	Positive Agreement (%)	Positive/ Valid	ID	Positive Agreement (%)	Positive/ Valid
Positive Cell Line Panel Members HPV16/18 Weak Positive (0.3 x LoD)											
HPV16 Weak Positive (0.3x LoD)	0.76	2.1	11	66.7	20/30	1	60.0	9/15	1	58.3	14/24
			21	76.7	23/30	2	73.3	11/15	2	54.2	13/24
			31	46.7	14/30	3	93.3	14/15	3	62.5	15/24
			32	60.0	18/30	4	60.0	9/15	4	83.3	20/24
						5	53.3	16/30	5	54.2	13/24
						6	53.3	16/30			

			Number of Positive Results / Total Number of Valid Results								
			Between-Site/Instrument			Betweeb-Operator/Run			Between-Day		
Panel Member	**Ct SD**	**Ct CV%**	**ID**	**Positive Agreement (%)**	**Positive/ Valid**	**ID[1]**	**Positive Agreement (%)**	**Positive/ Valid**	**ID**	**Positive Agreement (%)**	**Positive/ Valid**
HPV18 Weak Positive (0.3x LoD)	0.96	2.7	11	53.3	16/30	1	40.0	6/15	1	70.8	17/24
			21	60.0	18/30	2	66.7	10/15	2	66.7	16/24
			31	60.0	18/30	3	60.0	9/15	3	45.8	11/24
			32	70.0	21/30	4	60.0	9/15	4	70.8	17/24
						5	73.3	22/30	5	50.0	12/24
						6	56.7	17/30			
Positive Cell Line Panel Members HPV16/18 Low Positive (1 x LoD)											
HPV16 Low Positive (1x LoD)	0.47	1.3	11	96.7	29/30	1	100.0	15/15	1	95.8	23/24
			21	96.7	29/30	2	93.3	14/15	2	100.0	24/24
			31	100.0	30/30	3	93.3	14/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	95.8	23/24
						6	100.0	30/30			
HPV18 Low Positive (1x LoD)	0.63	1.9	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	96.7	29/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	95.8	23/24
						5	96.7	29/30	5	100.0	24/24
						6	100.0	30/30			
Positive Cell Line Panel Members HPV16/18 Positive (3 x LoD)[2]											
HPV16 Positive (3x LoD)	0.37	1.1	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	29/29	4	100.0	15/15	4	100.0	23/23
						5	100.0	30/30	5	100.0	24/24
						6	100.0	29/29			
HPV18 Positive (3x LoD)	0.40	1.2	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	29/29	4	100.0	15/15	4	100.0	23/23
						5	100.0	30/30	5	100.0	24/24
						6	100.0	29/29			
Positive Clinical Panel Members											
Pooled HPV16 Low Positive (1x LoD)	1.07	3.2	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV16 Positive (3x LoD)	0.89	2.7	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV18 Low Positive (1x LoD)	0.74	2.1	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	96.7	29/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24

			Number of Positive Results / Total Number of Valid Results								
			Between-Site/Instrument			Betweeb-Operator/Run			Between-Day		
Panel Member	**Ct SD**	**Ct CV%**	**ID**	**Positive Agreement (%)**	**Positive/ Valid**	**ID[1]**	**Positive Agreement (%)**	**Positive/ Valid**	**ID**	**Positive Agreement (%)**	**Positive/ Valid**
			32	100.0	30/30	4	93.3	14/15	4	95.8	23/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV18 Positive (3x LoD)	0.92	2.7	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV45 Low Positive (1x LoD)	1.80	5.6	11	96.7	29/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	93.3	14/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	95.8	23/24
						6	100.0	30/30			
Pooled HPV45 Positive (3x LoD)	1.54	5.2	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV39 Low Positive (!x LoD)	1.04	3.1	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			
Pooled HPV39 Positive (3x LoD)	1.45	4.6	11	100.0	30/30	1	100.0	15/15	1	100.0	24/24
			21	100.0	30/30	2	100.0	15/15	2	100.0	24/24
			31	100.0	30/30	3	100.0	15/15	3	100.0	24/24
			32	100.0	30/30	4	100.0	15/15	4	100.0	24/24
						5	100.0	30/30	5	100.0	24/24
						6	100.0	30/30			

Notes: Ct=Cycle Threshold; SD=Standard Deviation; CV=Coefficient of Variation.
[1]Operators 1 and 2 were at testing site 1; Operators 3 and 4 were at testing site 2; Operators 5 and 6 were at testing site 3.
[2]One replicate failed due to processing error and excluded from analysis.

Table 21: Overall mean, standard deviation, and coefficients of variation (%) for cycle threshold, estimated from positive panel members

			Standard Deviation, Coefficient of Variation (%)				
Panel Member	**N**	**Mean Ct**	**Between-Site/Instru-ment**	**Between-Operator/ Run**	**Between-Day**	**Within-Run**	**Total CV**
Positive Cell Line Panel Members							
HPV16/18 Weak Positive (0.3x LoD)							
HPV16 Weak Positive (0.3x LoD)	77	36.6	0.00, (0.00%)	0.00, (0.00%)	0.00, (0.00%)	0.76, (2.08%)	2.1
HPV18 Weak Positive (0.3x LoD)	74	35.3	0.00, (0.00%)	0.00, (0.00%)	0.12, (0.34%)	0.95, (2.69%)	2.7
HPV16/18 Low Positive (1x LoD)							
HPV16 Low Positive (1x LoD)	118	35.6	0.10, (0.27%)	0.00, (0.00%)	0.15, (0.43%)	0.43, (1.22%)	1.3
HPV18 Low Positive (1x LoD)	119	34.1	0.00, (0.00%)	0.09, (0.28%)	0.00, (0.00%)	0.63, (1.83%)	1.9
HPV16/18 Positive (3x LoD)							
HPV16 Positive (3x LoD)	119	34.7	0.05, (0.16%)	0.00, (0.00%)	0.11, (0.31%)	0.35, (1.01%)	1.1
HPV18 Positive (3x LoD)	119	32.9	0.05, (0.16%)	0.08, (0.25%)	0.00, (0.00%)	0.39, (1.19%)	1.2
Positive Clinical Panel Members							
Pooled HPV16 Low Positive (1x LoD)	120	33.6	0.25, (0.73%)	0.00, (0.00%)	0.00, (0.00%)	1.05, (3.11%)	3.2
Pooled HPV16 Positive (3x LoD)	120	33.1	0.30, (0.90%)	0.00, (0.00%)	0.00, (0.00%)	0.84, (2.53%)	2.7
Pooled HPV18 Low Positive (1x LoD)	119	35.1	0.00, (0.00%)	0.00, (0.00%)	0.11, (0.31%)	0.74, (2.09%)	2.1
Pooled HPV18 Positive (3x LoD)	120	34.0	0.56, (1.64%)	0.00, (0.00%)	0.21, (0.62%)	0.70, (2.06%)	2.7
Pooled HPV45 Low Positive (1x LoD)	120	31.9	0.56, (1.74%)	0.00, (0.00%)	0.00, (0.00%)	1.71, (5.37%)	5.6
Pooled HPV45 Positive (3x LoD)	120	29.7	0.00, (0.00%)	0.00, (0.00%)	0.60, (2.04%)	1.42, (4.79%)	5.2
Pooled HPV39 Low Positive (1x LoD)	120	33.4	0.20, (0.61%)	0.00, (0.00%)	0.33, (0.98%)	0.97, (2.90%)	3.1
Pooled HPV39 Positive (3x LoD)	120	31.5	0.00, (0.00%)	0.00, (0.00%)	0.62, (1.95%)	1.31, (4.15%)	4.6

11. Cross Contamination

A study was performed to evaluate the risk of producing a false positive result in either the same run (within run carry-over) or in a subsequent run (between run carry-over) on the cobas 6800/8800 Systems. Three runs were performed using a checkerboard pattern of HPV positive and negative samples, followed by a full run of negative samples. Each run was arranged in an alternating checkerboard pattern, with HPV negative samples (consisting of an HPV negative cell line (HCT-15) at 10,000 cells/mL) placed in an alternating pattern with samples consisting of an HPV 16 positive cell line spiked at a concentration targeting a Ct value of ≤ 20. This Ct value represents a signal that is stronger than 95% of the

positive results in the intended use population based on the clinical study. Both the sample to sample and run to run cross-contamination rates were 0% (0/288, 95% CI: 0.00, 1.27 and 0/187, 95% CI: 0.00, 1.95, respectively).

12. Reagent Stability

Expiration dating for the cobas HPV reagents has been established and approved at 18 months for the cobas HPV and cobas HPV Positive Control Kit and at 24 months for the cobas Buffer Negative Control Kit when stored at 2-8°C. The shelf lives of the cobas HPV reagents were established in a real-time stability study.

13. Specimen Stability

Specimen stability studies demonstrated that for the cobas HPV, cervical specimens can be stored in PreservCyt Solution at 2-30 °C for up to 3 months from the date of collection. The observed changes in Ct value between baseline and the different storage conditions did not change any reported results.

B. Animal Studies

Not Applicable

C. Additional Studies

Not Applicable

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed a clinical study to establish a reasonable assurance of safety and effectiveness of the cobas HPV for use on the 6800/8800 Systems in detecting high-risk HPV nucleic acid during routine cervical cancer screening in the US. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

A. Study Design

Subjects were enrolled between September 2017 to October 2018. The database for this PMA reflected specimen data collected through October 2018 and included 35,263 women. There were 32 clinical investigational sites in the United States.

A multicenter, prospective study (IMPACT study, IMproved Primary screening And Colposcopy Triage) was conducted to evaluate the performance of the cobas HPV on cobas 6800/8800 Systems (hereafter referred as cobas HPV) as a triage test to stratify women with ASC-US Pap cytology results for colposcopy, as an adjunctive test to cervical cytology to guide management decisions in women with NILM Pap cytology, and as a first-line primary test for cervical cancer screening.

In total, 35,263 women were enrolled from September 2017 to October 2018 at 32 clinical sites in the United States. Following written informed consent, demographic information and gynecologic histories were obtained. Approximately half of the women had one cervical sample collected using a brush/spatula while the other half had one cervical sample collected using the broom-type device. Cervical samples were collected for HPV testing and ThinPrep Pap Test liquid based cytology (LBC). Specimens were tested with two HPV devices: an FDA-approved HPV test and the cobas HPV for use on the 6800/8800 systems. Both tests were performed according to manufacturer's instructions. HPV testing was performed on pre-aliquoted samples in secondary vials prior to cytology processing at four testing laboratories. LBC testing was conducted at the same four laboratories. Cytology samples were classified according to the criteria of the 2001 Bethesda System. Results from pap cytology, the FDA-approved HPV test, and the cobas HPV were used to inform referral to colposcopy as per the study protocol.

To determine the clinical study endpoint, a subset of non pregnant women identified at the enrollment visit was selected to undergo colposcopy, where a biopsy/endocervical curettage (ECC) was collected. The subset included women aged 25-65 years with ≥ ASC-US cytology and women 25-65 years with positive HPV Test results by the FDA-approved HPV Test and/or cobas HPV. In addition, 59 women with unsatisfactory Pap cytology and HPV-negative results by both the FDA-approved and cobas HPV tests, and a randomly selected subset of subjects with NILM cytology and HPV-negative results by both the FDA-approved and cobas HPV tests (approximately 1:50) were referred to colposcopy. In order to avoid bias, study participants and colposcopists were blinded to all HPV test and cytology results until after the colposcopy procedure was completed.

Colposcopy was conducted according to a standardized protocol following the principles recommended by the American Society for Colposcopy and Cervical Pathology (ASCCP), which is as follows: biopsies were obtained on all visible lesions; ECC was performed in all patients in whom the squamocolumnar junction was not visualized, and a single random cervical biopsy was obtained if no lesions were visible. All biopsies were examined by a Central Pathology Review (CPR) panel consisting of three expert pathologists, and discordant results adjudicated according to a pre-defined protocol. The slides that were prepared from the biopsies were stained using conventional hematoxylin and eosin (H&E) staining and H&E with p16 IHC assay (CINtec Histology, Ventana Medical Systems, Inc.). Clinical performance of the cobas HPV is presented using interpretation of H&E-stained slides with adjunctive use of p16-stained slides in accordance with the 2012 Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) excluding ASC-US/HPV16+ as a LAST criterion (CPRH&E+p16 per LAST) at the clinical endpoints ≥CIN2 and ≥CIN3.

Subjects were recruited through general obstetrics and gynecology (OB/GYN) practices, and/or other healthcare facilities that routinely performed cervical cancer

screening, where either the same facility or an affiliated facility frequently performed colposcopy and cervical biopsy (hereafter referred to as "collection sites").

1. Clinical Inclusion and Exclusion Criteria

   Enrollment in the IMPACT study was limited to patients who met the following inclusion criteria:

   - Female 25-65 years of age presenting for routine cervical cancer screening
   - Intact cervix
   - Willing and able to undergo colposcopy and biopsy (and possibly ECC) within 12 weeks from the date of the cervical sample collection
   - Willing and able to provide written informed consent
   - Willing and able to participate in the 1-year Follow-Up Phase, should it be required

   Patients were not permitted to enroll in the IMPACT study if they met any of the following exclusion criteria:

   - Known pregnancy at enrollment
   - Current or planned participation in another cervical cancer screening study or in a cervical treatment or vaccine study
   - Incomplete informed consent
   - Any medical condition that, in the opinion of the Investigator, would have resulted in increased risk of bleeding at biopsy
   - Known history of ablative or excisional therapy (e.g., loop electrosurgical excision procedure (LEEP), cone biopsy) within the past 12 months
   - Known history of hysterectomy (including supracervical)

2. Clinical Endpoints

   With regard to safety, as an *in vitro* diagnostic test, the cobas HPV is performed on cervical cells collected during routine pelvic exam (i.e. cervical cytology) using an endocervical brush/spatula or broom. The test, therefore does not present any more safety hazard to an individual being tested than other tests where cervical cells are sampled in this manner (i.e., cervical cytology).

   With regard to effectiveness, the following clinical endpoints were used:

   For ASC-US Triage (25-65 years): The clinical performance of the cobas HPV was evaluated against CPR determined histologic diagnosis, with ≥CIN2 and ≥CIN3 as the disease endpoints.

   For Adjunctive screening (NILM 30-65 years): The clinical performance of the cobas HPV was evaluated against CPR determined histologic diagnosis, with ≥CIN2 and ≥CIN3 as the disease endpoints.

   For HPV Primary screening (25-65 years): The clinical performance of the cobas HPV was evaluated against CPR determined histologic diagnosis, with ≥CIN2 and ≥CIN3 as the disease endpoints. Risks for ≥CIN2 and ≥CIN3 were evaluated for

HPV genotypes based on the cobas HPV result, as well as different HPV genotype and cytology combinations.

