SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: CINtec *PLUS* Cytology

Device Trade Name: CINtec® *PLUS* Cytology

Device Procode: QKF

Applicant's Name and Address: Ventana Medical Systems, Inc.
1910 E Innovation Park Drive
Tucson, AZ 85755

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190024

Date of FDA Notice of Approval: March 10, 2020

II. INDICATIONS FOR USE

The CINtec® *PLUS* Cytology test is a qualitative immunocytochemical assay intended for the simultaneous detection of the $p16^{INK4a}$ and Ki-67 proteins in cervical specimens collected by a clinician using an endocervical brush/spatula or broom collection device and placed in the ThinPrep® Pap Test PreservCyt® Solution. The CINtec *PLUS* Cytology test includes a ready-to-use cocktail of primary antibodies which contains a mouse monoclonal antibody directed against human $p16^{INK4a}$ (p16) protein (clone E6H4), and a recombinant rabbit monoclonal antibody directed against human Ki-67 protein (clone 274-11AC3V1) for use on the BenchMark ULTRA instrument with 3,3-diaminobenzidine tetrahydrochloride (DAB) and Fast Red detection systems.

The CINtec *PLUS* Cytology test is indicated:

- To be used in women 25 - 65 years old with 12 Other High Risk (HR) HPV positive test results using the cobas® 4800 HPV Test in primary HPV screening, to determine the need for referral to colposcopy.

  To be used in women 25 - 65 years old with HPV16/18 positive test results using the cobas® 4800 HPV Test in primary HPV screening where the CINtec *PLUS* Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

- To be used in women 30 - 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and 12 Other HR HPV positive test results using the cobas 4800 HPV Test in adjunctive cervical cytology and HR HPV screening, to determine the need for referral to colposcopy.

  To be used in women 30 - 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and HPV16/18 positive test results using the cobas® 4800 HPV Test in adjunctive cervical cytology and HR HPV screening where the CINtec *PLUS* Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

Results from the CINtec *PLUS* Cytology test should be interpreted by a qualified pathologist.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

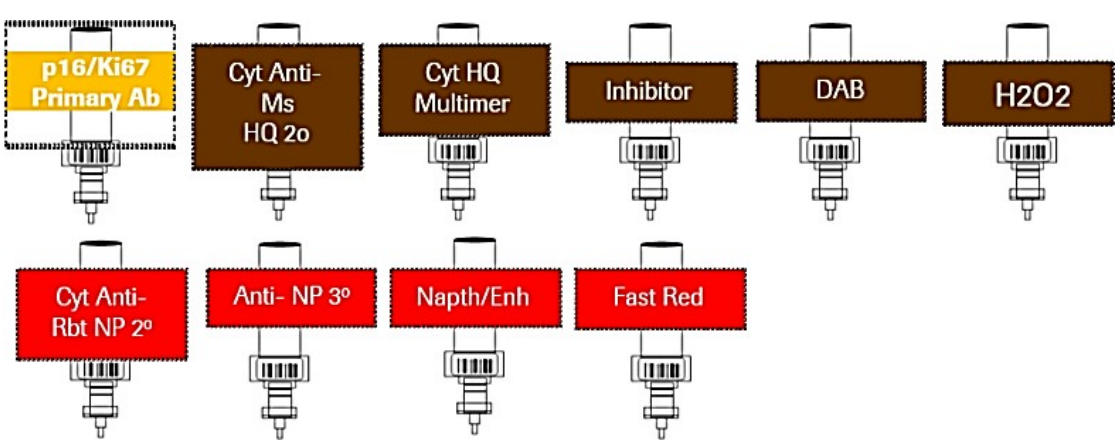
The warnings and precautions can be found in the CINtec® *PLUS* Cytology labeling.

V. DEVICE DESCRIPTION

CINtec *PLUS* Cytology is an immunocytochemistry test for use on the Benchmark ULTRA staining system for the simultaneous immunocytochemical detection of the $p16^{INK4a}$ (p16) and Ki-67 proteins in cytological specimens obtained from the uterine cervix. The proteins are detected using a ready-to-use cocktail of primary antibodies which contain a mouse monoclonal antibody directed against human $p16^{INK4a}$ a (p16) protein (clone E6H4), and a recombinant rabbit monoclonal antibody directed against human Ki-67 protein (clone 274-11AC3V1).

The CINtec *PLUS* Cytology test includes 10 FloLock dispensers filled with ready-to-use Reagents in the configuration as shown in the Figure below.

Figure 1: CINtec *PLUS* Cytology Configuration



As shown in Figure 1, the test kit includes 10 FloLock dispensers filled with ready-to-use reagents as listed in Table 1 below. Materials required but not provided with CINtec PLUS Cytology test kit are listed in Table 2.

Table 1: Components Included with CINtec *PLUS* Cytology

Component*	Description/Composition
Primary Antibody Cocktail (p16/Ki-67)	$p16^{INK4a}$ (E6H4) Mouse Monoclonal Primary Antibody, 4.0 µg/mL (0.004 mg/mL); Ki-67 (274-11AC3V1-IgG) Rabbit Recombinant Primary Antibody, 0.3 µg/mL (0.0003 mg/mL); Avidin Diluent with B5 blocker
Brown Detection System for p16	
HQ (hapten)-labeled Goat Anti-mouse IgG	Goat anti-mouse HQ Conjugate for Cytology, 35 µg/mL (0.035 mg/mL); Avidin Diluent with B5 blocker
Mouse monoclonal anti-HQ-labeled HRP Tertiary Antibody	Mouse anti HQ-HRP Conjugate for Cytology 7.5 µg/mL (0.0075 mg/mL); Avidin Diluent with B5 blocker

Hydrogen Peroxide (inhibitor)	3% Hydrogen Peroxide
DAB in Stabilizer Solution	Final density = 1.007 g/mL; DAB powder; deionized water; Imidazole; 2-hydroxypyridine; Liquid Brij 30%; Polyethylene Glycol 8000; Sodium Stannate Trihydrate; Sodium Metabisulfite; Diaminobenzidine Tetrahydrochloride; 6N HCl; 6N NaOH
Hydrogen Peroxide in Phosphate Buffer Solution	0.04% Hydrogen Peroxide; 77 mM Potassium Phosphate Dibasic; 23 mM Sodium Phosphate Monobasic, pH 7.3; 55 mM Sodium Chloride; 0.05 % Brij 35 (w/v)
Red Detection System for Ki-67	
NP-labeled Goat Anti-rabbit IgG	Goat anti-rabbit NP Conjugate for Cytology, 2.5 µg/mL (0.0025 mg/mL); Avidin Diluent with B5 blocker
Mouse monoclonal anti-NP-labeled AP Tertiary Antibody	Mouse anti-NP AP Conjugate 10 µg/mL (0.010 mg/mL)
Naphthol Phosphate	0.575% Naphthol AS-TR Phosphate Disodium Salt; 400 mM Tris; 17.2 mM L-Homoarginine Hydrochloride; 0.05% Brij-35; 0.05% ProClin 300; pH 7.5
Fast Red in Acetate Buffer	0.12% Fast Red KL Salt; 96.5 mM MgCl2; 11.2 mM Acetic Acid; 0.37% Brij-35; 0.05% ProClin 300

*Components are listed in the order of usage

Table 2: Materials Required but not Provided with CINtec *PLUS* Cytology

Component	Description
Reaction Buffer 10x	Tris based buffer solution (pH 7.6 ± 0.2) used to rinse slides between staining steps and provide a stable aqueous environment for reactions carried out on the BenchMark ULTRA instrument
ULTRA Liquid CoverSlip (LCS), predilute	Pre-diluted coverslip solution used as a barrier between aqueous reagents and air
ULTRA CC1 Cell Conditioning Solution (CC1)	Pre-diluted solution used as a pretreatment step in the processing of tissue samples on the BenchMark ULTRA instrument
Hematoxylin	Modified Mayer's hematoxylin used for staining cellular nuclei on slides containing cells from frozen tissue, formalin fixed and paraffin embedded (FFPE) tissue, or cytologic preparations
Bluing Reagent	Aqueous solution of buffered lithium carbonate used for bluing hematoxylin stained sections on glass slides
CC/Mount™ Aqueous mounting media (Diagnostic Biosystems; DBS)	Aqueous mounting medium with very high refractive index. When applied to the stained tissue sections, specimens can be permanently mounted without chromogens fading

Device Instrument and Software

The CINtec *PLUS* Cytology test is performed on the automated BenchMark ULTRA Advanced Staining System. It consists of four main modular components that work together as a system: 1) a stainer subassembly where all slide processing operations are performed and which contains a reagent carousel, dispenser mechanism, barcode readers, heating elements and other components; 2) an automated fluidics subassembly (AFS) that provides the compressed air and bulk fluids required by the stainer subassembly; 3) a waste bottle subassembly that collects the waste generated by the system during staining operations; and, 4) a personal computer (PC) running on a Microsoft Windows platform that controls and monitors the system through the Ventana System Software (VSS) host operating software (version 12.3). The VSS provides slide information such as patient and doctor identification, protocol, and dates. It also provides reagent information such as reagent name and number, expiration date, and tracks the number of dispensers remaining in the reagent packaging. The immunocytochemistry staining process is fully automated and the staining protocol is specific for the CINtec *PLUS* Cytology device.

Specimen Preparation

The CINtec *PLUS* Cytology test is intended for use on cervical cytology specimens collected by a clinician using an endocervical brush/spatula or broom collection device and placed in ThinPrep® Pap Test™ PreservCyt® Solution (Hologic, Inc.). Slides are prepared from these specimens using the FDA approved ThinPrep® 2000 or ThinPrep® 5000 automated slide processor (Hologic, Inc.) according to the labeling.

Cytologic samples in PreservCyt Solution (PC) intended for immunocytochemistry staining using CINtec *PLUS* Cytology test can be stored at room temperature (15°C to 30°C) for 6 weeks followed by 12 additional weeks refrigerated at 2°C to 8°C.

Dried (i.e., air-dried) slides can be stored at room temperature protected from light and should be stained with CINtec *PLUS* Cytology test within seven days after slide preparation.

Test Controls

Each staining run will include one control slide prepared from control material known to have both dual-stain positive and negative elements such as a high-grade intraepithelial lesion (HSIL) slide that has dual-stain positive epithelial cells. The superficial cells of the squamous epithelium which is known to be negative for the expression of both p16 and Ki-67 will serve as the negative control.

Principles of Procedure

CINtec *PLUS* Cytology staining will be performed by laboratory personnel trained in the use of the CINtec *PLUS* Cytology test and in operation of the BenchMark ULTRA instrument. The CINtec *PLUS* Cytology test requires one slide per case and one control slide per staining run. Following cell conditioning, inhibition of endogenous peroxidase activity and incubation with the primary antibody cocktail, the assay uses two ready-to-use detection systems optimized for use on cervical cytology specimens:

- a goat anti-mouse secondary antibody covalently attached to HQ haptens (proprietary hapten) and an anti-HQ hapten, horseradish peroxidase (HRP)-conjugated tertiary antibody, optimized for the detection of the monoclonal mouse antibody clone E6H4;
- a goat anti-rabbit secondary antibody covalently attached to NP haptens (proprietary hapten) and an anti-NP hapten, alkaline-phosphatase (AP)-conjugated tertiary antibody, optimized for the detection of the rabbit recombinant antibody clone 274-11AC3V1.

The chromogenic reactions are based on the HRP-mediated conversion of DAB resulting in a brown precipitate at the $p16^{INK4a}$ antigen site and the AP-mediated conversion of Fast Red with Naphthol Phosphate resulting in a red precipitate at the Ki-67 antigen site.

Hematoxylin counterstains the cytoplasm and nuclei of all cells with a blue color. The cytoplasm and nuclei of the superficial squamous cells that do not stain with p16 and/or Ki-67 can be used as a reference to compare staining intensity of other cells with specific p16 and Ki-67 staining. After automated counterstaining (i.e. using hematoxylin and bluing reagent), a two-step mounting procedure is followed. First, the slide is mounted using an aqueous mounting medium. Subsequently, the slide is coverslipped using a permanent mounting medium. The staining results are evaluated by a cytotechnologist and/or a pathologist by light microscopy. See device package insert (PI) for additional details. The staining protocol is provided in Table 3 below.

Table 3: Staining Protocol for CINtec *PLUS* Cytology on the BenchMark ULTRA Instrument

Protocol Step	Incubation Time
Baking	Not applicable
Deparaffinization	Not applicable
Cell Conditioning – CC1	16 minutes
Primary Antibody Cocktail (p16/Ki-67)	16 minutes
DAB anti-Mouse HQ Linker	12 minutes
DAB HRP Multimer	8 minutes
DAB detection	8 minutes
Red anti-rabbit NP Linker	8 minutes
Red AP Multimer	8 minutes
Red detection	16 minutes
Hematoxylin	8 minutes
Bluing	4 minutes

Interpretation of CINtec *PLUS* Cytology Staining

Control Slide

After a CINtec *PLUS* Cytology staining run is complete and before case slides are evaluated, a cytotechnologist or pathologist will assess the run control slide to determine whether it is valid, as defined in Table 4 below.

Table 4: Assessment of Run Control Slides, CINtec *PLUS* Cytology Staining

Control	Valid	Invalid
Positive Elements	At least one cell has both specific red nuclear staining and specific brown cytoplasmic staining	No cells have specific red nuclear staining and specific brown cytoplasmic staining
Negative Elements	Cell types known to be negative for expression of p16 and of Ki-67 (such as superficial squamous epithelial cells) show neither non-specific red nuclear staining nor non-specific brown cytoplasmic staining that interferes with interpretation	Cell types known to be negative for expression of p16 and of Ki-67 (such as superficial squamous epithelial cells) show non-specific red nuclear staining and/or non-specific brown cytoplasmic staining that interferes with interpretation

If the control slide is valid, then case slides stained on that run can be evaluated. If the control slide fails to demonstrate appropriate positive staining elements, but the individual case slides have internal positive cells showing specific red Ki-67 and/or brown p16 staining, that case slide will be considered valid for evaluation. If the control slide fails to demonstrate appropriate positive staining elements, individual case slides that do not contain internal positive cells shall be re-tested to confirm a negative result. If the

control slide shows unacceptable non-specific staining of negative elements, then troubleshooting of certain staining factors such as instrument performance should be investigated. Individual case slides contain internal negative elements which allow for assessment of appropriate staining. Individual case slides should be evaluated and those with unacceptable staining of negative elements that interferes with interpretation should be re-tested.

Definition of a Satisfactory/Unsatisfactory Slide

Similar to screening Pap cytology slides, CINtec *PLUS* Cytology slides should be assessed for specimen adequacy similar to the criteria described in The Bethesda System for Reporting Cervical Cytology. This assessment should be done in the context of definitions of satisfactory and unsatisfactory slides specific for CINtec *PLUS* Cytology, which are given in Table 5 below.

Table 5: Definitions of Satisfactory and Unsatisfactory CINtec *PLUS* Cytology Slides

Satisfactory Slide	Unsatisfactory Slide
All of the following will apply: 1. Slides exhibit satisfactory squamous cellularity* OR a dual-stained cell is present on the slide AND 2. Less than or equal to 75% of squamous cells are obscured (e.g., bacteria, mucus, etc. interferes with interpretation) OR a dual-stained cell is present on the slide AND 3. Background is graded as acceptable	Any of the following apply: 1. Slide does not exhibit satisfactory squamous cellularity* AND no dual-stained cell has been identified OR 2. More than 75% of squamous cells are obscured (e.g., bacteria, mucus, etc. interferes with interpretation) AND no dual-stained cell has been identified OR 3. Background is graded as unacceptable

* Adequate CINtec *PLUS* Cytology slides should have an estimated minimum of 5,000 total well-visualized/ well-preserved squamous epithelial cells

Identification of Dual-Stained Cells

CINtec *PLUS* Cytology-stained slides with acceptable background will then be assigned a CINtec *PLUS* Cytology test result of positive, negative, or unsatisfactory according to the criteria defined in Table 6 below.

Table 6: Criteria to Assess p16/Ki-67 Dual-Staining Using CINtec *PLUS* Cytology

CINtec *PLUS* Cytology Test Result	Staining Description
Positive	Presence of at least one dual-stained cervical epithelial cell Dual-stained cervical epithelial cells may be present in sheets, overlapping clusters, or isolated single cells
Negative	Sample meets squamous cellularity criteria* -AND- Cervical epithelial cells staining: 1. Only brown for p16 (nuclear and/or cytoplasmic), or 2. Only red for Ki-67 (nuclear) 3. Only blue counterstain; but do not show dual p16 and Ki-67 staining in the same cell

CINtec *PLUS* Cytology Test Result	Staining Description
Unsatisfactory	Sample does not meet squamous cellularity criteria* and no dual-stained cells are identifiable -OR- More than 75% of squamous cells are obscured (e.g., bacteria, mucus, etc. interferes with interpretation) and no dual-stained cells are identifiable -OR- Sample has unacceptable background that interferes with staining interpretation

* Adequate CINtec *PLUS* Cytology slides should have an estimated minimum of 5,000 total well-visualized/ well-preserved squamous epithelial cells. See CINtec *PLUS* Cytology Interpretation guide for additional information

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunocytochemistry assay available for the simultaneous detection of the p16 and Ki-67 proteins in alcohol-fixed cervical cytology specimens.