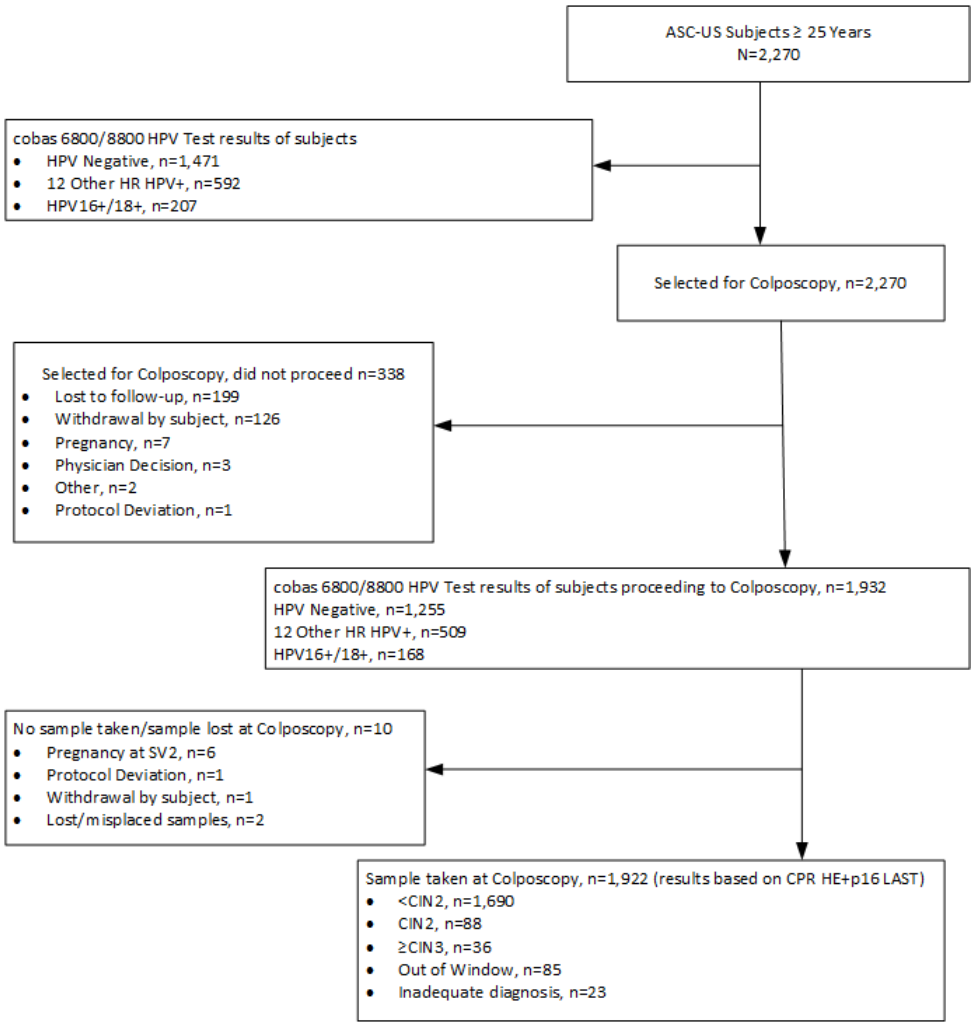
B. Accountability of PMA Cohort

Of 35,263 subjects enrolled in this study, 98.7% (34,807) of the subjects had HPV test results that were available for analysis at the completion of the study. Age and cytology results were used to group subjects into the following intended use populations:

ASC-US Population (25-65 years)

Eligible women who had an ASC-US Pap cytology result and valid cobas HPV results were considered evaluable for the analyses of the ASC-US triage. Of the 34,807 evaluable women, 2,270 had ASC-US Pap cytology (6.5%) and valid cobas 6800/8800 HPV test results. Of these, 1,932 (85.1%) proceeded to colposcopy, with a total of 1,814 subjects having valid biopsy results. The flow of ASC-US subjects (25-65 years) is shown in the Figure 2 below.

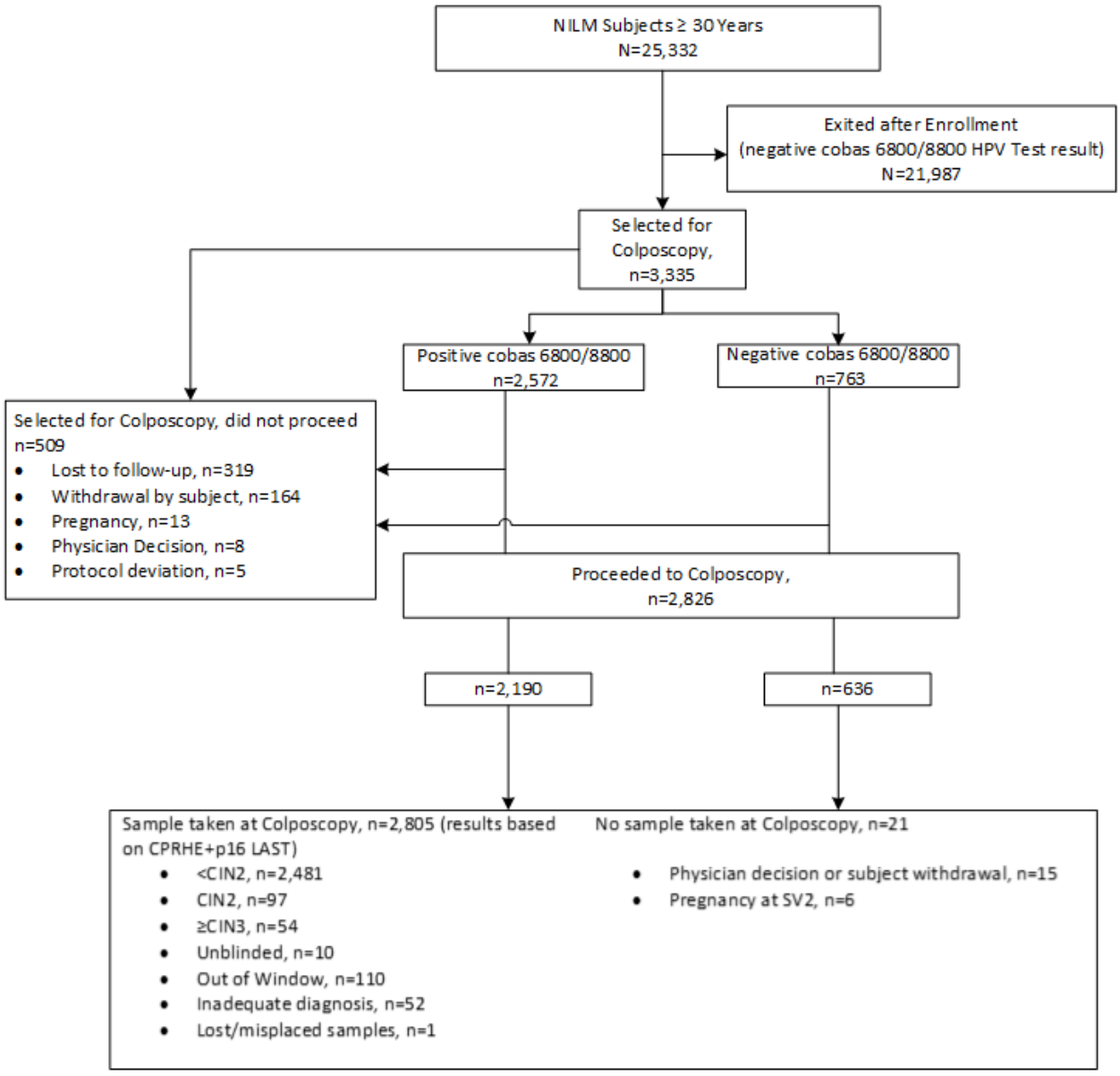
Figure 2: Subject disposition: Evaluated ASC-US population 25-65 years



Adjunct Population (NILM 30-65 years)

Women 30-65 years old with NILM Pap cytology results were evaluated for Adjunctive screening. Of the 34,807 evaluable women, 25,322 women had NILM Pap cytology and were 30-65 years of age. Among these, a total of 3,335 (13.17%) women were referred to colposcopy according to the study protocol, and 21,987 (86.82%) women were exited after enrollment. Of the 3,335 women referred to colposcopy, a total of 2,826 proceeded to colposcopy and of these, 2,632 subjects had valid biopsy results. The flow of NILM subjects (≥30 years) is shown in Figure 3 below.

Figure 3: Subject disposition: Evaluated Adjunct Population (NILM 30-65 years)

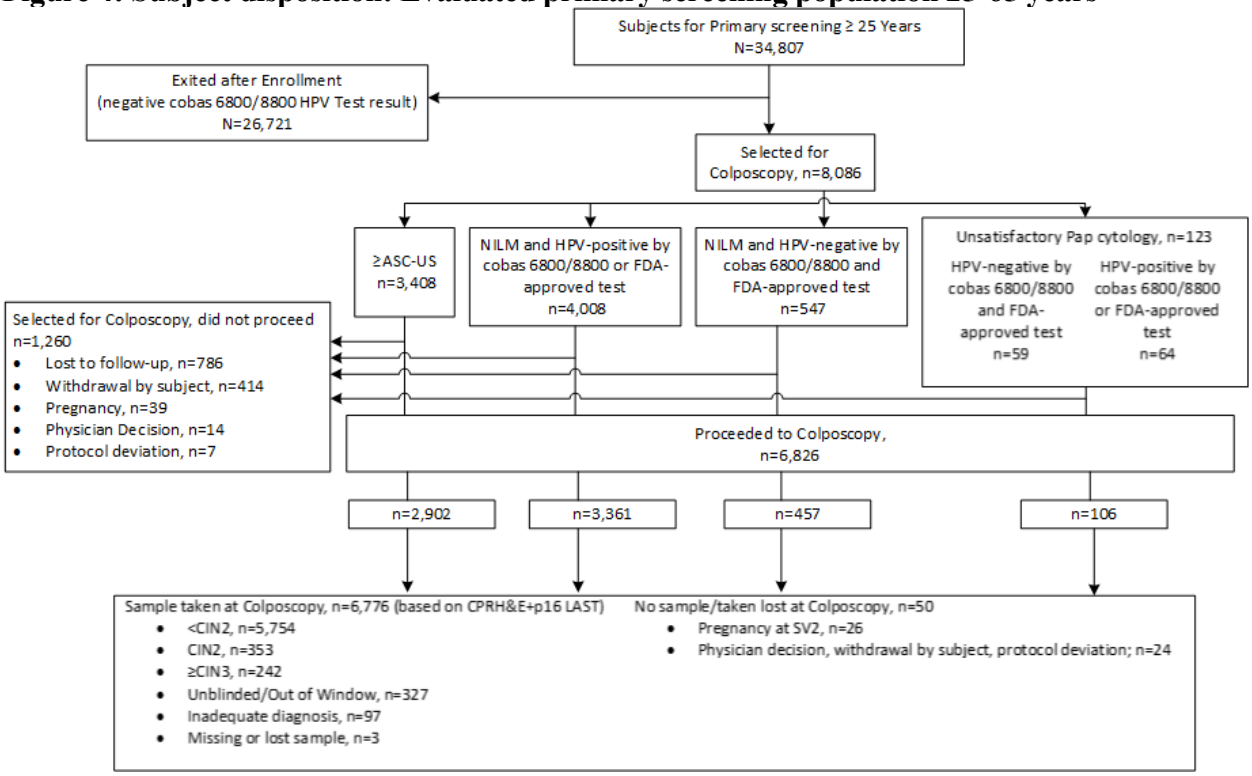


Primary Screening Population (25-65 years)

Of the 34,807 evaluable subjects, a total of 8,086 subjects were referred to colposcopy according to the study protocol, which included 3,408 subjects with abnormal Pap cytology results (≥ASC-US), 4,008 subjects with positive HPV results by either the cobas or FDA-approved HPV Test and NILM Pap cytology, 64 subjects with positive HPV results by either test and unsatisfactory Pap cytology, and 547 randomly selected subjects with NILM Pap cytology and negative HPV results by either test. An additional 59 subjects with unsatisfactory Pap cytology results and negative HPV results by either test were also selected sequentially.

Of 8,086 subjects referred to colposcopy according to the study protocol, 6,826 proceeded to colposcopy and of these, 6,776 subjects completed the colposcopy procedure. Of these, biopsy samples for three subjects were lost/misplaced during the transport. A total of 353 subjects had CPR diagnoses of CIN2 and 242 had CPR diagnoses of ≥CIN3. The flow of the primary screening subjects through the study is shown in Figure 4 below.

Figure 4: Subject disposition: Evaluated primary screening population 25-65 years



C. Study Population Demographics and Baseline Parameters

The demographics of the study population are shown in Table 24. The demographics of the study population are typical for a study of HPV molecular test.

Table 24: Demographics by intended use population

Characteristics	Primary Screening Population N = 34,807	NILM Population N = 25,322	ASC-US Population N = 2,270
Age (Years) at Enrollment			
Mean	40.5	43.7	39.3
Standard deviation	10.9	9.7	10.6
Median	39.0	42.0	37.0
(Min, Max)	(25, 65)	(30, 65)	(25, 65)

Characteristics	Primary Screening Population N = 34,807	NILM Population N = 25,322	ASC-US Population N = 2,270
Age Group (Years): n (%)			
25-29	6,530 (18.8)	10,482 (41.4)	512 (22.6)
30-39	11,826 (34.0)	7,397 (29.2)	754 (33.2)
40-49	8,271 (23.8)	7,443 (29.4)	538 (23.7)
50-65	8,180 (23.5)		466 (20.5)
Race: n (%)			
American Indian / Alaskan Native	118 (0.3)	84 (0.3)	13 (0.6)
Asian	703 (2.0)	499 (2.0)	33 (1.5)
Black / African American	7,124 (20.5)	4,993 (19.7)	529 (23.3)
Native Hawaiian / Pacific Islander	109 (0.3)	68 (0.3)	6 (0.3)
White	25,353 (72.8)	18,738 (74.0)	1,582 (69.7)
Other	905 (2.6)	627 (2.5)	75 (3.3)
Unknown/Not Reported	495 (1.4)	313 (1.2)	32 (1.4)
Ethnicity: n (%)			
Hispanic or Latino	8,165 (23.5)	5,819 (23.0)	655 (28.9)
Not Hispanic or Latino	26,260 (75.4)	19,260 (76.1)	1,593 (70.2)
Unknown/Not Reported	382 (1.1)	243 (1.0)	22 (1.0)
Education: n (%)			
Elementary	1,222 (3.5)	946 (3.7)	97 (4.3)
High School (or GED)	8,373 (24.1)	6,011 (23.7)	628 (27.7)
Vocational/Some College	8,657 (24.9)	6,117 (24.2)	570 (25.1)
College Degree	11,173 (32.1)	8,221 (32.5)	662 (29.2)
Some Graduate Work	696 (2.0)	481 (1.9)	47 (2.1)
Graduate Degree(Master's or Higher)	3,808 (10.9)	3,021 (11.9)	202 (8.9)
Unknown/Not Reported	878 (2.5)	525 (2.1)	64 (2.8)

The overall HPV positivity rate was 35.2% in the ASC-US (25-65 years) population, 10.2% in the NILM (30-65 years) population and 15.1% in the Primary Screening (25-65 years) population, as shown in Table 25 below.