According to current interim guidelines for primary HPV (Human Papilloma virus) screening for cervical cancer in the US and the FDA-approved use of the cobas HPV Test (P100020) in primary HPV screening, an alternative approach for the triage of women ≥25 years with positive HPV test results is referral of HPV Type 16 genotype positive (HPV16+) or HPV Type 18 genotype positive (HPV18+) women to colposcopy, with Pap cytology triage of women positive for 12 Other HR HPV genotypes.

According to current consensus guidelines for managing abnormal cervical cancer screening tests, there are two alternative management approaches for women ≥30 years with normal Pap cytology and positive HPV test results in the Pap/HPV co-testing setting: (i) repeat co-testing at 12 months, or (ii) HPV DNA genotyping with referral of HPV16+ or HPV18+ women directly to colposcopy and repeat co-testing at 12 months in 12 Other HR HPV+ women. Both are considered acceptable patient management approaches.

The patient's age, medical history (including past HPV and Pap cytology status), and thorough physical examination will provide further information on a patient's risk of cervical disease, as well as the need for referral to colposcopy. The CINtec *PLUS* Cytology test should only be used in conjunction with this clinical information.

VII. MARKETING HISTORY

The product is currently distributed/marketed in fifty nine countries. The product has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently improper patient management decisions in cervical cancer.

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. Analytical Specificity

The CINtec *PLUS* Cytology consists of the p16^{INK4a} (E6H4) mouse monoclonal primary antibody which targets amino acids 144 to 151 of human p16 protein and the Ki-67 (274-11AC3V1-IgG) rabbit recombinant primary antibody which targets the epitope -LAGFKELF- of human Ki-67 protein.

The following studies were conducted with the p16^{INK4a} (E6H4) and the Ki-67 (274-11AC3V1-IgG) antibodies to establish antibody specificity.

a. Western Blot

p16 Primary Antibody: Western blot analysis was performed on whole cell lysates from four cell lines representing a range of staining intensities when stained with anti- p16^{INK4a}, and purified recombinant p16^{INK4a} and Trefoil Factor 3 (TFF3) proteins (negative control). The cell lines were as follows: High expression cell lines: HeLa (4+) and SK Mel 28 (3+); Moderate expression cell line: DU145 (2+); Negative expression cell line: MDA MB 231 (0+). The Western blot assay tested the ability of the antibody to specifically bind denatured p16^{INK4a} recombinant and endogenous proteins immobilized on a Polyvinylidene difluoride (PVDF) membrane.

Anti- p16^{INK4a} antibody detected one ~17 kD band corresponding to the expected molecular weight for endogenous p16INK4a protein in all three immunohistochemistry (IHC) positive cell lines. The intensity of the ~17 kD bandcorrelated with the IHC staining score, showing the strongest signal strength in HeLa (4+), decreasing in SK Mel 28 (3+), and the lowest in DU 145 (2+). No band was observed in the IHC negative cell line MDA MB 231. The antibody bound specifically to purified recombinant p16INK4a protein and not to an equivalent amount of unrelated recombinant protein TFF3.

Ki-67 Primary Antibody: Western blot analysis was performed on whole cell lysates prepared from L428 (a Hodgkin's lymphoma positive for Ki-67 antigen, DSMZ ATCC 197) and LNCaP (a prostate carcinoma cell line with low expression of Ki-67 protein, ATCC CRL-1740) cell lines, a purified recombinant TFF3 protein (negative control) and a Ki-67 protein fragment.

The Ki-67 primary antibody detected a ~350kD band corresponding to endogenously expressed Ki-67 protein in the Ki-67 high-expressing L428 cell lysate. No band in this size range was detected by Western blot in the Ki-67 low-expressing cell line LNCaP. A single band of ~95kD was detected in the lane loaded with recombinant Ki-67 protein fragment when probed with Ki-67 primary antibody consistent with the expected molecular weight for the recombinant Ki-67 protein fragment. No band was observed in the TFF3 lane.

b. Peptide inhibition studies

Analytical specificity of CINtec *PLUS* Cytology test for its corresponding epitope-specific peptides was evaluated to demonstrate decreased staining of p16^{INK4a} in the presence of a p16 epitope-specific peptide and decreased staining of Ki-67 in the presence of a Ki-67 epitope-specific peptide when compared to a no-peptide control. The primary antibody cocktail was diluted at a 1:1 volume ratio with p16-specific and Ki-67 specific peptide solutions with concentrations of molar ratios of approximately 1-fold, 10-fold, 100-fold, 1,000-fold and 10,000-fold molar excess of peptide compared to the final concentration of anti-p16 antibody in the solution ($2.6x10^{-8}$ M) and anti-Ki-67 antibody in the solution ($2x10^{-9}$ M). Two control solutions were used in the study. Undiluted primary antibody cocktail (optimal) and primary antibody cocktail diluted at a ratio 1:1 with the antibody diluent, because all peptide solutions contained only 50% of the primary antibody cocktail.

Fifty-two (52) slides were stained using the CINtec *PLUS* Cytology test kit on a BenchMark ULTRA instrument as follows:

- One slide from each specimen (three cervical cytology specimen pools and one CaSki cells) was stained with each solution (ten specific peptide solutions, two control solutions).

- One slide from each specimen was incubated with diluent only, which served as a negative control.

The slides were evaluated for stain intensity and background by a qualified reader. The p16 and Ki-67 staining intensities determined for the cervical cytology specimens and CaSki slides stained with diluted primary antibody cocktail solution were used as a reference, since this solution contains the same amount of both antibodies as the solutions containing p16-specific or Ki-67-specific peptides (50%).

The specificity of the anti-p16 antibody for the p16 protein and the Ki-67 antibody for the Ki-67 protein was demonstrated by decreased p16 and Ki-67 staining intensities when the slides were stained with primary antibody cocktail containing p16-specific and Ki-67-specific peptides. Complete inhibition of the p16 or Ki-67 staining was observed when the corresponding peptides were present in the solution. No inhibition of the p16 or Ki-67 staining was seen when the unrelated peptides were present in the solution.

2. Robustness

a. Guardbanding studies

Studies were performed to evaluate the robustness (guardbanding) of the CINtec *PLUS* Cytology device to variations in the staining protocol, instrument settings, and concentrations of manufactured reagents. Study sample characteristics are provided in Table 7 below.

Table 7: Guardbanding Studies - Sample Characteristics

Sample Category	Pap Category	Number of Samples for Low testing	Number of Samples for High Testing
Intermediate	NILM	10	10
	ASCUS	10	10
	LSIL	10	10
Positive	HSIL	30	30

One reader team was used for evaluation of guardbanding and robustness slides. A cytotechnologist completed a primary read including adequate cellularity, signal intensity, background, CINtec *PLUS* Cytology status, and whether a positive slide had <10 dual-stained cells. A pathologist completed the confirmation read by confirming all entries. Any responses that the confirmation reader disagreed with included a comment and a consensus discussion between the readers.

The conditions and parameters tested are provided in Table 8 below.

Table 8: Guardbanding Studies – Conditions and Parameters Tested

Test Category	Variable	Low Condition	Reference Condition	High Condition
Staining Protocol	Cell conditioning time	16 minutes	16 minutes	24 minutes
	Primary antibody time	12 minutes	16 minutes	20 minutes

Test Category	Variable	Low Condition	Reference Condition	High Condition
	Hematoxylin incubation time	4 minutes	8 minutes	12 minutes
Instrument Specifications	Reaction buffer (RB) volume*	300 µL	270 µL	240 µL
	Mixing flow rate	450 mL/min	480 mL/min	510 mL/min
Reagent Concentration	Reagent formulations	-2% of target concentrations	Target concentrations	+2% of target concentrations
Potential impact to functional staining		Reduced signal intensity (possible loss in sensitivity) Weak counterstain leading to perceived high background staining	n/a	Increased signal intensity Decreased specificity (increase in background) Nucleus appears black in color and poor staining quality of Ki-67 (red nuclear staining)

*Reaction buffer volume refers to the amount of reaction buffer applied to the slide prior to the reagent (in dispenser) application. The reagent is applied directly into this reaction buffer "puddle" and therefore is diluted based on the volume of reaction buffer in this specification. For this reason, a larger reaction buffer volume will give a small effective concentration of the reagent being dispensed and therefore is included in the "low" robustness setting.

Study results are provided in Tables 9 and 10 below.

Table 9: Pass Rates for HSIL Sample Category

Test Condition	Test Group	Metric	No. of Passing Slides	No. of Svaluable Slides	Pass Rate (CI)
Protocol Setting	Low test condition	p16 signal intensity	31	31	100 (89.0, 100)
		DAB background	31	35	88.6 (74.0, 95.5)
		Ki-67 signal intensity	31	31	100 (89.0, 100)
		Red background	31	31	100 (89.0, 100)
		Status	30	30	100 (88.6, 100)
	High test condition	p16 signal intensity	35	35	100 (90.1, 100)
		DAB background	35	35	100 (90.1, 100)
		Ki-67 signal intensity	35	35	100 (90.1, 100)

Test Condition	Test Group	Metric	No. of Passing Slides	No. of Svaluable Slides	Pass Rate (CI)
		Red background	35	36	97.2 (85.8, 99.5)
		Status	35	35	100 (90.1, 100)
Instrument Setting	Low test condition	p16 signal intensity	34	34	100 (89.8, 100)
		DAB background	34	35	97.1 (85.5, 99.5)
		Ki-67 signal intensity	34	34	100 (89.8, 100)
		Red background	34	34	100 (89.8, 100)
		Status	33	33	100 (89.6, 100)
	High test condition	p16 signal intensity	35	35	100 (90.1, 100)
		DAB background	35	36	97.2 (85.8, 99.5)
		Ki-67 signal intensity	35	35	100 (90.1, 100)
		Red background	35	35	100 (90.1, 100)
		Status	35	35	100 (90.1, 100)
Reagent Formulation	Low test condition	p16 signal intensity	35	35	100 (90.1, 100)
		DAB background	35	35	100 (90.1, 100)
		Ki-67 signal intensity	35	35	100 (90.1, 100)
		Red background	35	35	100 (90.1, 100)
		Status	34	34	100 (89.8, 100)
	High test condition	p16 signal intensity	36	36	100 (90.4, 100)
		DAB background	36	36	100 (90.4, 100)
		Ki-67 signal intensity	36	36	100 (90.4, 100)
		Red background	36	36	100 (90.4, 100)
		Status	36	36	100 (90.4, 100)

Table 10: Pass Rates for Intermediate Sample Category

Test Condition	Test Group	Metric	No. of Passing Slides	No. of Svaluable Slides	Pass Rate (CI)
Protocol setting	Low test condition	p16 signal intensity	24	24	100 (86.2, 100)
		DAB background	24	29	82.8 (65.5, 92.4)
		Ki-67 signal intensity	24	24	100 (86.2, 100)
		Red background	24	24	100 (86.2, 100)
		Status	20	23	87.0 (67.9, 95.5)
	High test condition	p16 signal intensity	31	31	100 (89.0, 100)
		DAB background	31	31	100 (89.0, 100)
		Ki-67 signal intensity	31	31	100 (89.0, 100)
		Red background	31	31	100 (89.0, 100)
		Status	29	31	93.5 (79.3, 98.2)
Instrument Setting	Low test condition	p16 signal intensity	28	28	100 (87.9, 100)
		DAB background	28	29	96.6 (82.8, 99.4)
		Ki-67 signal intensity	28	28	100 (87.9, 100)
		Red background	28	29	96.6 (82.8, 99.4)
		Status	23	27	85.2 (67.5, 94.1)
		p16 signal intensity	30	30	100 (88.6, 100)

Test Condition	Test Group	Metric	No. of Passing Slides	No. of Svaluable Slides	Pass Rate (CI)
	High test condition	DAB background	30	32	93.8 (79.9, 98.3)
		Ki-67 signal intensity	30	30	100 (88.6, 100)
		Red background	30	31	96.8 (83.8, 99.4)
		Status	26	30	86.7 (70.3, 94.7)
		P16 signal intensity	30	30	100 (88.6, 100)
Reagent Formulation	Low test Condtion	DAB background	30	30	100 (88.6, 100)
		Ki-67 signal intensity	30	30	100 (88.6, 100)
		Red background	30	30	100 (88.6, 100)
		Status	26	29	89.7 (73.6, 96.4)
	High test condition	p16 signal intensity	32	32	100 (89.3, 100)
		DAB background	32	32	100 (89.3, 100)
		Ki-67 signal intensity	32	32	100 (89.3, 100)
		Red background	32	33	97.0 (84.7, 99.5)
		Status	25	32	78.1 (61.2, 89.0)

b. Slide Preparation and Coverslipping Robustness Studies

This study was performed to demonstrate the following:

- Ability of CINtec *PLUS* Cytology to achieve equivalent performance on at least two slide types (Arcless ThinPrep slides and Superfrost *PLUS* slides).
- Acceptable performance of various pre-staining drying times (60 minutes [recommended], 45 min and 30 min) for test slide thas is prepared before performing the CINtec *PLUS* Cytology test.
- The robustness of post-processing aqueous mounting medium drying time post CINtec *PLUS* Cytology test (60 min [recommended], 50 min, 40 min and 30 min) to provide acceptable performance.

Stained ThinPrep slides were assessed for signal intensity, cellularity adequacy, background, and CINtec *PLUS* Cytology test status (positive or negative), by a single cytotechnologist reader. The sample size and study characteristics are provided in Table 11 below.

Table 11: Number of Cases and Slides for Each Test Condition

Number of Cases	Test condition	Number of slides
20	Slide type assessment (ThinPrep Arcless)	20
	Slide type assessment (Superfrost *PLUS*)	20
	Cover-slipping robustness for 60 minutes pre-staining	20
	Cover-slipping robustness for 45 minutes pre-staining	20
	Cover-slipping robustness for 30 minutes pre-staining	20
20	Cover-slipping robustness for 60 minutes post-processing	20
	Cover-slipping robustness for 50 minutes post-processing	20
	Cover-slipping robustness for 40 minutes post-processing	20
	Cover-slipping robustness for 30 minutes post-processing	20

Results are as follows:

Recommended Condition Pass Rate for ThinPrep Arcless Slides:

The recommended condition of drying the ThinPrep Arcless slide for 60 minutes at room temperature after fixation in ethanol provided a passing rate of 100% (20/20). The post-process method of coverslipping using CC/Mount™ aqueous mounting medium (CC/Mount) and then drying for 60 minutes at 60°C in the oven provided a passing rate of 90.0%

(18/20). The two slides that failed at the recommended condition were due to higher DAB background which is not an anticipated or plausible failure mode for the conditions being tested.

Superfrost *PLUS* Slides Pass Rate:
The coverslipping robustness pass rate for the Superfrost *PLUS* Slides was 95.0% (19/20). The one failed slide that failed was due to DAB background, which is not an anticipated or plausible failure mode for the conditions being tested.

Pre-processing Slide Equivalency for ThinPrep Arcless Slides:
Drying the slide for 30 minutes or 45 minutes at room temperature afterfixation in ethanol were the two test conditions for pre-processing. There were 20 ThinPrep Arcless slides prepared and stained for each of the two testing conditions. Both test conditions had an equivalency rate of 95.0 % (19/20).

Post-processing Slide Equivalency for ThinPrep Arcless Slides:
Three testing conditions post-processing were considered as follows: (1) dry the slide for 30 minutes, (2) 40 minutes, and, (3) 50 minutes at 60°C after processed with CC/Mount. The 30-minute post-processing had an equivalency rate of 88.9% (16/18) and did not meet the passing endpoint of 90%, 40-minute post-processing equivalency rate was 94.4% (17/18), and 50-minute post-processing equivalency rate was 100% (18/18).

CINtec *PLUS* Result Concordance Between the Recommended and Test Conditions: From all the testing conditions, 30-minute pre-staining had a concordance of 100% (20/20) for CINtec *PLUS* result. The 40 and 50-minute poststaining concordance were 100% (20/20). The 45-minute pre-staining dry time was 95% (19/20) and the 30-minute post-staining was 99.4% (17/18) for CINtec *PLUS* result concordance.