Table 25: cobas HPV positivity by testing sites and study population

Testing Site	Evaluable ASC-US Population (25-65Years)	Evaluable NILM Population (30-65Years)	Evaluable Primary Screening Population (25-65Years)
1	38.28% (116/303)	8.83% (454/5,139)	13.29% (910/6,846)
2	39.53% (204/516)	10.47% (636/6,074)	15.10% (1,229/8,138)
3	32.40% (335/1,034)	10.78% (902/8,364)	16.85% (2,051/12,171)
4	34.53% (144/417)	10.10% (580/5,745)	13.85% (1,060/7,652)
Overall	35.20% (799/2,270)	10.16% (2,572/25,322)	15.08% (5,250/34,807)

HPV positivity decreased with age in each study population. In the ASC-US population, HPV positivity decreased from 52.1% in the 25-29 age group to 38.2% in the 30-39 age group, and remained ~24% in women 40-65 years old. In the NILM population, HPV positivity was 12.7% in 30-39 age group and remained ~8% in the 40-65 age group. In the primary screening population, HPV positivity decreased from 24.0% in the 25-29 age group to 16.4% in the 30-39 age group, and remained relatively constant at ~10-11% in the 40-65 age group. HPV positivity by age is shown in Table 26 below.

Table 26: cobas HPV positivity by age and study population

	cobas HPV Result		
Age Group (Years)	**ASC-US Population (≥ 25 Years)**	**NILM Population (≥ 30 Years)**	**Primary Screening Population (≥ 25 Years)**
25-29	52.1% (267/512)	Not Applicable	24.0% (1,568/6,530)
30-39	38.2% (288/754)	12.7% (1,328/10,482)	16.4% (1,944/11,826)
40-49	24.0% (129/538)	8.5% (632/7,397)	11.1% (914/8,271)
50-65	24.7% (115/466)	8.2% (612/7,443)	10.1% (824/8,180)
Overall	35.2% (799/2,270)	10.2% (2,572/25,322)	15.1% (5,250/34,807)

The cobas HPV results stratified by age groups are outlined in Table 27. In all populations, 12 Other HR HPV positive results were more frequent than HPV16 and HPV18 positive results in general and within age groups.

Table 27: cobas HPV results by age group for the evaluable populations

	cobas HPV Result				
Age Group (Years)	**HPV16 Positive n (%)**	**HPV18 Positive n (%)**	**12 Other HR HPV Positive n (%)**	**HPV Negative n (%)**	**Total N**
ASC-US Population (25-65 Years)					
Overall	6.34% (144/2,270)	2.78% (63/2,270)	26.08% (592/2,270)	64.80% (1,471/2,270)	2,270
25-29	8.01% (41/512)	2.34% (12/512)	41.80% (214/512)	47.85% (245/512)	512

	cobas HPV Result				
Age Group (Years)	**HPV16 Positive n (%)**	**HPV18 Positive n (%)**	**12 Other HR HPV Positive n (%)**	**HPV Negative n (%)**	**Total N**
30-39	7.43% (56/754)	3.18% (24/754)	27.59% (208/754)	61.80% (466/754)	754
40-49	5.02% (27/538)	2.42% (13/538)	16.54% (89/538)	76.02% (409/538)	538
50-65	4.29% (20/466)	3.00% (14/466)	17.38% (81/466)	75.32% (351/466)	466
NILM Population (30-65 Years)					
Overall	2.20% (556/25,322)	1.21% (306/25,322)	6.75% (1,710/25,322)	89.84% (22750/25,322)	25,322
30-39	2.57% (269/10,482)	1.43% (150/10,482)	8.67% (909/10,482)	87.33% (9,154/10,482)	10,482
40-49	2.16% (160/7,397)	1.04% (77/7,397)	5.34% (395/7,397)	91.46% (6,765/7,397)	7,397
50-65	1.71% (127/7,443)	1.06% (79/7,443)	5.45% (406/7,443)	91.78% (6,831/7,443)	7,443
	Primary Screening Population (25-65 Years)				
Overall	3.06% (1,064/34,807)	1.42% (493/34,807)	10.61% (3,693/34,807)	84.92% (29557/34,807)	34,807
25-29	3.61% (236/6,530)	1.23% (80/6,530)	19.17% (1,252/6,530)	75.99% (4,962/6,530)	6,530
30-39	3.57% (422/11,826)	1.70% (201/11,826)	11.17% (1,321/11,826)	83.56% (9,882/11,826)	11,826
40-49	2.88% (238/8,271)	1.27% (105/8,271)	6.90% (571/8,271)	88.95% (7,357/8,271)	8,271
50-65	2.05% (168/8,180)	1.31% (107/8,180)	6.71% (549/8,180)	89.93% (7,356/8,180)	8,180

D. Safety and Effectiveness Results

1. Safety Results

As an *in vitro* diagnostic test, the cobas HPV involves sampling cells from the cervix using an endocervical brush/spatula combination or broom. The test, therefore, presents no more safety hazard to an individual being tested than other tests where cervical cells are sampled in this manner (such as cervical cytology or HPV testing).

Adverse effects that occurred in the PMA clinical study:

There were no adverse effects of the device reported during the study.

The Principal Investigators or designee reported all study-related adverse events during the IMPACT study. Events such as pain, discomfort, or minor bleeding from cervical procedures, which are anticipated as part of a normal gynecological exam, colposcopy, and biopsy, were not recorded and monitored.
Each adverse event was categorized according to its severity (mild, moderate, and severe) by the Principal Investigator or designee. Each adverse event was also evaluated for determination as a serious adverse event by the Principal Investigator or designee. During the clinical study, 23 adverse events were reported, of which 7 were serious adverse events.

The 16 non-serious adverse events included one subject who presented with cervicitis (one); three subjects with expulsion of intrauterine device (IUD); three subjects with heavy bleeding; two subjects with vaginal bleeding (one with cramping); one subject with prolapsing uterine fibroid with dysfunctional uterine bleeding; one subject with chronic and acute cholecystitis and cholelithiasis; one subject with miscarriage; one subject with trichomoniasis and gonorrhea; one subject with pelvic pain; one subject with acute vaginitis; one subject with and abdominal pain, cramping and chills following loop electrosurgical excision procedure (LEEP) procedure.

The seven serious adverse events included one subject presenting with brain tumor; one subject with headache, neck pain, skin abscess, and vasovagal near syncope; one subject with intractable nausea, vomiting, and diarrhea; one subject with uterine fibroid with dysfunctional uterine bleeding; one subject with endometriosis; one subject with microcytic anemia; and one subject with focal segmental glomerulosclerosis. These serious adverse events were all determined by decision of both the Principal Investigators involved and the Safety Board not to be related to the investigational device.

2. Effectiveness Results
   The analysis of effectiveness was based on the data in the following sections. The clinical performance data in this section are based on histologically determined diagnosis by central pathology review panel using H&E+p16 assistance for slides meeting the LAST-adapted criteria as reference diagnoses for the clinical endpoints ≥CIN2 and ≥CIN3. The clinical performance using H&E alone as the histology endpoint for all slides was also analyzed. No significant difference in performance was apparent when using either histology endpoint.

Performance characteristics in the ASC-US population (25-65 years)
Study enrollment was completed in October 2018. In the IMPACT study, 2,270 subjects aged 25-65 had ASC-US cytology results and cobas HPV results, of which 1,922 were evaluable for the analyses.

Table 28 summarizes the cobas HPV results stratified by histology diagnoses. Among the evaluable ASC-US population, prevalence of ≥CIN2 was 6.8% (104 of 1,814). The majority of cases occurred among HPV-positive women (15.9%) compared with HPV-negative women (1.4%). Similarly, the majority of the ≥CIN3 cases (4.7%)

occurred among women with HPV-positive results compared with women with HPV-negative results (0.3%).

Table 28: cobas HPV results and central pathology review diagnoses in the evaluable ASC-US population (25-65 years)

cobas HPV Result	Central Pathology Review Diagnoses					Total
	Undetermined[1]	Normal[2]	CIN1	CIN2	≥CIN3	
HPV Positive	42	440	84	75	32	673
HPV Negative	66	1,101	65	13	4	1,249
Total	108	1,541	149	88	36	1,922

[1]Undetermined includes: biopsy sample inadequate for analysis, subject/colposcopist unblinded to HPV or Pap cytology result at colposcopy visit, or biopsy sample taken out of window.
[2]Normal includes: Negative or normal histology and atypical squamous cells or glandular changes indefinite for neoplasia.

The performance of the cobas HPV and the FDA-approved HPV test in detecting high grade cervical disease (≥CIN2 and ≥CIN3) is presented in Table 29. The sensitivity for detecting ≥CIN2 was 86.29% (95% CI: 79.14, 91.26) for the cobas 6800/8800 HPV test, similar to the performance of the FDA-approved HPV test (86.18%, 95% CI: 78.98, 91.19). The specificity for detecting ≥CIN2 was 68.99% (95% CI: 66.75, 71.15) for the cobas HPV and 69.47% (95% CI: 67.23, 71.62) for the FDA-approved HPV test.

The sensitivity for detecting ≥CIN3 was 88.89% (95% CI: 74.69, 95.59) for the cobas HPV, and 86.11% (95% CI: 71.34, 93.92) for the FDA-approved HPV test. The specificity for detecting ≥CIN3 was 66.31% (95% CI: 64.08, 68.47) for the cobas HPV, and 66.74% (95% CI: 64.52, 68.89) for the FDA-approved HPV test.

Table 29: Performance of the cobas HPV and the FDA-approved HPV test in the evaluable ASC-US population (25-65 years)

Performance Parameters	≥CIN2 Prevalence (95% CI) = 6.84% (124/1814) (5.76, 8.09)		≥CIN3 Prevalence (95% CI) = 1.98% (36/1814) (1.44, 2.74)	
	cobas HPV	FDA-approved HPV Test	cobas HPV	FDA-approved HPV Test
Sensitivity (%) (95% CI)	86.29 (107/124) (79.14, 91.26)	86.18 (106/123) (78.98, 91.19)	88.89 (32/36) (74.69, 95.59)	86.11 (31/36) (71.34, 93.92)
Specificity (%) (95% CI)	68.99 (1166/1690) (66.75, 71.15)	69.47 (1174/1690) (67.23, 71.62)	66.31 (1179/1778) (64.08, 68.47)	66.74 (1186/1777) (64.52, 68.89)
PPV (%) (95% CI)	16.96 (107/631) (15.60, 18.41)	17.04 (106/622) (15.66, 18.52)	5.07 (32/631) (4.47, 5.75)	4.98 (31/622) (4.33, 5.73)
NPV (%) (95% CI)	98.56 (1166/1183) (97.78, 99.07)	98.57 (1174/1191) (97.80, 99.08)	99.66 (1,179/1,183) (99.15, 99.87)	99.58 (1,186/1,191) (99.06, 99.81)
PLR (95% CI)	2.78 (107/124) / (524/1690) (2.52, 3.08)	2.82 (106/123) / (516/1690) (2.55, 3.12)	2.64 (32/36) / (599/1778) (2.31, 3.01)	2.59 (31/36) / (591/1777) (2.24, 3.00)

Performance Parameters	≥CIN2 Prevalence (95% CI) = 6.84% (124/1814) (5.76, 8.09)		≥CIN3 Prevalence (95% CI) = 1.98% (36/1814) (1.44, 2.74)	
	cobas HPV	FDA-approved HPV Test	cobas HPV	FDA-approved HPV Test
NLR (95% CI)	0.20 (17/124) / (1166/1690) (0.13, 0.31)	0.20 (17/123) / (1174/1690) (0.13, 0.31)	0.17 (4/36) / (1179/1778) (0.07, 0.42)	0.21 (5/36) / (1186/1777) (0.09, 0.47)

PPV=Positive predictive value; NPV=Negative predictive value; PLR=Positive likelihood ratio; NLR= Negative likelihood ratio

The performance of the cobas HPV for detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Table 30. The sensitivity for detecting ≥CIN2 ranged from 80.77% to 89.36% for the cobas HPV and from 84.62% to 89.36% for the FDA-approved HPV test; the specificity ranged from 51.35% to 77.72% for the cobas HPV and from 52.25% to 78.43% for the FDA-approved HPV test.

The sensitivity for ≥CIN3 of both the cobas HPV and the FDA-approved HPV test ranged from 66.67% to 93.33%; the specificity ranged from 47.95% to 76.22% for the cobas HPV and from 48.77% to 76.79% for the FDA-approved HPV test.

Table 30: Performance of the cobas HPV and the FDA-approved HPV test in detecting ≥CIN2 and ≥CIN3 in the evaluable ASC-US population, stratified by age group

	cobas HPV	FDA-approved HPV test	cobas HPV	FDA-approved HPV test	cobas HPV	FDA-approved HPV Test
	25-29 Years		30-39 Years		40-65 Years	
≥CIN2						
Prevalence (%) (95% CI)	12.37 (47/380) (9.43, 16.06)		8.63 (51/591) (6.62, 11.17)		3.08 (26/843) (2.11, 4.48)	
Sensitivity (%) (95% CI)	89.36 (42/47) (77.41, 95.37)	89.36 (42/47) (77.41, 95.37)	86.27 (44/51) (74.28, 93.19)	84.00 (42/50) (71.49, 91.66)	80.77 (21/26) (62.12, 91.49)	84.62 (22/26) (66.47, 93.85)
Specificity (%) (95% CI)	51.35 (171/333) (46.00, 56.67)	52.25 (174/333) (46.89, 57.56)	66.67 (360/540) (62.59, 70.51)	66.54 (360/541) (62.46, 70.39)	77.72 (635/817) (74.74, 80.44)	78.43 (640/816) (75.48, 81.12)
PPV (%) (95% CI)	20.59 (42/204) (18.27, 23.11)	20.90 (42/201) (18.53, 23.47)	19.64 (44/224) (17.21, 22.32)	18.83 (42/223) (16.38, 21.56)	10.34 (21/203) (8.42, 12.65)	11.11 (22/198) (9.20, 13.36)
NPV (%) (95% CI)	97.16 (171/176) (93.69, 98.75)	97.21 (174/179) (93.79, 98.77)	98.09 (360/367) (96.27, 99.03)	97.83 (360/368) (95.96, 98.84)	99.22 (635/640) (98.30, 99.64)	99.38 (640/644) (98.48, 99.75)
≥CIN3						
Prevalence (%) (95% CI)	3.95 (15/380) (2.41, 6.41)		2.54 (15/591) (1.54, 4.15)		0.71 (6/843) (0.33, 1.54)	
Sensitivity (%) (95% CI)	93.33 (14/15) (70.18, 98.81)	93.33 (14/15) (70.18, 98.81)	93.33 (14/15) (70.18, 98.81)	86.67 (13/15) (62.12, 96.26)	66.67 (4/6) (30.00, 90.32)	66.67 (4/6) (30.00, 90.32)

	cobas HPV	FDA-approved HPV test	cobas HPV	FDA-approved HPV test	cobas HPV	FDA-approved HPV Test
	25-29 Years		**30-39 Years**		**40-65 Years**	
Specificity (%) (95% CI)	47.95 (175/365) (42.87, 53.07)	48.77 (178/365) (43.68, 53.88)	63.54 (366/576) (59.53, 67.37)	63.54 (366/576) (59.53, 67.37)	76.22 (638/837) (73.22, 78.98))	76.79 (642/836) (73.81, 79.53)
PPV (%) (95% CI)	6.86 (14/204) (5.87, 8.01)	6.97 (14/201) (5.95, 8.14)	6.25 (14/224) (5.31, 7.34)	5.83 (13/223) (4.71, 7.20)	1.97 (4/203) (1.11, 3.46))	2.02 (4/198) (1.14, 3.55)
NPV (%) 95% CI (%)	99.43 (175/176) (96.33, 99.91)	99.44 (178/179) (96.39, 99.92)	99.73 (366/367) (98.22, 99.96)	99.46 (366/368) (98.05, 99.85)	99.69 (638/640) (99.04, 99.90)	99.69 (642/644) (99.04, 99.90)

PPV=Positive predictive value; NPV=Negative

ASC-US (25-65 years) Population – Likelihood Ratios:

Table 31 presents CPR panel diagnosis by all possible cobas HPV results in the evaluable ASC-US population.

Table 31: All possible cobas HPV results and central pathology review diagnoses in the evaluable ASC-US population (25-65 years)

	Central Pathology Review Diagnoses					
cobas HPV Result (12 Other HR HPV; HPV16; HPV18)	**Undetermined[1]**	**Normal[2]**	**CIN1**	**CIN2**	**≥CIN3**	**Total**
12 Other HR HPV Negative; HPV16 Negative; HPV18 Negative	66	1,101	65	13	4	1,249
12 Other HR HPV Negative; HPV16 Negative; HPV18 Positive	1	18	5	2	1	27
12 Other HR HPV Negative; HPV16 Positive; HPV18 Negative	3	31	4	10	9	57
12 Other HR HPV Negative; HPV16 Positive; HPV18 Positive	0	1	0	1	0	2
12 Other HR HPV Positive; HPV16 Negative; HPV18 Negative	31	344	67	49	13	504
12 Other HR HPV Positive; HPV16 Negative; HPV18 Positive	2	15	3	3	0	23
12 Other HR HPV Positive; HPV16 Positive; HPV18 Negative	5	28	4	10	9	56
12 Other HR HPV Positive; HPV16 Positive; HPV18 Positive	0	3	0	0	0	3

	Central Pathology Review Diagnoses					
cobas HPV Result (12 Other HR HPV; HPV16; HPV18)	**Undetermined[1]**	**Normal[2]**	**CIN1**	**CIN2**	**≥CIN3**	**Total**
12 Other HR HPV Positive; Invalid; Invalid	0	0	1	0	0	1
Overall	108	1,541	149	88	36	1,922

[1]Undetermined includes: biopsy sample inadequate for analysis, subject/colposcopist unblinded to HPV or Pap cytology result at colposcopy visit, or biopsy sample taken out of window.
[2]Normal includes: Negative or normal histology and atypical squamous cells or glandular changes indefinite for neoplasia.

Likelihood ratios (LRs) for the cobas HPV are presented in Table 32 for the ASC-US (25-65 years) population.

The likelihood ratios for the overall HR HPV positive results associated with ≥CIN2 and ≥CIN3 was 2.78 and 2.64, respectively, indicating an overall increased probability of disease in women with HPV positive results. For ≥CIN2, the likelihood ratio for HPV16 positive and/or HPV18 positive results was 5.48, indicating that an HPV16 positive and/or an HPV18 positive result is ~5.5 times more likely to occur in a subject with ≥ CIN2 than in a subject without. The likelihood ratio of a negative cobas HPV result was 0.20, indicating that a negative result was 5 times more likely to occur in a subject without <CIN2 than in a subject with ≥CIN2. For ≥CIN3, likelihood ratio of HPV16 positive and/or HPV18 positive was 6.80, and the likelihood ratio of an HPV negative result was 0.17.

Table 32: Likelihood ratios of disease (≥CIN2 and ≥CIN3) by the cobas HPV results in the evaluable ASC-US population (25-65 years)

cobas HPV Result	**Likelihood Ratio (95% CI)**	
	≥CIN2 vs <CIN2	**≥CIN3 vs <CIN3**
HPV Positive	2.78 (2.52, 3.08)	2.64 (2.31, 3.01)
HPV16 Positive	7.49 (5.30, 10.58)	9.66 (6.59, 14.17)
HPV18 Positive	1.99 (0.86, 4.61)	1.07 (0.15, 7.57)
HPV16/18 Positive	5.48 (4.08, 7.35)	6.80 (4.80, 9.63)
12 Other HR HPV Positive	2.05 (1.69, 2.49)	1.39 (0.90, 2.17)
HPV Negative	0.20 (0.13, 0.31)	0.17 (0.07, 0.42)

ASC-US (25-65 years) Population – Risks Estimates:

The absolute risk of disease among women with positive HPV results was 16.96% and 5.07% for ≥CIN2 and ≥CIN3, respectively (Table 33). For both ≥ CIN2 and ≥ CIN3, the risk of disease was highest for women with HPV positive results, HPV16 and/or HPV18 positive results, and 12 Other HR HPV positive results and lowest for an HPV negative result.

Table 33: Absolute risk of disease (≥CIN2 and ≥CIN3) by HPV genotype from the cobas HPV in the evaluable ASC-US population (25-65 years)

cobas HPV Result	Absolute Risk % (n/N) (95% CI)	
	≥CIN2	≥CIN3
HPV Positive	16.96 (107/631) (14.23, 20.08)	5.07 (32/631) (3.61, 7.07)
HPV16/18 Positive	28.66 (45/157) (22.17, 36.18)	12.10 (19/157) (7.89, 18.13)
HPV16 Positive	35.45 (39/110) (27.14, 44.75)	16.36 (18/110) (10.61, 24.39)
HPV18 Positive	12.77 (6/47) (5.98, 25.17)	2.13 (1/47) (0.38, 11.11)
12 Other HR HPV Positive	13.08 (62/474) (10.34, 16.41)	2.74 (13/474) (1.61, 4.64)
HPV Negative	1.44 (17/1183) (0.90, 2.29)	0.34 (4/1183) (0.13, 0.87)

The absolute risk of ≥CIN2 and ≥CIN3 for cobas HPV results stratified by age group in the evaluable ASC-US population is presented in Table 34. For all age groups, absolute risks were higher for women with any HPV positive results and lowest for an HPV negative result.

Table 34: Absolute risk of disease (≥CIN2 and ≥CIN3) by HPV Genotype from the cobas HPV in the evaluable ASC-US population (25-65 years), stratified by age group

cobas HPV Result	Absolute Risk,% (95% CI)	
	≥CIN2	≥CIN3
25 – 29 Years		
HPV Positive	20.59 (15.61, 26.66)	6.86 (4.13, 11.19)
HPV16/18 Positive	34.21 (21.21, 50.11)	15.79 (7.44, 30.42)
HPV16 Positive	38.71 (23.73, 56.18)	19.35 (9.19, 36.28)
HPV18 Positive	14.29 (2.57, 51.31)	0.00 (0.00, 35.43)
12 Other HR HPV Positive	17.47 (12.45, 23.96)	4.82 (2.46, 9.22)
HPV Negative	2.84 (1.22, 6.48)	0.57 (0.10, 3.15)
30 – 39 Years		
HPV Positive	19.64 (14.97, 25.34)	6.25 (3.76, 10.22)
HPV16/18 Positive	40.00 (28.57, 52.63)	18.33 (10.56, 29.92)
HPV16 Positive	46.51 (32.51, 61.08)	23.26 (13.15, 37.74)
HPV18 Positive	23.53 (9.55, 47.26)	5.88 (1.05, 26.98)
12 Other HR HPV Positive	12.20 (8.03, 18.09)	1.83 (0.62, 5.24)
HPV Negative	1.91 (0.93, 3.88)	0.27 (0.05, 1.53)
40 – 65 Years		
HPV Positive	10.34 (6.87, 15.30)	1.97 (0.77, 4.96)
HPV16/18 Positive	13.56 (7.03, 24.54)	3.39 (0.93, 11.54)
HPV16 Positive	19.44 (9.75, 35.03)	5.56 (1.54, 18.14)
HPV18 Positive	4.35 (0.77, 20.99)	0.00 (0.00, 14.31)
12 Other HR HPV Positive	9.03 (5.35, 14.83)	1.39 (0.38, 4.92)
HPV Negative	0.78 (0.33, 1.82)	0.31 (0.09, 1.13)

Use of the cobas HPV in ASC-US triage of women ≥21 years

The performance of the cobas HPV in 21 to 24 years old women with ASC-US Pap cytology was evaluated using residual cervical samples. 140 refrigerated residual cervical samples collected in PreservCyt Solution were identified from participants in the ATHENA study (**A**ddressing **the N**eed for **A**dvanced HPV Diagnostics study, evaluated the performance of the cobas HPV test on the cobas 4800 System) who were 21-24 years old and diagnosed with ASC-US Pap cytology. Samples were tested in 2018 using both cobas HPV and the FDA-approved HPV test. One sample had an invalid result by the FDA-approved HPV test, so the number of evaluable samples was 139. Agreements for HPV16, HPV18, and 12 Other HR HPV are shown in Table 35, respectively.

Table 35: Cross-tabulation of cobas HPV results and the FDA-approved HPV test results using residual ATHENA study samples

cobas HPV Result	FDA-approved HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	HPV Negative	
HPV16 Positive	33	0	2	0	35
HPV18 Positive	0	8	3	1	12
12 Other HR HPV Positive	0	0	81	0	81
HPV Negative	0	0	2	9	11
Total	33	8	88	10	139

cobas HPV Result	FDA-approved HPV Test Result				Total
	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	HPV Negative	
Genotype Specific PPA (95% CI)	100.00% (33/33) (89.57, 100.00)	100.00% (8/8) (67.55, 100.00)	92.05% (81/88) (84.48, 96.09)		
14 HR HPV Percent Agreement (95% CI)	PPA: 98.45% (127/129) (94.52, 99. 57)			NPA: 90.00% (9/10) (59.50, 98.21)	

PPA: Positive percent agreement; NPA: Negative percent agreement.
Note: HPV16 positive implies HPV16 positive, HPV18 positive or negative and 12 Other HR HPV positive or negative.
HPV18 positive implies HPV16 negative, HPV18 Positive and 12 Other HR HPV positive or negative.
12 Other HR HPV positive implies HPV16 negative, HPV18 negative and 12 Other HR HPV positive.

Performance characteristics in the Adjunct population (NILM 30-65 years)

Subjects with NILM cytology results that were 30-65 years old were evaluated in support of the adjunct claim. Of the total women enrolled and evaluable in IMPACT study, 25,322 subjects were 30-65 years of age and had NILM cytology results. All subjects with a positive HPV result from either the FDA-approved HPV test or cobas HPV (2,572) and a subset of subjects with negative HPV results from both test results (763) were invited to proceed to colposcopy, for a total of 3,335 subjects. Of these subjects, 2,826 proceeded to colposcopy and 2,804 subjects completed the procedure. 2,632 had a valid histological diagnoses and HPV result.

Verification bias adjustment was performed for subsequent analyses to account for the study design in which not all subjects were referred to colposcopy. This was accomplished by calculating the likely number of diseased cases that would have been found if all the subjects in a given subgroup had undergone colposcopy.

The cobas HPV results compared to CPR adjudicated histology in the adjunct population are summarized in Table 36. A total of 151 subjects were diagnosed with ≥CIN2 by CPR, including 54 cases with ≥CIN3.

Table 36: cobas HPV results and central pathology review diagnoses in the evaluable NILM population (30-65 years)

cobas HPV Result	Central Pathology Review Diagnoses				Unknown Disease Status[2]	Total
	Normal[1]	CIN1	CIN2	≥ CIN3		
HPV Positive	1,797	100	94	54	527	2,572
HPV Negative	571	13	3	0	22,163	22,750
Total	2,368	113	97	54	22,690	25,322

[1]Normal includes: Negative or normal histology, and atypical squamous cells or glandular changes indefinite for neoplasia.
[2]Unknown disease status includes: biopsy sample inadequate for analysis, subject/colposcopist unblinded to HPV or Pap cytology result at colposcopy visit, biopsy sample taken out of window, or subjects not selected for colposcopy.

Unadjusted and adjusted performance characteristics for the NILM (30-65) population are shown in Table 37. The unadjusted estimates of sensitivity and specificity for detection of ≥CIN2 were 98.01% (95% CI: 94.32, 99.32) and 23.54% (95% CI: 21.91, 25.25) respectively; for detection of ≥CIN3 estimates were 100% (95% CI: 93.36, 100) and 22.77% (95% CI: 21.19, 24.43). The verification bias

adjusted sensitivities for ≥CIN2 and ≥CIN3 were 72.51% and 100%, respectively; adjusted specificities were 90.48% and 90.08%, respectively. The adjusted estimates of PPV for ≥CIN2 and ≥CIN3 were 7.20% and 2.61%, respectively; NPVs were 99.69% and 100%, respectively. The adjusted estimates of prevalence for ≥CIN2 and ≥CIN3 were 1.01% and 0.27%, respectively.

Table 37: Performance of the cobas HPV in the evaluable NILM population (30-65 years)

Performance Parameters	Central Pathology Review Diagnoses			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Sensitivity (%) (95% CI)	98.01 (148/151) (94.32, 99.32)	72.51 (44.28, 100.00)	100.00 (54/54) (93.36, 100.00)	100.00 (93.36, 100.00)
Specificity (%) (95% CI)	23.54 (584/2481) (21.91, 25.25)	90.48 (90.10, 90.85)	22.77 (587/2578) (21.19, 24.43)	90.08 (89.72, 90.45)
PPV (%) (95% CI)	7.24 (148/2045) (6.19, 8.44)	7.20 (6.10, 8.34)	2.64 (54/2045) (2.03, 3.43)	2.61 (1.93, 3.30)
NPV (%) (95% CI)	99.49 (584/587) (98.51, 99.83)	99.69 (99.02, 100.00)	100.00 (587/587) (99.35, 100.00)	100.00 (99.35, 100.00)
Prevalence (%) (95% CI)	5.74 (151/2632) (4.91 ,6.69)	1.01 (0.65, 1.62)	2.05 (54/2632) (1.58, 2.67)	0.27 (0.19, 0.34)

PPV=Positive predictive value; NPV=Negative predictive value.

NILM (30-65 years) Population – Likelihood Ratios:

Table 38 presents all the possible cobas HPV results in the NILM (30-65 years) evaluable subjects together with the CPR panel diagnosis.