Since the 30-minute dry time failure, it is recommended that the pre- and post-staining drying conditions remain at 60 minutes in a 60°C oven pre-staining (or overnight at room temperature), and 60 minutes at room temperature for post-staining.

c. Effectiveness of Recommended Control Sample

This study was performed to evaluate the effectiveness of control samples to detect assay-related failures. The control samples are ThinPrep patient samples that are known to be positive for CINtec *PLUS* Cytology and have acceptable background. The ThinPrep samples may be used as controls either as individual cases or in a sample pool where multiple patient cases are pooled together for the purpose of creating more slides per sample. Twenty (20) control sample pools created from qualified individual patient samples were used in this study. The test condition levels and paramenters assessed are shown in Table 12 below:

Table 12: Test Condition Levels, Settings and Number of Pools

Category	Sub-category	Low Severe (10 pools)	Low Moderate (20 pools)	Target (20 pools)	High Moderate (20 pools)	High Severe (10 pools)
Instrument	Rate of Mixing	7 mL/min	225 mL/min	480 mL/min	805 mL/min	1100 mL/min
	Puddle Volume	0.500g	0.400g	0.270g	0.190g	0.190g

Category	Sub-category	Low Severe (10 pools)	Low Moderate (20 pools)	Target (20 pools)	High Moderate (20 pools)	High Severe (10 pools)
Instrument	Reaction buffer (RB) formulation change	Not Tested	Not Tested	Bulk RB formulate to design	Half water and Half Reaction Buffer	Water only (in place of RB)
Reagent Dispense	Dispense	Missed dispense (0 µL)	Partial dispense (50 µL)	1 complete dispense (100 µL)	2 dispenses (200 uL)	4 dispenses (400 uL)

- Five severe conditions were tested using slides prepared from 10 pools, five moderate conditions were tested using slides prepared from 20 pools.
- One test slide made from each pool was evaluated using each condition. In addition, one slide from each pool was stained using recommended target conditions (reference slide).
  The results showed that three of the five severe conditions caused assay failure and the other two conditions were not severe enough to drive the assay to failing results despite being at the uppermost limits of the instrument.
- All 10 pools tested under the instrument category for the high/severe reaction buffer condition (RB) had a failing rate of 100% (10/10).
- All 10 pools tested under the instrument category for the mixers/puddle volume at high/severe conditions had a failing rate of 100% (10/10).
- All 10 pools tested under the reagent dispense category for low/severe conditions had a failing rate of 100% (10/10).
- For two out of the five severe conditions, the 10 pools passed and had appropriate positivity of CINtec *PLUS* Cytology stain and background acceptability. These included high/severe conditions for mixers/puddle volume and high/severe conditions for reagent dispense.

All 20 pools tested under target and moderate conditions had a passing rate of 100% (20/20).

3. Interference

The effect of exogenous and endogenous substances on the performance of the CINtec *PLUS* Cytology device was evaluated. One hundred twenty (120) ThinPrep (TP) Pap test samples qualified for this study (qualified as HSIL, satisfactory for cellularity, acceptable for background, and acceptable for obscuring elements) were treated with different potentially interfering substances and/or re-processed with glacial acetic acid (GAA) in order to assess the effect on CINtec *PLUS* Cytology test results. From the 120 individual patient samples included in the study, a total of 60 cases were selected for testing with whole blood and 10 cases were selected for testing with each of the other 6 interfering substances specified in Table 13 below. Each case was tested with CINtec *PLUS* cytology prior to the addition of the interfering substance as a reference for assay/sample performance. Once each case had a reference slide prepared and stained with CINtec *PLUS* Cytology device, its respective interfering substance was added to the sample and a slide was then prepared from each vial using the TP 2000 processor and stained with the CINtec *PLUS* Cytology device.

Table 13: Interfering Substances Sample Set

Pap Cytology	Interfering Substance (amount added to vial)	Number of Sample Cases	Number of slides (includes 1 test slide and 1 Reference slide per sample)	Number of Reprocessed Slides	Total Number of slides
HSIL	Whole blood (1% of vial volume)	60	120	60	180
HSIL	Mucus (250µL)	10	20	10	30
HSIL	Leukocytes (1% of vial volume)	10	20	NA	20
HSIL	Vaginal Douche (75µL)	10	20	NA	20
HSIL	anti-Fungal Cream (18mg-22mg)	10	20	NA	20
HSIL	Vaginal Lubricant (30mg-35mg)	10	20	NA	20
HSIL	Vaginal Deodorant (30mg-35mg)	10	20	NA	20

Results are provided in Table 14 below.

Table 14: Interfering Substance Study Results

Interfering Substance		Number of Slides				Pass Rate (Two-sided 95% CI)
		Total	Positive	Negative	Invalid	
Whole blood	Before GAA treatment	60	19	0	41	31.7% (21.3, 44.2)
	After GAA treatment	60	60	0	0	100% (94.0, 100)
Mucus	Before GAA treatment	10	0	0	10	0% (0, 27.75)
	After GAA treatment	10	4	0	6	40% (16.82, 68.73)
Leukocytes		10	8	0	2	80% (49.02, 94.33)
Vaginal Douche		10	0	0	0	100% (72.25, 100)

Interfering Substance	Number of Slides				Pass Rate (Two-sided 95% CI)
	Total	Positive	Negative	Invalid	
anti-Fungal Cream	10	0	0	0	100% (72.25, 100)
Vaginal Lubricant	10	9	0	1	90% (59.58, 98.21)
Vaginal Deodorant	10	8	0	2	80% (72.25, 100)

Based on the above, the presence of leukocytes, mucus or vaginal deodorant in the sample are a limitation to the performance of the CINtec *PLUS* Cytology device.

4. Precision

The precision of the CINtec *PLUS* Cytology device was evaluated on a cohort of pooled ThinPrep Pap test samples (ThinPrep pools) that represent the possible biological variability that can be observed in clinical practice. The device precision was assessed by testing duplicate slides from each cervical cytology sample pool (within-run), over five non-consecutive days on one BenchMark ULTRA instrument (between-day) and with multiple lots of reagents (between-lot). The resulting slides were evaluated for CINtec *PLUS* Cytology test status (positive or negative) by three teams (one cytotechnologist and 1 pathologist per team) of readers (between-reader). A subset of these slides were read a second time by the same teams of readers (within-reader precision) after a two week wash out period.

Twelve (12) ThinPrep pools were created (Table 15) to test the precision of the device. Nine (9) ThinPrep pools were created from individual patient samples and 3 pools were created from T24 cell lines prepared in PreservCyt® solution (i.e. ThinPrep Pap test collection media). Six (6) different intermediate pools were created (see Table 15). In order to prepare an adequate volume for multiple replicates (slides) in this study, 8 individual patient samples were pooled together to create each pool in the intermediate and true positive pool categories. Therefore these pools may have additional variability beyond the variability from CINtec *PLUS* Cytology preparation and reading. The patient samples were selected based on ThinPrep Pap test and cobas® 4800 HPV test status. Specimens that demonstrated adequate cellularity and the absence of obscuring elements on Pap test were included in the study.

Table 15: Sample Pools, Precision Study

Cell Type/ Pap Cytology Status	HR-HPV (cobas) Status	Pool Category	Number of Pools
T24 Cell line	Negative	True Negative	3
NILM	Negative	Intermediate (Patient-specimen Derived Negative)	3
NILM	Positive	Intermediate	1
ASC-US	Positive		1
LSIL	Positive		1
HSIL	Positive	True Positive	3

NILM - Negative for intraepithelial lesion or malignancy; ASC-US - Atypical squamous cells of undetermined significance; LSIL - Low-grade intraepithelial lesion; HSIL - High grade intraepithelial lesion

A total of 30 slides were prepared across multiple days from each pool with 10 slides allocated to each of the 3 CINtec *PLUS* Cytology reagent lots. Three runs were performed on each day and each run included 2 replicates stained with one of 3 randomly selected lots.

Each of 30 slides was read by 3 reader teams consisting of 1 cytotechnologist and one pathologist in randomized order. Each cytotechnologist evaluated background acceptability, indicated test result as positive or negative for the presence of p16/Ki-67 dual-stain cell(s) and the number of dual-stained cells present up to a count of 50 cells.

Analysis was performed on each cervical cytology pools by determining the pool-level mode across the sources of variability tested (majority call), then calculating the percent of results same as majority call. Each source of variability was studied independently and total imprecision was also calculated. In addition, background acceptability was calculated across all slides stained and dual-stained (positive) cells were counted in all pools from all categories in order to characterize any relationship between Pap/HPV category and CINtec *PLUS* Cytology dual-stained cell counts.

Within laboratory precision study results are provided in the Tables 16 through 19 below.

Table 16: Within-Laboratory Precision Study - Overall Precision

Pool Category	Number of Slides/reads	Mode of CINtec *PLUS* Cytology Results (majority call)	Percent of Results Same as Majority Call	95% Confidence Interval
T24 Cell Line	270	Negative	100%	(98.6, 100)
NILM/HPV-	270	Negative	94.1%	(90.7, 97.0)
NILM/HPV+	90	Positive	61.1%	(47.2, 74.4)
ASCUS/HPV+	90	Positive	93.3%	(88.9, 97.8)
LSIL/HPV+	90	Positive	100%	(95.9, 100)
HSIL/HPV+	270	Positive	98.9%	(96.7, 100)

Table 17: Within-Laboratory Precision Study - Between-Day Precision

Pool Category	Number of Slides/reads	Mode of CINtec *PLUS* Cytology Results (majority call)	Percent of Results Same as Majority Call				
			Day 1	Day 2	Day3	Day 4	Day 5
T24 Cell Line	270	Negative	100%	100%	100%	100%	100%
NILM/HPV-	270	Negative	88.9%	90.7%	98.1%	98.1%	94.4%
NILM/HPV+	90	Positive	44.4%	50.0%	77.6%	66.7%	66.7%
ASCUS/HPV+	90	Positive	94.4%	88.9%	88.9%	94.4%	100%
LSIL/HPV+	90	Positive	100%	100%	100%	100%	100%
HSIL/HPV+	270	Positive	100%	94.4%	100%	100%	100%

Table 18: Within-Laboratory Precision Study – Between-Lot Precision

Pool Category	Number of Slides/reads	Mode of CINtec *PLUS* Cytology Results (majority call)	Percent of Results Same as Majority Call		
			Lot 1	Lot 2	Lot 3
T24 Cell Line	270	Negative	100%	100%	100%
NILM/HPV-	270	Negative	92.2%	93.3%	96.7%
NILM/HPV+	90	Positive	46.7%	70.0%	66.7%
ASCUS/HPV+	90	Positive	90.0%	96.7%	93.3%
LSIL/HPV+	90	Positive	100%	100%	100%
HSIL/HPV+	270	Positive	100%	100%	96.7%

Table 19: Within-Laboratory Precision Study - Between-Reader Precision

Pool Category	Number of Slides/reads	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call		
			Reader Team 1	Reader Team 2	Reader Team 3
T24 Cell Line	270	Negative	100%	100%	100%
NILM/HPV-	270	Negative	95.6%	91.1%	95.6%
NILM/HPV+	90	Positive	70.0%	63.3%	50.0%
ASCUS/HPV+	90	Positive	100%	90.0%	90.0%
LSIL/HPV+	90	Positive	100%	100%	100%
HSIL/HPV+	270	Positive	98.9%	98.9%	98.9%

5. Reproducibility

The primary objective of this study was to evaluate the reproducibility of the CINtec *PLUS* Cytology test in the detection of p16/Ki-67 dual-staining of cervical epithelial cells in liquid based cytology (LBC) specimens at 3 different sites.

The study used a specimen pooling strategy in which individual, de-identified, residual ThinPrep liquid-based cytology specimens of the same HPV status (positive vs negative) and same Pap cytology result (NILM, ASC-US, LSIL, and HSIL) were combined to make specimen pools that had adequate volume to support the study design. Fourteen (14) different intermediate pools were created. In order to prepare an adequate volume for multiple replicates (slides) in this study, 15 individual patient samples were pooled together to create each pool in the intermediate and true positive categories. Therefore these pools may have additional variability beyond the variability from CINtec *PLUS* Cytology preparation and reading. The study included 21 specimen pools (Table 20) and 6 distinct cultures of T24 cells (a cell line that is negative for p16/Ki-67 dual-staining), for a total sample size of 27.

Table 20: Specimen Pool Characteristics

Sample Type	Dual-Staining Category	Pap Cytology	HPV Status	Anticipated CINtec *PLUS* Cytology Test Result	Number
Cell Line	True Negative (T24 cells)	N/A	N/A	Negative	6 cultures
LBC Specimens	Intermediate	NILM	Negative	Positive or Negative	3 pools
		NILM	Positive	Positive or Negative	3 pools
		ASC-US	Positive	Positive or Negative	4 pools
		LSIL	Positive	Positive or Negative	4 pools
LBC Specimens	True Positive	HSIL	Positive	Positive	7 pools

Aliquots of sufficient volume of each specimen pool (21 in total), T24 cell culture (6 in total), was created for testing at each site. Two study sites prepared a single slide from each specimen pool, T24 culture (27 slides in total) on a single slide processor (Site 1: ThinPrep 2000 Processor and Site 2: ThinPrep 5000 Processor). At Site 3, one set of slides was prepared on ThinPrep 2000 processor and one set of slides was prepared on ThinPrep 5000 processor. The reproducibility study was conducted for 5 days. On each day, each site stained one set of 27 slides using a BenchMark ULTRA instrument. The 5 staining days were non-consecutive and spanned at least 20 days.

At each site, 2 reader-teams, each consisting of a cytotechnologist and a pathologist, independently evaluated the slides stained at their site for the presence or absence of dual staining and assigned the slide a CINtec *PLUS* Cytology test result of positive, negative, or invalid. The reader-teams were blinded to any prior determination of HPV status, Pap cytology status, CINtec *PLUS* Cytology test results from other reader teams, or other clinical information.

Results of the reproducibility studies are provided in the Tables 21and 22 below.

Table 21: Percent Same Result by Pool Category

Pool Category	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent Same Result as Majority Call		
		%	(n/N)	95%
T24 Cells (True Negative)	Negative	100%	240/240	(98.4, 100)
NILM/HPV-	Negative	94.2%	113/120*	(90.0, 100)
NILM/HPV+	Positive	65.8%	79/120	(50.0, 77.5)
ASC-US/HPV-	Positive	83.5%	132/158	(71.3, 96.2)
LSIL/HPV+	Positive	88.1%	140/159	(81.9, 96.2)
HSIL/HPV+ (True Positive)	Positive	100%	280/280	(98.6, 100.0)

* After all slides at a site had been assigned a CINtec *PLUS* Cytology test result, the slides were shipped back to the Sponsor. A Sponsor's reader was provided with all evaluable slides from the 3 NILM/HPV-negative pools stained at the sites. The Sponsor's reader evaluated these slides to record the number of dual-stained cells present on each slide. Four out of 60 slides prepared from the 3 NILM/HPVnegative pools had dual stained cells; Three slides had one dual-stained cell and 1 slide had 2 dual-stained cells.

Table 22: Between-Site Reproducibility Study Results

Pool Category	Number of Slides/reads	Mode of CINtec *PLUS* Cytology Results (majority call)	Percent of Results Same as Majority Call		
			Site 1	Site 2	Site 3
T-24 Cell Line	270	Negative	100%	100%	100%
NILM/HPV-	270	Negative	100%	86.7%	95.0%
NILM/HPV+	90	Positive	60.0%	60.0%	71.7%
ASCUS/HPV+	90	Positive	72.5%	82.5%	89.7%
LSIL/HPV+	90	Positive	85.0%	77.5%	94.8%
HSIL/HPV+	270	Positive	100%	100%	100%

6. Stability Studies

a. Sample stability

This study was performed to evaluate the stability of the epitopes in cervical cytology specimens collected and transported in ThinPrep vials containing PreservCyt media and stored for at least 18 weeks, including 6 weeks at room temperature (15°C to 30°C) followed by 12 additional weeks refrigerated at 2°C to 8°C.

Twenty (20) ThinPrep LBC cervical specimens with adequate cellularity, acceptable backgrounds for both biomarkers and positive CINtec *PLUS* Cytology test were included in the study. One slide from each specimen was prepared and tested at the following testing time points: day zero, 6-week, 19-week. Stained slides were assessed by a qualified reader to determine acceptable cellularity, acceptable background for both biomarkers, positive CINtec *PLUS* Cytology test result. Sample stability study results are provided in Table 23 below.

Table 23 : Sample Stability Study Results

Assessment Parameter	6-wk Testing Time Point	19-wk Testing Time Point
CINtec *PLUS* result (same as for day 0 slide)	100%	100%
Background (score within 0.25 of day 0 slide	100%	95%
Staining intensity (score within 0.5 of day 0 slide	100%	100%
Adequate cellularity (per Bethesda System 2014)	100%	100%

The study supports sample stability for CINtec *PLUS* Cytology staining that has been stored for 6 weeks at room temperature (15 to 30ºC) followed by 12 weeks at 2-8ºC.

b. Prepared Slide stability

This study was performed to evaluate the ability of the CINtec *PLUS* Cytology test to achieve equivalent staining on freshly prepared ThinPrep slides (reference slides) and on ThinPrep slides stored protected from light (in slide folders with flaps closed) at room temperature for 7 days before staining with the CINtec *PLUS* Cytology device.