Table 38: All possible cobas HPV results and central pathology review diagnoses in the evaluable NILM population (30-65 years)

	Central Pathology Review Diagnoses					
cobas HPV Result (12 Other HR HPV; HPV16; HPV18)	**Undetermined[1]**	**Normal[2]**	**CIN1**	**CIN2**	**≥CIN3**	**Total**
12 Other HR HPV Negative; HPV16 Negative; HPV18 Negative	22,163	571	13	3	0	22,750
12 Other HR HPV Negative; HPV16 Negative; HPV18 Positive	45	189	5	5	5	249
12 Other HR HPV Negative; HPV16 Positive; HPV18 Negative	98	304	13	6	20	441
12 Other HR HPV Negative; HPV16 Positive; HPV18 Positive	3	9	1	0	0	13

	Central Pathology Review Diagnoses					
cobas HPV Result (12 Other HR HPV; HPV16; HPV18)	**Undetermined[1]**	**Normal[2]**	**CIN1**	**CIN2**	**≥CIN3**	**Total**
12 Other HR HPV Positive; HPV16 Negative; HPV18 Negative	351	1,193	73	70	22	1,709
12 Other HR HPV Positive; HPV16 Negative; HPV18 Positive	10	39	4	3	1	57
12 Other HR HPV Positive; HPV16 Positive; HPV18 Negative	19	56	4	10	6	95
12 Other HR HPV Positive; HPV16 Positive; HPV18 Positive	0	7	0	0	0	7
12 Other HR HPV Positive ; Invalid; Invalid	1	0	0	0	0	1
Overall	22,690	2,368	113	97	54	25,322

[1]Undetermined includes: biopsy sample inadequate for analysis, subject/colposcopist unblinded to HPV or Pap cytology result at colposcopy visit, biopsy sample taken out of window, or subjects not identified for colposcopy
[2]Normal includes: Negative or normal histology and atypical squamous cells or glandular changes indefinite for neoplasia.

Unadjusted and adjusted estimates of likelihood ratios along with 95% CIs for HPV16, 18, 12 Other HR HPV, and HR HPV negative for the NILM (30-65 years) population are presented in Table 39. Adjusted likelihood ratios of HR HPV positive results associated with ≥CIN2 and ≥CIN3 were 7.62 and 10.08, respectively, indicating an overall increased probability of disease associated with HPV positive result.

For ≥CIN3, positive HPV16 results had the highest positive likelihood ratios of 23.78 (adjusted), indicating that a positive HPV16 result is approximately 23 times more likely to come from those with ≥CIN3 than without. There were no cases of ≥CIN3 observed among women with a negative cobas HPV result. Similar patterns of high positive likelihood associated with HPV positive results and low negative likelihoods associated with HPV negative results were observed for ≥CIN2.

Table 39: Likelihood ratios of disease (≥ CIN2 and ≥ CIN3) by the cobas HPV results in the evaluable NILM population (30-65 years)

cobas HPV Result	Likelihood Ratio (95% CI)			
	≥CIN2 vs <CIN2		≥CIN3 vs <CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
HPV Positive	1.28 (1.24 ,1.32)	7.62 (4.63, 10.78)	1.29 (1.27, 1.32)	10.08 (9.64, 10.50)
HPV16 Positive	1.75 (1.33, 2.30)	10.14 (5.34, 16.58)	3.03 (2.26, 4.05)	23.78 (13.94, 30.90)
HPV18 Positive	0.97 (0.58, 1.62)	5.78 (2.19, 11.65)	1.17 (0.54, 2.51)	8.82 (1.44, 16.05)
HPV16/18 Positive	1.46 (1.17, 1.81)	8.55 (5.07, 12.89)	2.33 (1.85, 2.94)	18.34 (12.49, 22.22)
12 Other HR HPV Positive	1.19 (1.04, 1.36)	7.15 (4.37, 11.05)	0.79 (0.57,1.09)	6.05 (4.02, 8.95)
HPV Negative	0.08 (0.03, 0.24)	0.30 (0.00, 0.60)	0.00* (0.00, 0.29)	0.00* (0.00, 0.29)

*No ≥CIN3 cases observed among women with negative cobas HPV results

NILM (30-65 years) Population – Risk Estimates:

Estimates of absolute risk of ≥CIN2 and ≥CIN3 for the cobas HPV results are presented in Table 40. The adjusted absolute risk of ≥CIN2 was 7.19% among women with a positive HPV test result. Risk was highest in women with a positive HPV16 result (9.35%), followed by women with 12 Other HR HPV result (6.78%), and women with an HPV18 positive result (5.56%). The adjusted absolute risk of ≥CIN3 was 2.60% among women with a positive HPV test result. Adjusted risk was highest in women with a positive HPV16 result (5.94%%), followed by women with a positive HPV18 result (2.29%), and women with 12 Other HR HPV result (1.58%). The risks of ≥CIN2 (0.31%) and ≥CIN3 (0.15%) were low among women with HPV negative results.

Table 40: Absolute risk of disease (≥CIN2 and ≥CIN3) by HPV genotype from the cobas HPV in the evaluable NILM population (30-65 years)

cobas HPV Result	Absolute Risk % (95% CI)			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
HPV Positive	7.24 (148/2045) (6.19, 8.44)	7.19 (6.10, 8.33)	2.64 (54/2045) (2.03, 3.43)	2.60 (1.93, 3.31)
HPV16 Positive	9.63 (42/436) (7.21, 12.77)	9.35 (6.66, 12.09)	5.96 (26/436) (4.10, 8.59)	5.94 (3.60, 8.19)
HPV18 Positive	5.58 (14/251) (3.35, 9.14)	5.56 (2.68, 8.40)	2.39 (6/251) (1.10, 5.12)	2.29 (0.63, 4.38)
HPV16/18 Positive	8.15 (56/687) (6.33, 10.44)	8.00 (5.99, 10.06)	4.66 (32/687) (3.32, 6.50)	4.64 (3.11, 6.29)

cobas HPV Result	Absolute Risk % (95% CI)			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
12 Other HR HPV Positive	6.77 (92/1358) (5.56, 8.24)	6.78 (5.54, 8.21)	1.62 (22/1358) (1.07, 2.44)	1.58 (0.96, 2.30)
HPV Negative	0.51 (3/587) (0.17, 1.49)	0.31 (0.00, 0.98)	0.09 (0.5*/587) (0.01, 0.81)	0.15 (0.00; 0.18)

*No ≥CIN3 cases observed among women with negative cobas HPV results, 0.5 case was used in order to estimate risk.

The absolute risks of disease (≥CIN2 and ≥CIN3) by cobas HPV results stratified by age group in the evaluable NILM (30-65 years) population are presented in Table 41 and Table 42. For all age groups, absolute risks were higher for women with any HPV positive results and lowest for an HPV negative result.

Table 41: Absolute risk of disease (≥CIN2 and ≥CIN3) by HPV genotype from the cobas HPV in the evaluable NILM population (30-39 years)

cobas HPV Result	Absolute Risk % (95% CI)			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
HPV Positive	9.44 (98/1038) (7.81, 11.37)	9.26 (7.64,10.93)	3.47 (36/1038) (2.52, 4.76)	3.39 (2.31,4.44)
HPV16 Positive	16.27 (34/209) (11.88, 21.87)	15.61 (11.21,20.38)	9.57 (20/109) (6.28, 14.32)	9.29 (5.55,13.25)
HPV18 Positive	5.74 (7/122) (2.81, 11.37)	5.33 (2.03,9.83)	1.64 (2/122) (0.45, 5.78)	1.33 (0.00,4.21)
HPV16/18 Positive	12.39 (41/331) (9.26, 16.37)	11.93 (8.95,15.35)	6.65 (22/331) (4.43, 9.86)	6.44 (4.00,9.10)
12 Other HR HPV Positive	8.06 (57/707) (6.27, 10.30)	8.03 (6.11,9.95)	1.98 (14/707) (1.18, 3.30)	1.98 (1.04,2.96)
HPV Negative	0.42 (1/236) (0.07, 2.36)	0.74 (0.00,2.55)	0.21 (0.5/236) (0.02, 2.00)	0.37 (0.00,0.43)

Table 42: Absolute risk of disease (≥ CIN2 and ≥ CIN3) by HPV genotype from the cobas HPV in the evaluable NILM population (40-65 years)

cobas HPV Result	Absolute Risk % (95% CI)			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
HPV Positive	4.97 (50/1007) (3.79, 6.49)	4.98 (3.78,6.43)	1.79 (18/1007) (1.13, 2.81)	1.85 (0.97,2.65)

cobas HPV Result	Absolute Risk % (95% CI)			
	≥CIN2		≥CIN3	
	Unadjusted	Adjusted	Unadjusted	Adjusted
HPV16 Positive	3.52 (8/227) (1.80, 6.80)	3.48 (1.44,6.11)	2.64 (6/227) (1.22, 5.65)	2.79 (0.66,5.03)
HPV18 Positive	5.43 (7/129) (2.65, 10.78)	5.77 (1.95,9.90)	3.10 (4/129) (1.21, 7.70)	3.21 (0.56,6.62)
HPV16/18 Positive	4.21 (15/356) (2.57, 6.83)	4.29 (2.23,6.47)	2.81 (10/356) (1.53, 5.09)	2.93 (1.12,4.59)
12 Other HR HPV Positive	5.38 (35/651) (3.89, 7.39)	5.37 (3.83,7.08)	1.23 (8/651) (0.62, 2.41)	1.25 (0.50,2.11)
HPV Negative	0.57 (2/351) (0.16, 2.05)	0.02 (0.00,0.05)	0.14 (0.5/351) (0.01, 1.35)	0.01 (0.00,0.01)

Performance characteristics in the primay screening population (25-65 years)

The primary screening strategy used for the performance evaluation utilizes HPV genotype differentiation of HPV16 and HPV18 with reflex cytology. Int his algorithm, women who test negative for high risk HPV types by the cobas HPV are followed up in accordance with the physician's assessment of screening and medical history, other risk factors, and professional guidelines. Women who test positive for HPV genotypes 16 and/or HPV 18 by the cobas HPV are referred to colposcopy. Women who test high risk HPV positive and 16/18 negative by the cobas HPV (i.e., 12 Other HR HPV positive) are evaluated by cervical cytology to determine the need for referral to colposcopy. Women with ≥ASCUS cytology are referred to colposcopy. Women with NILM cytology are referred to follow up. A diagram of the primary screening strategy used for the performance evaluation is presented below in Figure 5 and Table 43.

Figure 5: Primary screening algorithm

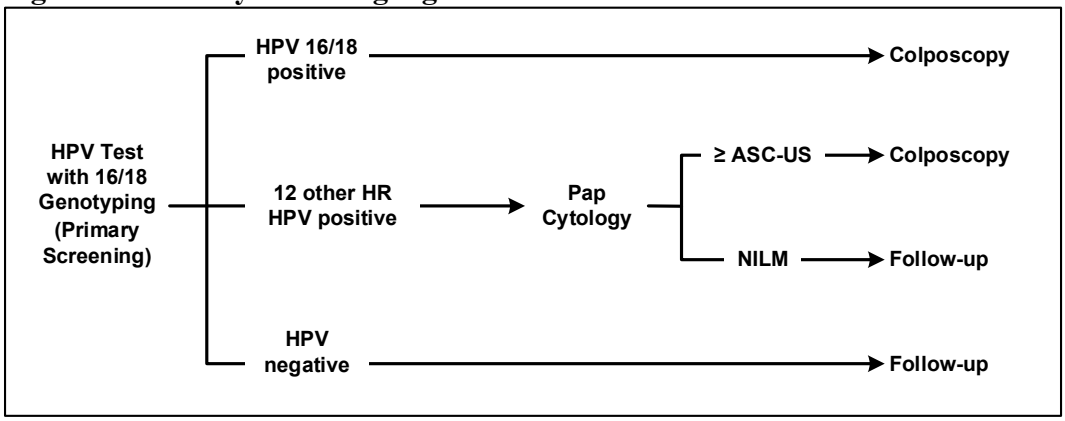


Table 43: Definition of positive and negative results based on the primary ccreening algorithm

cobas HPV Results	Cytology			
	>ASC-US	ASC-US	NILM	
			≥30	25-29
HPV16 /18 Positive	Positive			
12 Other HR HPV Positive	Positive		Negative	
HPV Negative	Negative			

Positive results are defined as women referred immediately to colposcopy as per the algorithm used in the performance evaluation.

The primary screening algorithm was compared to two other clinical screening algorithms. The first algorithm is cytology screening alone,
and is independent of any HPV test result. This algorithm is used as a benchmark to represent clinically acceptable performance levels for the evaluation. This algorithm is outlined in Figure 6 and Table 44.

Figure 6: Cytology alone algorithm

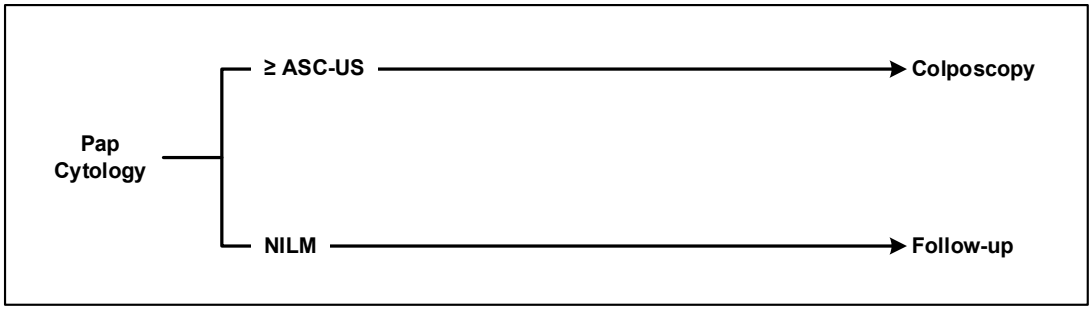


Table 44: Definition of positive and negative results based on the cytology alone algorithm

cobas HPV result	Cytology			
			NILM	
	>ASC-US	ASC-US	≥30	25-29
HPV16 /18 Positive	Positive		Negative	
12 Other HR HPV Positive	Positive		Negative	
HPV Negative	Positive		Negative	

Positive results are defined as women referred immediately to colposcopy as per the algorithm.

The second algorithm is consistent with the currently used standard of care practice in the US. It corresponds to the 2012 American Society of Colposcopy and Cervical Pathology guidelines. In this algorithm, women 25- to 29-years old with >ASC-US cytology or with ASC-US/HR HPV positive results are referred to colposcopy. In addition, women who are ≥30 years old with >ASC-US regardless of HPV status, and

women with NILM cytology and HPV16/18 positive results are also referred to colposcopy. This screening algorithm is outlined in Figure 7 and Table 45.