Twenty (20) ThinPrep liquid based cytology (LBC) cervical specimens that were categorized as HSIL/HPV+ were included in the study. Three slides were prepared from each specimen on Day 0 using the ThinPrep 2000 processor. Slides were then incubated in 99% ethanol solution for 15 minutes before being dried for 60 minutes at room temperature. Overall, 60 slides were created for the study. The first and third slides made from each specimen were stained on Day 0 with the CINtec *PLUS* Cytology test on the BenchMark ULTRA instrument. These slides were then evaluated by a qualified reader to determine if they met the following inclusion criteria for the study:

- Acceptable cellularity according to Bethesda-based method, acceptable backgrounds for both biomarkers within 0.25 point of each other
- Staining intensity scores for both biomarkers within 0.5 point of each other
- Positive CINtec *PLUS* Cytology test result

This approach provided assurance that if the readings for first and last slides made from each specimen are equivalent on Day 0, then any different result obtained for the test slides (stained at Day 8) are likely due to slide aging. The second slide from each specimen was stored in a controlled environment protected from light (in slide folders with flaps closed) at room temperature and stained with CINtec Cytology *PLUS* on Day 8. This slide was evaluated by the qualified reader and compared with the first slide that served as reference slide.

The results from this study demonstrated the following:

- For all 20 cases, staining performance was equivalent between slides stained after being stored for 7 days compared to reference slides stained on Day 0.
- All 20 slides tested after being stored for 7 days after slide preparation showed the same CINtec *PLUS* Cytology test result as reference slides,
  acceptable background within 0.25 point from reference slides, and
  staining intensities within 0.5 point for both biomarkers compared to reference slides and adequate cellularity.

Dried slides can be stored at room temperature protected from light and must be stained with the CINtec PLUS Cytology test within 7 days of preparation.

c. Reagent stability

Reagent stability study was performed to evaluate the interim reagent shelf life and in-use stability of the CINtec *PLUS* Cytology device. Testing evaluated staining performance (signal intensity and background) of the test on LBC cervical specimens using 3 final lots of the device which were held at the following storage and ship stress conditions and tested at specified intervals until 26 months or failure, whichever occured first:

- Intended Storage Condition: Test kits from each of the three lots placed at 2-8°C for the duration of testing
- Ship Stress:
  - Hot (Category A): Test kits from each of the three lots held at 33±3°C for 192 hours, then placed at 2-8°C for the duration of the study
  - Hot (Category B): Test kits from each of the three lots held at 18±3°C for 192 hours, then placed at 2-8°C for the duration of the study
  - Cold (Freeze/Thaw): Test kits from each of the three lots held at -20±5°C for 192 hours, then placed at 2-8°C for the duration of the study

At each specified time point, one reference slide and one test slide is created from each enrolled case. The reference slide is stained using a previously released lot of CINtec *PLUS* Cytology within expiration dating. Stained reference and test slides are then evaluated by qualified readers for adequate cellularity, signal intensity of both biomarkers (p16 and Ki-67), and background. Real-time stability study results are provided in Table 24 below.

Table 24: Real-Time Stability Testing Results

All Conditions			
Time Point	**Lot 1 (Pass/Fail/Invalid)**	**Lot 2 (Pass/Fail/Invalid)**	**Lot 3 (Pass/Fail/Invalid)**
Day 0	Positive (100%)	Positive (100%)	Positive (100%)
Month 10	Positive (100%)	Positive (100%)	Positive (100%)
Month 15	Positive (100%)	Positive (100%)	Positive (100%)

The study results support interim stability dating of 12 months and current shipping category B for the CINtec *PLUS* Cytology device.

B. Animal Studies

None

C. Additional Studies

ThinPrep 2000/ThinPrep 5000 Equivalency Study

The objective of this study was to verify the ability of the CINtec *PLUS* Cytology test to achieve equivalent staining on ThinPrep slides prepared on the ThinPrep 2000 and ThinPrep 5000 Processors. All samples were residual ThinPrep liquid-based cytology (LBC) cervical specimens. Specimens were acquired from previously approved external clinical laboratories. Three hundred forty-eight (348) specimens were enrolled in the study and 271 specimens were used for analysis. Samples were enrolled based on a cobas HPV test and a Pap cytology stain with results of NILM/HPV- or HSIL/HPV+. For half of the specimens tested, slide one was made using a T2000 processor and slide two was made on a T5000 processor. For the other half of specimens tested, slide one was made using a T5000 processor and slide two was made on a T2000 processor. A control slide was included in each CINtec *PLUS* Cytology run to ensure that the system worked as expected. The control slides were produced from known CINtec *PLUS* Cytology positive specimen pools and evaluated for a positive test result (positive control elements) and acceptable background (negative control elements) to determine staining run validity.

Study slides were evaluated by a reader team consisting of a cytotechnologist and a pathologist. The following parameters were assessed: slide background (non-specific staining), slide cellularity per the Bethesda System criteria, assessement for the presence of obscuring elements (≤75% or >75%), including mucus, bacteria, and inflammatory cells, which may interfere with the interpretation of the assay and assessement for the CINtec *PLUS* Cytology result. A CINtec *PLUS* Cytology test result was positive if a minimum of one p16/Ki-67 dual-stained cell was present. Study results are provided in the Table 25 below:

Table 25: CINtec *PLUS* Cytology Agreement Between Slide Processors

T5000 Slide CINtec *PLUS* *PLUS* Result	**T2000 Slide CINtec *PLUS***			**Agreement**		
	Positive	**Negative**	**Total**	**Measure**	**% (n/N)**	**95% CI**
Positive	133	7	140	PPA	91.1 (133/146)	(85.4, 94.7)
Negative	13	118	131	NPA	94.4 (118/125)	(88.9, 97.3)
Total	146	125	271			

X. SUMMARY OF PRIMARY CLINICAL STUDY

The applicant performed a clinical study in the US to establish a reasonable assurance of safety and effectiveness of the CINtec *PLUS* Cytology device for the following indications:

(a) To be used in women 25 - 65 years old with 12 Other high-risk (HR) HPV positive test results using the cobas® 4800 HPV Test in primary HPV screening, to determine the need for referral to colposcopy.

To be used in women 25 - 65 years old with HPV16/18 positive test results using the cobas® 4800 HPV Test in primary HPV screening where the CINtec PLUS Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

(b) To be used in women 30 - 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and 12 Other HR HPV positive test results using the cobas 4800 HPV Test in adjunctive cervical cytology and HR HPV screening, to determine the need for referral to colposcopy.

To be used in women 30- 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and HPV16/18 positive test results using the cobas® 4800 HPV Test in adjunctive cervical cytology and HR HPV screening where the CINtec PLUS Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

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

A multicenter, prospective study (IMPACT trial, IMproved Primary Screening And Colposcopy Triage) was conducted to evaluate the performance of the CINtec *PLUS* Cytology test as a triage test to stratify high-risk (HR) HPV-positive (HPV+) women 25–65 years old in primary HPV cervical cancer screening using the cobas 4800 HPV Test for referral to colposcopy. Furthermore, the study evaluated the performance of the CINtec *PLUS* Cytology test as a triage test to stratify women 30 - 65 years with NILM (negative for intraepithelial lesion or malignancy) Pap cytology and positive HPV test results by either the cobas 4800 HPV Test in adjunctive cervical cytology and HPV screening for referral to colposcopy.

Baseline Phase

The study enrolled 35,263 women 25 - 65 years undergoing routine cervical cancer screening in the US from September 2017 to October 2018 at 32 clinical sites in the Baseline Phase. A total of 34,914 women were eligible to participate in the study.

All women had one cervical sample collected in PreservCyt media at Study Visit 1 (SV1). The specimens were collected using a spatula/brush or broom-type device; approximately half of the specimens were collected with each device. Pap cytology and HPV testing by cobas 4800 HPV Test (Roche Molecular Systems, Inc.), were performed on the PreservCyt samples for all subjects at four testing sites. Pap cytology results were classified according to the criteria of The Bethesda System for Reporting Cervical Cytology. CINtec *PLUS* Cytology testing was performed on all women with cobas 4800 HPV positive results using the residual volume remaining in the PreservCyt cervical sample collected at SV1.