Figure 7: ASC-US triage and co-testing algorithm

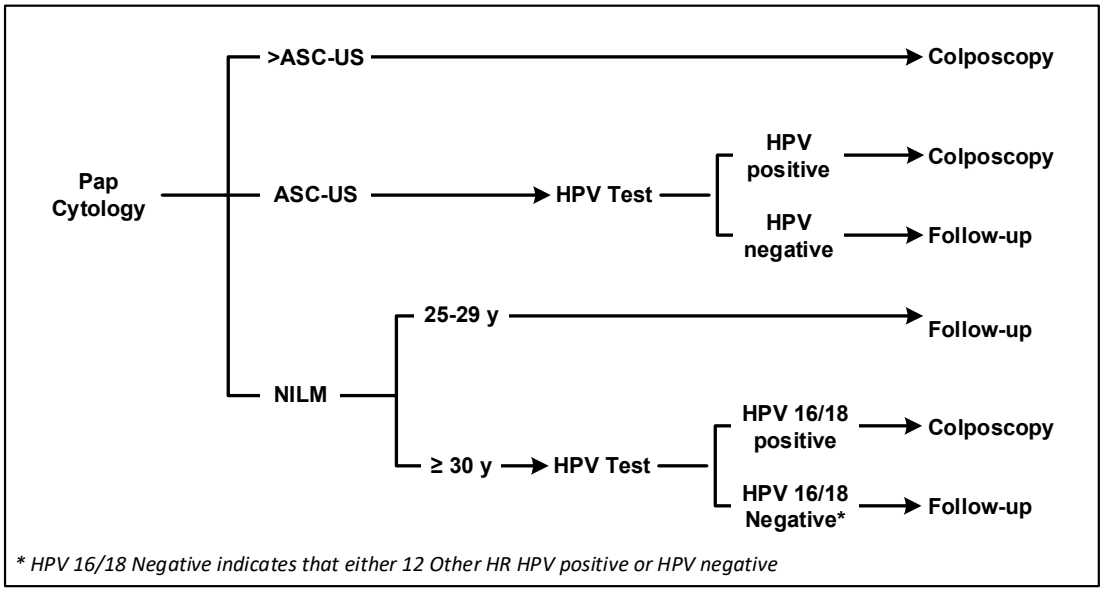


Table 45: Definition of positive and negative results based on the ASC-US triage and co-testing algorithm

cobas HPV test Results	Cytology			
	>ASC-US	ASC-US	NILM	
			≥30	25-29
HPV16 /18 Positive	Positive			Negative
12 Other HR HPV Positive	Positive		Negative	
HPV Negative	Positive	Negative		

Positive results are defined as women referred immediately to colposcopy as per the algorithm.

Definition of positive and negative results and their interpretations:

As described above, "positive" results for the screening algorithms are defined as women who would be referred immediately to colposcopy. "Negative" results for the screening algorithms indicate a woman who would not be sent immediately to colposcopy. Any additional follow-up procedures are not directly assessed. Therefore, the cobas HPV primary screening algorithm is being evaluated regarding its performance in directing immediate follow-up decisions. Longer-term follow-up decisions (i.e. subsequent screening visits) are not directly assessed.

Note that algorithm positive and negative results are distinct from the "disease positive" and "disease negative" results referred to in the clinical performance

sections below, which are defined as women diagnosed with or without high grade CIN, respectively (results are presented for both ≥CIN2 and ≥CIN3). Therefore, when probability (i.e., risk) of disease is described in this document it refers to the probability that a woman has disease at the time of HPV testing.

Performance evaluation in the primary screening population:

A total of 35,263 women 25-65 years of age were enrolled in the IMPACT study, of which, 34,914 (99%) met the study eligibility criteria. Out of 34,914 eligible subjects, 34,807 (99.7%) had valid cobas HPV results and constituted the evaluable population.

A total of 6,826 women proceeded to colposcopy, and of these 6,776 subjects completed the colposcopy procedure. Of these, biopsy samples for 3 subjects were lost/misplaced during transport. Diagnosis of ≥CIN2 (by CPR) was observed in 595 of 6,773 (8.8%) women who went to colposcopy. The number of women with adjudicated histology results for each combination of the cobas HPV and cytology results are shown in Table 46.

Table 46: Number of subjects with adjudicated histology, Pap cytology, and cobas HPV results in the evaluable primary screening population (25-65 years)

cobas HPV Result	Number of Subjects	Pap Cytology				Total
		NILM	ASC-US	>ASC-US	Unsatisfactory	
HPV16/18 Positive	Total	1,061	207	270	19	1,557
	With adjudicated colposcopy	884	168	230	12	-
12 Other HR HPV Positive	Total	2,518	592	545	38	3,693
	With adjudicated colposcopy	2,099	506	447	26	-
HPV Negative	Total	27,326	1,471	323	437	29,557
	With adjudicated colposcopy	804	1,250	285	65	-
Total	-	30,905	2,270	1,138	494	34,807

A correction of verification bias was applied due to the different rate of disease adjudication in each category, in particular the NILM/HPV negative category.

Performance of the primary screening algorithm and the two comparator algorithms were evaluated in the primary screening population by estimating the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), prevalence, positive predictive value (PPV), and negative predictive value (NPV) in the identification of high-grade cervical disease (≥CIN2 and ≥CIN3). Results

comparing the primary screening algorithm to the cytology alone and ASC-US triage and co-testing algorithms are presented in Table 47 and 48, respectively.

The performance of the primary screening algorithm was significantly better than the cytology alone algorithm. For both ≥CIN2 and ≥CIN3 clinical endpoints, the primary screening algorithm had significantly higher sensitivity, specificity, PPV, NPV and PLR, and also significantly lower NLR compared with the cytology alone algorithm. Additionally, the primary screening algorithm required 2.05% fewer colposcopy referrals compared to the cytology alone algorithm.

Table 47: Adjusted performance of the primary screening and cytology alone algorithms in the evaluable primary screening population (25-65 years)

	≥CIN2 Prevalence (95% CI) = 2.34 (2.03, 2.83)			**≥CIN3 Prevalence (95% CI) = 0.87 (0.77, 0.98)**		
Performance Parameters	**Primary Screening Algorithm**	**Cytology Alone Algorithm**	**Difference**	**Primary Screening Algorithm**	**Cytology Alone Algorithm**	**Difference**
Sensitivity (%)	62.41	56.39	6.02	79.93	66.12	13.82
(95% CI)	(52.39, 70.24)	(47.27, 64.21)	(2.85, 9.21)	(74.36, 84.80)	(59.71, 72.37)	(8.42, 19.46)
Specificity (%)	93.57	91.32	2.24	92.90	90.71	2.19
(95% CI)	(93.31, 93.85)	(91.04, 91.63)	(1.93, 2.54)	(92.62, 93.19)	(90.41, 91.00)	(1.89, 2.50)
PPV (%)	18.86	13.47	5.39	9.02	5.90	3.12
(95% CI)	(17.15, 20.55)	(12.13, 14.85)	(4.35, 6.23)	(7.90, 10.20)	(5.06, 6.81)	(2.51, 3.81)
NPV (%)	99.05	98.87	0.18	99.81	99.67	0.14
(95% CI)	(98.57, 99.32)	(98.39, 99.16)	(0.09, 0.25)	(99.75, 99.86)	(99.60, 99.74)	(0.09, 0.19)
PLR	9.70	6.50	3.20	11.25	7.11	4.14
(95% CI)	(8.09, 11.11)	(5.38, 7.47)	(2.51, 3.86)	(10.35, 12.12)	(6.42, 7.83)	(3.37, 4.92)
NLR	0.40	0.48	-0.08	0.22	0.37	-0.16
(95% CI)	(0.32, 0.51)	(0.39, 0.58)	(-0.11, -0.04)	(0.16, 0.28)	(0.31, 0.44)	(-0.22, -0.10)
Colposcopy Referral (%)	7.74	9.79	-2.05	7.74	9.79	-2.05
(95% CI)	(7.45, 8.02)	(9.48, 10.09)	(-2.35, -1.74)	(7.45, 8.02)	(9.48, 10.09)	(-2.35, -1.74)

PPV=Positive predictive value; NPV=Negative predictive value; PLR=Positive likelihood ratio; NLR= Negative likelihood ratio.

The primary screening algorithm had significantly higher specificity, PPV, and PLR for both ≥CIN2 and ≥CIN3 endpoints compared with the ASC-US Triage and co-testing algorithm. For detecting ≥CIN2 and ≥CIN3, sensitivity, NPV, and NLRs were similar between the two algorithms. Additionally, the primary screening algorithm required 0.35% fewer colposcopy referrals compared to the ASC-US triage and co-testing algorithm.

Table 48: Adjusted performance of the primary screening algorithm and the ASC-US triage and co-testing algorithm in the evaluable primary screening population (25-65 years)

	≥CIN2 Prevalence (95% CI) = 2.34% (2.03, 2.83)			≥CIN3 Prevalence (95% CI) = 0.87% (0.77, 0.98)		
Performance Parameters	**Primary Screening Algorithm**	**ASC-US Triage & Co-testing Algorithm**	**Difference**	**Primary Screening Algorithm**	**ASC-US Triage & Co-testing Algorithm**	**Difference**
Sensitivity (%)	62.41	62.53	-0.12	79.93	77.63	2.30
(95% CI)	(52.39, 70.24)	(52.65, 70.78)	(-2.18, 1.55)	(74.36, 84.80)	(71.90, 82.69)	(-0.96, 5.95)
Specificity (%)	93.57	93.21	0.36	92.90	92.52	0.37
(95% CI)	(93.31, 93.85)	(92.96, 93.50)	(0.21, 0.48)	(92.62, 93.19)	(92.25, 92.80)	(0.24, 0.50)
PPV (%)	18.86	18.08	0.78	9.02	8.38	0.64
(95% CI)	(17.15, 20.55)	(16.51, 19.83)	(0.14, 1.26)	(7.90, 10.20)	(7.34, 9.57)	(0.27, 1.05)
NPV (%)	99.05	99.05	0.00	99.81	99.79	0.02
(95% CI)	(98.57, 99.32)	(98.57, 99.33)	(-0.05, 0.04)	(99.75, 99.86)	(99.72, 99.84)	(-0.01, 0.06)
PLR	9.70	9.21	0.49	11.25	10.38	0.87
(95% CI)	(8.09, 11.11)	(7.71, 10.56)	(0.09, 0.81)	(10.35, 12.12)	(9.52, 11.20)	(0.36, 1.40)
NLR	0.40	0.40	-0.00	0.22	0.24	-0.03
(95% CI)	(0.32, 0.51)	(0.31, 0.51)	(-0.02, 0.02)	(0.16, 0.28)	(0.19, 0.30)	(-0.07, 0.01)
Colposcopy Referral (%)	7.74	8.09	-0.35	7.74	8.09	-0.35
(95% CI)	(7.45, 8.02)	(7.80, 8.38)	(-0.48, -0.22)	(7.45, 8.02)	(7.80, 8.38)	(-0.48, -0.22)

PPV=Positive predictive value; NPV=Negative predictive value; PLR=Positive likelihood ratio; NLR= Negative likelihood ratio.

The clinical performance for detection of ≥CIN3 for all three screening algorithms stratified by age is summarized in Table 49.

Table 49: Adjusted performance of the primary screening algorithm, cytology alone algorithm and ASC-US triage and co-testing algorithm for detection of ≥ CIN3, stratified by age group

Performance Parameters	**Sensitivity (%) (95% CI)**	**Specificity (%) (95% CI)**	**PPV (%) (95% CI)**	**NPV (%) (95% CI)**	**PLR (95% CI)**	**NLR (95% CI)**	**Colposcopy Referral (%) (95% CI)**
25 – 29 Years Prevalence (%) (95% CI) =1.50 (1.19,1.87)							
Primary Screening Algorithm	76.53 (65.77, 85.86)	89.60 (88.81,90.35)	10.08 (7.55, 12.71)	99.60 (99.38, 99.78)	7.36 (6.18, 8.42)	0.26 (0.16, 0.38)	11.39 (10.64,12.20)
Cytology Alone Algorithm	65.31 (54.12, 75.77)	87.66 (86.87, 88.49)	7.46 (5.54, 9.56)	99.40 (99.15, 99.61)	5.29 (4.36, 6.22)	0.40 (0.28, 0.52)	13.14 (12.33, 13.97)
ASC-US Triage & Co-Testing Algorithm	64.29 (52.94, 73.96)	91.45 (90.73, 92.15)	10.28 (7.61, 13.27)	99.41 (99.15, 99.61)	7.52 (6.04, 8.99)	0.39 (0.28, 0.52)	9.39 (8.68, 10.11)

Performance Parameters	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	PLR (95% CI)	NLR (95% CI)	Colposcopy Referral (%) (95% CI)
30 – 39 Years Prevalence (%) (95% CI) = 1.23 (1.02, 1.44)							
Primary Screening Algorithm	84.14 (77.23, 90.48)	92.30 (91.78, 92.79)	11.94 (9.70, 14.29)	99.79 (99.69, 99.88)	10.92 (9.84, 12.12)	0.17 (0.10, 0.25)	8.64 (8.15, 9.17)
Cytology Alone Algorithm	68.28 (59.41, 76.34)	90.75 (90.24, 91.27)	8.39 (6.62, 10.02)	99.57 (99.42, 99.70)	7.38 (6.31, 8.40)	0.35 (0.26, 0.45)	9.98 (9.46, 10.51)
ASC-US Triage & Co-Testing Algorithm	86.21 (79.43, 92.39)	91.30 (90.76, 91.80)	10.96 (8.90, 13.05)	99.81 (99.71, 99.90)	9.91 (8.95, 10.85)	0.15 (0.08, 0.23)	9.65 (9.13, 10.19)
40 – 65 Years Prevalence (%) (95% CI) = 0.37 (0.27, 0.47)							
Primary Screening Algorithm	77.05 (64.81, 87.67)	94.62 (94.27, 94.95)	5.06 (3.66, 6.58)	99.91 (99.85, 99.95)	14.33 (11.91, 16.67)	0.24 (0.13, 0.37)	5.64 (5.32, 6.00)
Cytology Alone Algorithm	62.30 (50.75, 76.36)	91.87 (91.46, 92.30)	2.77 (1.96, 3.79)	99.85 (99.78, 99.91)	7.67 (6.22, 9.53)	0.41 (0.26, 0.54)	8.33 (7.89, 8.75)
ASC-US Triage & Co-Testing Algorithm	80.33 (68.94, 90.65)	93.82 (93.47, 94.17)	4.61 (3.39, 6.02)	99.92 (99.87, 99.97)	13.00 (11.09, 15.00)	0.21 (0.10, 0.33)	6.46 (6.11, 6.83)

PPV=Positive predictive value; NPV=Negative predictive value; PLR=Positive likelihood ratio; NLR= Negative likelihood ratio.

Primary Screening Population (25-65 years) – Risk Estimates:

The risks of high-grade cervical disease using the primary screening algorithm are presented in Table 50. Women positive for HPV16 and/or HPV18 (representing 4.47% of the popualtion) and 12 Other HR HPV positive with ≥ASC-US cytology (representing 3.27% of the population) are referred for immediate colposcopy by the primary screening algorithm. The risks of ≥ CIN2 were 18.63% (95% CI: 16.60, 20.70) for HPV16 and/or HPV18 positive and 19.09% (95% CI: 16.60, 21.69) for 12 Other HR HPV positive with ≥ ASC-US cytology. Women with 12 Other HR HPV positive and Normal cytology had a ≥CIN2 risk of 7.47% . The majority of women (84.92%) were HPV-negative and had a risk of 0.39% for ≥CIN2.

Table 50: Adjusted risk of disease in HPV and cytology categories by the primary screening algorithm (25-65 years)

cobas HPV Result	Proportion of women with Result (%)	Risk of ≥ CIN2 (%) (95% CI)	Risk of ≥ CIN3 (%) (95% CI)
HPV Positive	15.08	13.30 (12.26, 14.39)	5.56 (4.91, 6.18)
HPV16/18 Positive	4.47	18.63 (16.60, 20.70)	10.85 (9.27, 12.44)
HPV16 Positive	3.06	22.65 (19.99, 25.41)	14.00 (11.78, 16.09)
HPV18 Positive	1.42	9.94 (7.25, 12.99)	4.06 (2.42, 6.22)
12 Other HR HPV Positive and ≥ASC-US	3.27	19.09 (16.60, 21.69)	6.60 (5.11, 8.31)
12 Other HR HPV Positive and Normal Cytology	7.34	7.47 (6.25, 8.71)	1.88 (1.27, 2.52)
HPV Negative	84.92	0.39 (0.15, 0.88)	0.05 (0.02, 0.08)

The risks of high-grade cervical disease using the primary screening algorithm stratified by age group are presented in Table 51. The risks of ≥CIN2 were all above 10% in each age group for women with HPV16 and/or HPV18 positive results and women with 12 Other HR HPV positive result with ≥ASC-US cytology. The risk of ≥CIN3 was no more than 0.10% in each age group for women with a HPV negative test result (ranged from 0.03 to 0.10%).

Table 51: Risk of disease in HPV and cytology categories by the primary screening algorithm, stratified by age group

cobas 6800/8800 HPV Test Result	Proportion of women with Result (%)	Risk of ≥CIN2 (%) (95% CI)	Risk of ≥CIN3 (%) (95% CI)
25-29 Years			
HPV Positive	24.01	15.18 (13.30, 17.26)	5.99 (4.71, 7.41)
HPV16/18 Positive	4.84	22.15 (17.49, 27.60)	12.97 (8.87, 17.33)
HPV16 Positive	3.61	27.12 (20.80, 34.03)	16.95 (11.60, 22.68)
HPV18 Positive	1.23	7.50 (1.39, 15.79)	1.25 (0.00, 5.59)
12 Other HR HPV Positive and ≥ ASC-US	6.55	22.43 (17.81, 26.97)	7.94 (5.09, 11.23)
12 Other HR HPV Positive and Normal Cytology	12.62	8.74 (6.65, 11.02)	2.31 (1.13, 3.55)
HPV Negative	75.99	0.40 (0.22, 0.61)	0.10 (0.02, 0.22)
30-39 Years			
HPV Positive	16.44	15.74 (13.86, 17.65)	7.25 (6.04, 8.58)
HPV16/18 Positive	5.27	24.08 (20.45, 28.09)	14.93 (11.92, 18.15)
HPV16 Positive	3.57	29.62 (25.00, 35.02)	19.19 (15.10, 23.65)

cobas 6800/8800 HPV Test Result	Proportion of women with Result (%)	Risk of ≥CIN2 (%) (95% CI)	Risk of ≥CIN3 (%) (95% CI)
HPV18 Positive	1.70	12.44 (7.23, 17.47)	5.97 (2.78, 9.61)
12 Other HR HPV Positive and ≥ ASC-US	3.37	20.30 (15.97, 24.68)	7.27 (4.45, 10.26)
12 Other HR HPV Positive and Normal Cytology	7.80	8.24 (6.29, 10.25)	2.06 (1.10, 3.14)
HPV Negative	83.56	0.82 (0.13, 2.26)	0.04 (0.00, 0.08)
40-65 Years			
HPV Positive	10.56	8.86 (7.46, 10.34)	3.28 (2.42, 4.17)
HPV16/18 Positive	3.76	11.33 (8.67, 13.84)	5.66 (3.90, 7.75)
HPV16 Positive	2.47	12.81 (9.48, 16.23)	6.90 (4.40, 9.77)
HPV18 Positive	1.29	8.49 (4.52, 12.53)	3.30 (0.91, 5.97)
12 Other HR HPV Positive and ≥ ASC-US	1.88	12.90 (9.10,17.47)	3.87 (1.84, 6.46)
12 Other HR HPV Positive and Normal Cytology	4.92	5.31 (3.74, 7.21)	1.23 (0.50, 2.12)
HPV Negative	89.44	0.10 (0.05, 0.17)	0.03 (0.01, 0.07)

The absolute risks of ≥CIN2 and ≥CIN3 for women with NILM Pap cytology, HPV-negative results, and NILM with HPV-negative results are presented in Table 52. The risk of ≥CIN3 in women with NILM Pap cytology was 0.33% compared with 0.05% among women with negative cobas HPV results. This indicates that women with NILM Pap cytology have a 6.6 (0.33/0.05) times higher risk of ≥CIN3 compared to women with HPV-negative results. The addition of a NILM cytology result to a negative cobas HPV result marginally decreased ≥CIN3 risk.

Table 52: Adjusted risk of disease in women with NILM cytology and negative cobas HPV results

Cytology and cobas HPV Result	Proportion of women with Result (%)	Risk of ≥CIN2 (%) (95% CI)	Risk of ≥CIN3 (%) (95% CI)
NILM	90.21% (31,399/34,807)	1.13 (0.84, 1.61)	0.33 (0.26, 0.40)
HPV Negative	84.92% (29,557/34,807)	0.39 (0.15, 0.88)	0.05 (0.02, 0.08)
NILM with HR HPV Negative	79.76% (27,763/34,807)	0.25 (0.01, 0.76)	0.00 (0.00, 0.01)

Primary Screening Population (25-65 years) – Benefit and Risk of Screening Strategies:

The benefit (number of CIN2 and ≥CIN3 cases detected) and risk (number of CIN2 or ≥CIN3 cases missed and number of <CIN2 sent to colposcopy) per 10,000 women using the primary screening, cytology alone, and ASC-US triage and co-testing algorithms are presented in Table 53. Per 10,000 women, the primary screening algorithm correctly identified the highest number of true positive ≥CIN3 cases (70) compared to the cytology alone algorithm (58) and ASC-US triage and co-testing algorithm (68). The primary screening algorithm was associated with fewer colposcopies compared to the cytology alone algorithm and the ASC-US triage and co-testing algorithm (775 vs. 980 and 810, respectively). Fewer cases of ≥CIN3 high grade disease were missed by the primary screening algorithm compared to the cytology alone and ASC-US Triage and co-testing algorithms (17 vs. 29 and 19, respectively), and fewer false positives (i.e., <CIN2 referred to colposcopy) were identified with the primary screening algorithm compared with cytology alone and ASC-US Triage and co-testing algorithms (628 vs. 848 and 663, respectively).

Table 53: Benefit and risk of using the primary screening, cytology alone, and ASC-US triage and co-testing algorithms in the primary screening population (25-65 years) per 10,000 women

				Benefit		Risk		
	Number of Tests and Procedures			True Positives		False Negatives		False Positives
Algorithm	Pap Cytology	cobas HPV	Colposcopy	≥CIN3	CIN2	≥CIN3	CIN2	<CIN2
Primary Screening	1,061	10,000	775	70	77	17	70	628
Cytology Alone	10,000	0	980	58	74	29	73	848
ASC-US Triage & Co-testing	10,000	8,043	810	68	79	19	68	663

The benefit and risk per 100 colposcopies for the different screening algorithms were evaluated and are presented in Table 54. The number of screening tests that had to be performed to select 100 women for colposcopy were 1,427 (137+1,290) for the primary screening algorithm, 1,020 for the cytology alone algorithm, and 2,228 (1,235+993) for the ASC-US triage and co-testing algorithm. The number of patients with ≥CIN2 identified by the primary screening algorithm was 19 per 100 colposcopies compared to 14 for cytology alone, and 18 for the ASC-US triage and co-testing algorithm. The probability of ≥CIN3 among women not referred to colposcopy was 0.17% (2/1,190) by the primary screening algorithm, 0.30% (3/920) by the cytology alone algorithm and 0.18% (2/1,135) by the ASC-US triage and co-testing algorithm.

Table 54: Benefit and risks of the primary screening, cytology alone, and ASC-US triage and co-testing algorithms in the primary screening population (25-65 years) per 100 colposcopy procedures

				Benefit		Risk		
	Number of Tests and Procedures			True Positives		False Negatives		False Positives
Algorithm	Pap Cytology	cobas HPV	Colposcopy	≥CIN3	CIN2	≥CIN3	CIN2	<CIN2
Primary Screening	137	1,290	100	9	10	2	9	81
Cytology Alone	1,020	0	100	6	8	3	7	86
ASC-US Triage/Co-testing	1,235	993	100	8	10	2	8	82

Agreement between the cobas HPV results and the FDA-approved HPV Test results for women 25-65 years of age

The agreement between the cobas HPV and FDA-approved HPV test results was evaluated for women 25-65 years of age. These data are presented in Table 55 and Table 56. Genotype specific percent agreements were as follows: PPA for HPV16 positivity was 97.07% (95% CI: 95.64, 98.04); PPA for HPV18 positivity was 97.21% (95% CI: 94.60, 98.658), PPA for 12 Other HR HPV positivity was 85.96% (95% CI: 84.8, 87.00), and NPA for HPV negative was 97.73% (95% CI: 97.55, 97.89).

Table 55: Cross-tabulation of cobas HPV results and the FDA-approved HPV test results

	FDA Approved HPV Test Result				
cobas HPV Result	HPV16 Positive	HPV18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV16 Positive	762	6	46	250	1,064
HPV18 Positive	2	279	47	165	493
12 Other HR HPV Positive	13	1	3,409	260	3,683
HPV Negative	8	1	464	29,011	29,484
Total	785	287	3,966	29,686	34,724

Note: HPV16 positive implies HPV16 positive, HPV18 positive or negative and 12 Other HR HPV positive or negative.
HPV18 positive implies HPV16 negative, HPV18 Positive and 12 Other HR HPV positive or negative.
12 Other HR HPV positive implies HPV16 negative, HPV18 negative and 12 Other HR HPV positive.

Table 56: Agreement between the cobas HPV results and the FDA-approved HPV test results for the detection of HPV genotypes

HPV Genotypes	Positive Percent Agreement % (n/N; 95% CI)	Negative Percent Agreement % (n/N; 95% CI)
HPV16 Positive	97.07% (762/785; 95.64%, 98.04%)	99.11% (33,637/33,939; 99.00%, 99.20%)
HPV18 Positive	97.21% (279/287; 94.60%, 98.58%)	99.38% (34,223/34,437; 99.29%, 99.46%)

HPV Genotypes	Positive Percent Agreement % (n/N; 95% CI)	Negative Percent Agreement % (n/N; 95% CI)
12 Other HR HPV Positive	85.96% (3,409/3,966; 84.84%, 87.00%)	99.11% (30,484/30,758; 99.00%, 99.21%)
14 HR HPV Positive	90.61% (4,565/5,038; 89.77%, 91.39%)	97.73% (29,011/29,686; 97.55%, 97.89%)

Note: HPV16 positive implies HPV16 positive, HPV18 positive or negative and 12 Other HR HPV positive or negative.
HPV18 positive implies HPV16 negative, HPV18 Positive and 12 Other HR HPV positive or negative.
12 Other HR HPV positive implies HPV16 negative, HPV18 negative and 12 Other HR HPV positive.

Agreement with a composite comparator in the ASC-US (25-65 years) and the NILM (30-65 years) populations

Representative cervical samples selected from two subsets of women from the IMPACT study were analyzed using a composite comparator. The two subsets of women included women 25-65 years of age who had ASC-US Pap cytology results (n = 590) and women 30-65 years of age with NILM Pap cytology results (n = 3,167). The composite comparator was comprised of two components: 1. An FDA-approved HPV test and 2) Next-generation HPV DNA sequencing.

The analytical agreement of the cobas HPV results were compared with the composite comparator for the detection of 14 HR HPV genotypes and the positive percent agreement (PPA), negative percent agreement (NPA) and 95% confidence intervals (CIs) were calculated. The composite comparator for the detection of 14 HR HPV was indeterminate if results were discordant between HPV DNA sequencing result and the FDA-approved HPV DNA test result. Table 57 summarizes the agreement between the cobas HPV with the composte comparator for both population.

Table 57: Agreement between the cobas HPV results and the composite comparator for the detection of 14 HR HPV

Population	cobas HPV Result	HPV Composite Comparator			Total	Agreement (%) (95% CI)
		Positive	Negative	Indeterminate		
ASC-US ≥ 25 Years	Positive	420	0	12	432	PPA: 98.36% (420/427) (96.66%, 99.20%)
	Negative	7	134	17	158	NPA: 100.0% (134/134) (97.21%, 100.00%)
	Total	427	134	29	590	-
NILM ≥ 30 Years	Positive	1153	31	79	1263	PPA: 90.57% (1153/1273) (88.84%, 92.06%)
	Negative	120	1635	149	1904	NPA: 98.14% (1635/1666) (97.37%, 98.69%)
	Total	1273	1666	228	3167	-

PPA=positive percent agreement, NPA=negative percent agreement.

The agreement of the cobas HPV genotyping results were compared with the composite comparator. The percent agreements (PA) along with corresponding 95% confidence intervals (CIs) for HPV 16, HPV 18, 12 Other HR HPV, and HPV negative were calculated for the ASC-US (25-65 years) and NILM (30-65 years) populations as presented in Table 58 and Table 59, respectively. The composite comparator for HPV genotyping was indeterminate if results were discordant between HPV DNA sequencing result and the FDA-approved HPV test result.

Table 58: Agreement between the cobas HPV results and composite comparator in the ASC-US Population (25-65 Years)

	Composite Comparator for HPV Genotyping					
cobas HPV	FDA Approved = HPV 16 Positive, DNA Sequencing = HPV 16 Positive	FDA Approved = HPV18 Positive, DNA Sequencing = HPV18 Positive	FDA Approved = 12 Other HR HPV Positive, DNA Sequencing =12 Other HR HPV Positive	FDA Approved = HPV Negative DNA Sequencing= HPV Negative	Indeterminate	Total
HPV 16 Positive	68	0	2	0	6	76
HPV 18 Positive	0	21	2	0	8	31
12 Other HR HPV Positive	0	0	317	0	8	325
HPV Negative	0	0	7	134	17	158
Total	68	21	328	134	39	590
Percent Agreement (95% CI)	100.0% (68/68) (94.65%, 100.0%)	100.0% (21/21) (84.54%, 100.0%)	96.65% (317/328) (94.10%, 98.12%)	100.0% (134/134) (97.21%, 100.0%)		

Note: Indeterminate includes results where FDA approved and DNA Sequencing results are discordant.

Table 59: Agreement between the cobas HPV results and composite comparator in the NILM Population (Age 30-65 Years)

	Composite Comparator for HPV Genotyping					
cobas HPV	FDA Approved = HPV 16 Positive, DNA Sequencing = HPV 16 Positive	FDA Approved = HPV 18 Positive, DNA Sequencing = HPV 18 Positive	FDA Approved = 12 Other HR HPV Positive, DNA Sequencing = 12 Other HR HPV Positive	FDA Approved = HPV Negative, DNA Sequencing = HPV Negative	Indeterminate	Total
HPV 16 Positive	171	1	13	19	30	234
HPV 18 Positive	0	74	11	5	15	105
12 Other HR HPV Positive	0	0	853	7	64	924
HPV Negative	1	1	113	1635	154	1904
Total	172	76	990	1666	263	3167

	Composite Comparator for HPV Genotyping					
cobas HPV	FDA Approved = HPV 16 Positive, DNA Sequencing = HPV 16 Positive	FDA Approved = HPV 18 Positive, DNA Sequencing = HPV 18 Positive	FDA Approved = 12 Other HR HPV Positive, DNA Sequencing = 12 Other HR HPV Positive	FDA Approved = HPV Negative, DNA Sequencing = HPV Negative	Indeterminate	Total
Percent Agreement (95% CI)	99.42% (171/172) (96.78%, 99.90%)	97.37% (74/76) (90.90%, 99.28%)	86.16% (853/990) (83.87%, 88.17%)	98.14% (1635/1666) (97.37%, 98.69%)		

Note: Indeterminate includes results where FDA approved and DNA Sequencing results are discordant.

3. Subgroup Analyses

The following characteristics were evaluated for potential association with outcomes:

Performance by vaccination status

The clinical sites enrolled both vaccinated and unvaccinated women. 12.3% of the enolled women reported receiving the HPV vaccine. Since the first HPV vaccine was introduced in 2006 and the IMAPCT study occurred from 2018-2019, a majority of the vaccinated women in the study were expected to be under the age of 30. Therefore, the performance of the cobas HPV was evaluated in the 25-29 year age group. The performance of cobas HPV in unvaccinated and vaccinated women with ASC-US cytology (25-29 years old) and the primary screening (25-29 years old) stratified by self-repoted vaccinateion status is presented in Table 60 and Table 61 respectively.

Table 60: Performance of the cobas HPV in detecting disease, stratified by HPV vaccination status in the ASC-US population (25-29 years)

	Overall	Vaccinated	Unvaccinated
≥CIN2			
Sensitivity (%) (95% CI)	89.13 (41/46) (76.96, 95.27)	78.57 (11/14) (52.41, 92.43)	93.75 (30/32) (79.85, 98.27)
Specificity (%) (95% CI)	51.35 (171/333) (46.00, 56.67)	52.76 (67/127) (44.12, 61.23)	50.49 (104/206) (43.71, 57.24)
PPV (%) (95% CI)	20.20 (41/203) (15.25, 26.25)	15.49 (11/71) (8.88, 25.65)	22.73 (30/132) (16.41, 30.59)
NPV (%) (95% CI)	97.16 (171/176) (93.52, 98.78)	95.71 (67/70) (88.14, 98.53)	98.11 (104/106) (93.38, 99.48)
Prevalence (%)	12.14	9.93	13.45 (32/238)

(95% CI)	(46/379) (9.22 ,15.81)	(14/141) (6.01 ,15.98)	(9.69 ,18.36)
≥CIN3			
Sensitivity (%) (95% CI)	93.33 (14/15) (70.18, 98.81)	83.33 (5/6) (43.65, 96.99)	100.00 (9/9) (70.09, 100.00)
Specificity (%) (95% CI)	48.08 (175/364) (42.99, 53.20)	51.11 (69/135) (42.77, 59.40)	46.29 (106/229) (39.94, 52.75)
PPV (%) (95% CI)	6.90 (14/203) (4.15, 11.24)	7.04 (5/71) (3.05, 15.45)	6.82 (9/132) (3.63, 12.45)
NPV (%) (95% CI)	99.43 (175/176) (96.85, 99.90)	98.57 (69/70) (92.34, 99.75)	100.00 (106/106) (96.50, 100.00)
Prevalence (%) (95% CI)	3.96 (15/379) (2.41 ,6.43)	4.26 (6/141) (1.96 ,8.97)	3.78 (9/238) (2.00 ,7.03)

PPV=Positive predictive value; NPV=Negative predictive value.

Table 61: Performance of the cobas HPV in detecting disease, stratified by HPV vaccination status in the primary screening population (25-29 years)

	Unadjusted		**Adjusted**	
	Vaccinated	**Unvaccinated**	**Vaccinated**	**Unvaccinated**
≥CIN2				
Sensitivity (%) (95% CI)	55.17 (32/58) (42.45, 67.25)	69.12 (94/136) (60.92, 76.27)	54.67 (42.22, 68.01)	68.51 (60.98, 76.60)
Specificity (%) (95% CI)	75.56 (405/536) (71.75, 79.01)	64.21 (531/827) (60.88, 67.40)	93.04 (91.99, 94.06)	89.29 (88.24, 90.39)
PPV (%) (95% CI)	19.63 (32/163) (15.64, 24.35)	24.10 (94/390) (21.55, 26.85)	19.07 (13.67, 25.44)	23.48 (19.60, 27.86)
NPV (%) (95% CI)	93.97 (405/431) (92.10, 95.41)	92.67 (531/573) (90.73, 94.23)	98.56 (97.98, 99.07)	98.34 (97.85, 98.82)
PLR (95% CI)	2.26 (32/58) /(131/536) (1.71, 2.97)	1.93 (94/136) / (296/827) (1.67, 2.23)	7.85 (5.87, 10.51)	6.40 (5.51, 7.47)
NLR (95% CI)	0.59 (26/58) / (405/536) (0.44, 0.79)	0.48 (42/136) / (531/827) (0.37, 0.62)	0.49 (0.34, 0.62)	0.35 (0.26, 0.44)
Colposcopy Referral (%) (95% CI)	27.44 (163/594) (24.01, 31.17)	40.50 (390/963) (37.44, 43.63)	8.35 (7.34, 9.42)	13.35 (12.29, 14.48)
Prevalence (%) (95% CI)	9.76 (58/594) (7.63, 12.42)	14.12 (136/963) (12.07, 16.46)	2.91 (2.25, 3.65)	4.58 (3.88, 5.36)
≥CIN3				

Sensitivity (%) (95% CI)	65.38 (17/26) (46.22, 80.59)	81.63 (40/49) (68.64, 90.02)	66.67 (45.83, 83.87)	80.00 (69.78, 92.91)
Specificity (%) (95% CI)	74.30 (422/568) (70.55, 77.72)	61.71 (564/914) (58.51, 64.80)	92.41 (91.32, 93.42)	87.76 (86.68, 88.90)
PPV (%) (95% CI)	10.43 (17/163) (7.85, 13.73)	10.26 (40/390) (8.90, 11.79)	10.23 (5.94, 15.23)	9.85 (7.24, 13.19)
NPV (%) (95% CI)	97.91 (422/431) (96.50, 98.76)	98.43 (564/573) (97.20, 99.13)	99.53 (99.15, 99.79)	99.62 (99.39, 99.88)
PLR (95% CI)	2.54 (17/26) / (146/568) (1.86, 3.48)	2.13 (40/49) / (350/914) (1.82, 2.49)	8.78 (5.85, 11.63)	6.54 (5.62, 7.77)
NLR (95% CI)	0.47 (9/26) / (422/568) (0.27, 0.79)	0.30 (9/49) / (564/914) (0.16, 0.54)	0.36 (0.17, 0.59)	0.23 (0.08, 0.34)
Colposcopy Referral (%) (95% CI)	27.44 (163/594) (24.01, 31.17)	40.50 (390/963) (37.44, 43.63)	8.35 (7.34, 9.42)	13.35 (12.29, 14.48)
Prevalence (%) (95% CI)	4.38 (26/594) (3.00, 6.34)	5.09 (49/963) (3.87, 6.66)	1.28 (0.85, 1.83)	1.64 (1.21, 2.10)

Comparison of results in prequot vs. postquot clinical samples

An agreement study was performed to compare the performance of the cobas HPV with a cervical specimen aliquoted for testing prior to (prequot) or after (postquot) normal cytology processing. This analysis was performed on results from 3,753 paired prequot and postquot samples.

Agreement between the cobas HPV results from pre-quot and post-quot samples for overall HPV positivity and for genotype-specific results are presented in Table 62, Table 63, and Table 64 **Error! Reference source not found.**for each of the three study populations (ASC-US (25-65 years), NILM (30-65 years), and the primary screening population (25-65 years)), respectively. The resuls are stratified by disease (≥CIN2) and no disease (<CIN2).

Table 62: Agreement of cobas HPV results in prequot vs. postquot samples in the ASC-US population (25-65 years), stratified by CPR diagnosis

	Pre-quot Cytology Samples				
	≥CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	3	0	0	0	3
HPV 18 Positive	0	0	0	0	0
12 Other HR HPV Positive	0	0	2	0	2
HPV Negative	0	0	0	2	2

Total	3	0	2	2	7
Genotype Specific PPA (95 % CI)	100.0% (3/3) (43.85%, 100.0%)	NC*	100.0% (2/2) (34.24%, 100.0%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=100.0% (5/5) (56.55%, 100.0%)			NPA=100.0% (2/2) (34.24%, 100.0%)	
	<CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	6	0	1	1	8
HPV 18 Positive	0	3	0	0	3
12 Other HR HPV Positive	0	0	32	0	32
HPV Negative	0	0	4	121	125
Total	6	3	37	122	168
Genotype Specific PPA (95 % CI)	100.0% (6/6) (60.97%, 100.0%)	100.0% (3/3) (43.85%, 100.0%)	86.49% (32/37) (72.02%, 94.09%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=91.30% (42/46) (79.68%, 96.57%)			NPA=99.18% (121/122) (95.50%, 99.86%)	

*NC= not calculable

Table 63: Agreement of cobas HPV results in prequot vs. postquot samples in the NILM population (30-65 years), stratified by CPR diagnosis

	Pre-quot Cytology Samples				
	≥CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	6	0	0	0	6
HPV 18 Positive	0	0	0	0	0
12 Other HR HPV Positive	0	0	8	0	8
HPV Negative	0	0	0	1	1
Total	6	0	8	1	15
Genotype Specific PPA (95 % CI)	100.00% (6/6) (60.97%, 100.0%)	NC	100.0% (8/8) (67.56%, 100.0%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=100.0% (14/14) (78.47%, 100.0%)			NPA=100.0% (1/1) (20.65%, 100.0%)	

	<CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	41	0	0	2	43
HPV 18 Positive	0	17	1	0	18
12 Other HR HPV Positive	1	0	107	5	113
HPV Negative	8	2	19	58	87
Total	50	19	127	65	261
Genotype Specific PPA (95 % CI)	82.00% (41/50) (69.20%, 90.23%)	89.47% (17/19) (68.61%, 97.06%)	84.25% (107/127) (76.92%, 89.57%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=85.20% (167/196) (79.56%, 89.50%)			NPA=89.23% (58/65) (79.40%, 94.68%)	

NC= not calculable

Table 64: Agreement of cobas HPV results in prequot vs. postquot samples in the primary screening population (25-65 years), stratified by CPR diagnosis

	Pre-quot Cytology Samples				
	≥CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	18	0	0	0	18
HPV 18 Positive	0	5	0	0	5
12 Other HR HPV Positive	0	0	30	1	31
HPV Negative	0	0	0	5	5
Total	18	5	30	6	59
Genotype Specific PPA (95 % CI)	100.0% (18/18) (82.41%, 100.0%)	100.0% (5/5) (56.55%, 100.0%)	100.0% (30/30) (88.65%, 100.0%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=100.0% (53/53) (93.24%, 100.0%)			NPA=83.33% (5/6) (43.65%, 96.99%)	
	<CIN2				
Post-quot Cytology Samples	HPV 16 Positive	HPV 18 Positive	12 Other HR HPV Positive	HPV Negative	Total
HPV 16 Positive	67	0	2	4	73
HPV 18 Positive	0	28	1	0	29
12 Others Positive	1	0	218	7	226

HPV Negative	8	2	26	237	273
Total	76	30	247	248	601
Genotype Specific PPA (95 % CI)	88.16% (67/76) (79.00%, 93.64%)	93.33% (28/30) (78.68%, 98.15%)	88.26% (218/247) (83.65%, 91.70%)		
14 HR HPV Percent Agreement (95 % CI)	PPA=89.80% (317/353) (86.20%, 92.54%)			NPA=95.56% (237/248) (92.23%, 97.51%)	

Performance of cobas HPV using cervical samples from women with cervical cancer

A separate study was conducted to provide additional clinical evidence that the cobas HPV could detect HPV in cytology samples of women subsequently diagnosed with cancer. Pre-aliquoted de-identified ThinPrep cervical samples from 23 women who were subsequently diagnosed with histologically confirmed invasive cervical cancer were obtained from a separate study. The diagnosis of invasive cervical cancer in the samples was confirmed by the central pathology review panel. Of the 23 invasive cervical cancer cases, 21 were squamous cell carcinoma, one was adenocarcinoma, and one was small cell carcinoma.

Of the 23 invasive cervical cancer cases, 20 were cobas HPV positive, indicating a sensitivity of 87.0% (95% CI: 67.9, 95.5). Of the three HPV negative cases, one was adenocarcinoma, and two were squamous cell carcinomas.

The sensitivity of primary screening algorithm was 82.6% (19/23) (95% CI: 62.9, 93.0) compared to 56.5% (13/23) (95% CI: 36.8, 74.4) for the cytology alone algorithm.

4. Pediatric Extrapolation

   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included four investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Devices, 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. Effectiveness Conclusions

The effectiveness of the cobas HPV with cervical specimens collected by a physician using an endocervical brush/spatula or broom placed in the ThinPrep Pap Test PreservCyt Solution has been demonstrated for use in conjunction with cervical cytology in the following patient populations: women with ASC-US cytology 21-65 years of age, women with NILM cytology 30-65 years of age, and all women 25-65 years of age. 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.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory as well as data collected in a clinical study conducted to support PMA approval as described above. Based on the results of these studies, the cobas HPV, when used according to the provided directions and together with the physician's interpretation of cytology results, other risk factors, and professional guidelines, should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Determination

The probable benefits of the device are also based on data collected in a clinical study conducted to support PMA approval as described above. The cobas HPV has been shown to effectively assess a woman's risk for ≥CIN2 and ≥CIN3 by detecting high risk HPV nucleic acid.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support the claimed indications for use and the probable benefits outweigh the probable risks.

D. 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, reproducibility, and analytical specificity of the cobas HPV when used according to instructions for use, warnings and precautions, and limitations sections of the labeling. The clinical studies and performance analysis of the clinical data in this application have shown that the assay is safe and effective for use in routine cervical cancer screening for its approved indications when used according to the directions for use in the labeling.

XIV. CDRH DECISION

CDRH issued an approval order on April 3, 3020. The final clinical conditions of approval cited in the approval order are described below.

The applicant's manufacturing facilities have been 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.