Women 25 - 65 years with positive cobas HPV Test results (positive by the cobas 4800 HPV Test) were invited to undergo colposcopy.

Colposcopy was performed at Study Visit 2 (SV2) with colposcopists blinded to the CINtec *PLUS* Cytology results. Colposcopy was conducted following the principles recommended by the American Society for Colposcopy and Cervical Pathology (ASCCP) as follows. Biopsies were obtained on all visible lesions, and a single random cervical biopsy was obtained from the squamocolumnar junction (SCJ) if no lesions were visible. Endocervical curettage was performed in all patients in whom the SCJ was not visualized or only partially visualized. All biopsies were examined by a Central Pathology Review (CPR) process which included up to three expert pathologists. Discordant histology results were adjudicated according to a pre-defined protocol. The slides that were prepared from the biopsies and reviewed by the CPR panel were stained with hematoxylin and eosin (H&E) and CINtec Histology (p16 IHC assay). The expert pathologist first evaluated H&E-stained slides to establish the CPRH&E reference diagnosis, then evaluated both H&E- and p16 histology-stained slides for that case to establish the CPRH&E+p16 reference diagnosis

The clinical performance of the CINtec *PLUS* Cytology test in HPV+ (cobas 4800 HPV+) women was assessed using CPR histology results as reference diagnoses at the clinical endpoints ≥CIN2 and ≥CIN3

1. Clinical Inclusion and Exclusion Criteria

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

   a. Female 25 - 65 years presenting for routine cervical cancer screening
   b. Intact cervix
   c. Willing and able to undergo colposcopy and biopsy (and possibly ECC) within 12 weeks from the date of the cervical sample collection
   d. Willing and able to provide written informed consent
   e. Willing and able to participate in the 1-year Follow-up Phase if required

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

   a. Known pregnancy
   b. Current or planned participation in another cervical cancer screening study or in a cervical treatment or vaccine study
   c. Incomplete informed consent
   d. Any medical condition that, in the opinion of the investigator, would result in increased risk of bleeding at biopsy
   e. Known history of ablative or excisional therapy (eg, LEEP, cone biopsy) within the past 12 months
   f. Known history of hysterectomy (including supracervical)

2. Follow-up Schedule

   All women from SV2 who had a diagnosis of <CIN2 by at least one CPR method and did not undergo treatment during the Baseline Phase were invited to participate in the Year 1–Follow-up Phase (N=6,242). Women who proceeded to the Follow-up Phase underwent Pap cytology and cobas HPV testing at Year 1–Visit 1, approximately one year after Baseline SV1. All women with NILM Pap cytology and negative cobas HPV Test results (both cobas 4800 HPV from Year 1–Visit 1 exited the study. Colposcopy and biopsy/ECC were performed only in non-pregnant women who remained in the study from Year 1–Visit 1. Adverse events and complications were recorded at all visits.

3. Clinical Endpoints

   With regards to safety, the incidence of serious adverse events and their potential relation to the investigational device were recorded and monitored during the IMPACT clinical study, and are presented later in this section. Potential safety issues regarding sampling and false positive and false negative test results are discussed in Section VIII.

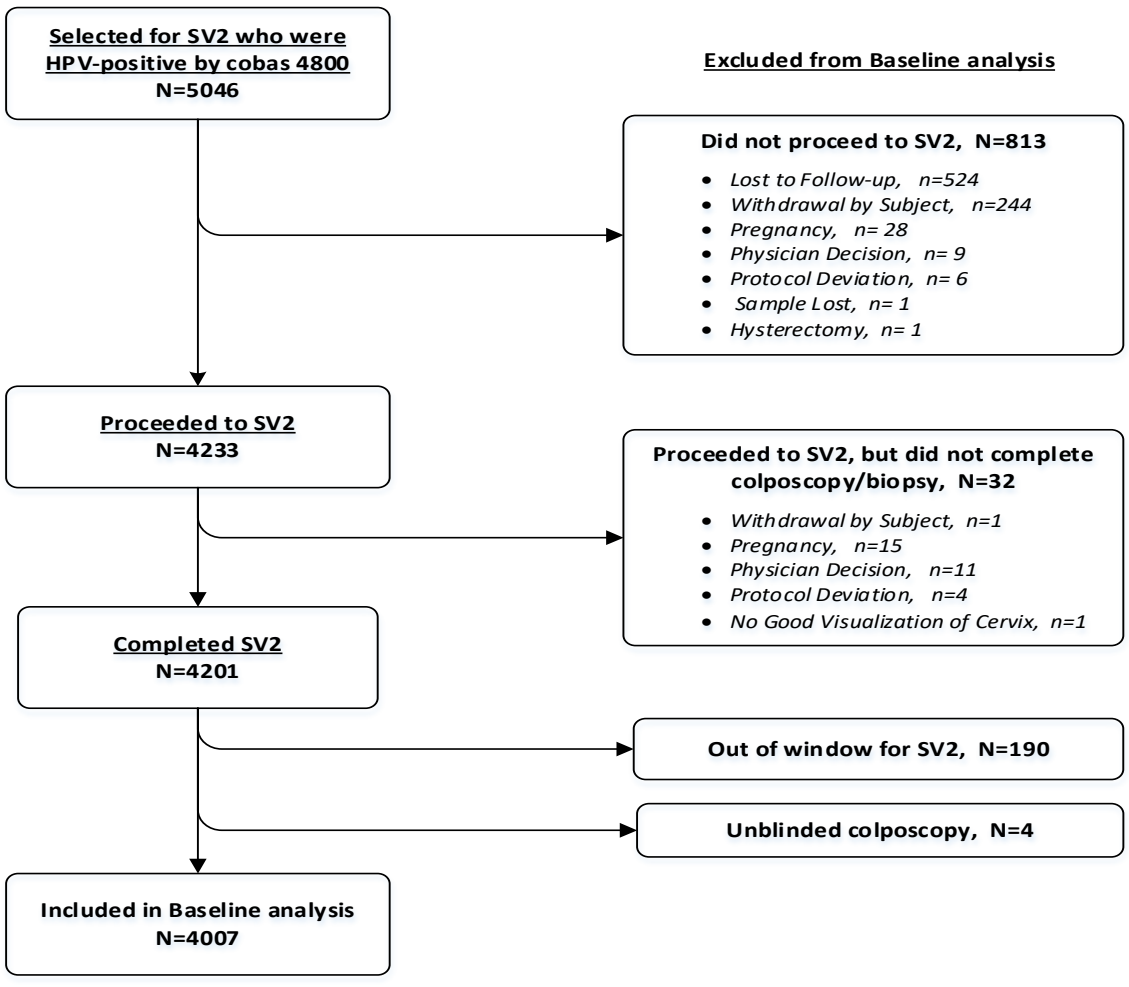
With regards to effectiveness, the clinical performance of CINtec *PLUS* Cytology in HR HPV+ women as tested by the cobas 4800 HPV test was assessed using central pathology review (CPR) histology results as reference diagnoses. The analyses were performed for the ≥CIN2 and ≥CIN3 disease thresholds, as determined by CPR.
With regard to an acceptable performance of CINtec *PLUS* Cytology in the IMPACT clinical study, performances of CINtec *PLUS* Cytology test was assessed by the risk (1−NPV) at the ≥CIN3 clinical endpoint, separately for women positive for HPV16/18 (HPV16/18+) and 12 Other HR HPV (12 Other HR HPV+).

B. Accountability of PMA Cohort

Of the 35,263 subjects enrolled in the study, 34,914 proceeded to Study Visit 1 (SV1). Of these subjects, 5,046 had cobas 4800 HPV positive resuts. These subjects were referred to colposcopy and and 4,201 proceeded to and completed Study Visit 2 (SV2).

The patient accountability chart is provided in Figure 2 below.

Figure 2: Baseline Analysis Population Disposition for cobas 4800 HPV+ Subjects



C. Study Population Demographics and Baseline Parameters

The demography of the study population is typical for a cervical cancer screening study performed in the US. Demographic characteristics of the intended use population is provided in the Table 26 below.

Table 26: Demographic Characteristics of Subjects in the Intended Use Populations

Demographic Characteristics	HPV+ women 25-65 years old	NILM/HPV+ women 30-65 years old
	cobas 4800 HPV+ (N=5046)	Cobas 4800 HPV+ (N=2212)
Age		
Mean (SD)	36.8 (10.3)	41.6 (9.4)
Median	34.0	39.0
Min, Max	25.0, 65.0	30.0, 65.0
Age Group		
25-29	1557 (30.9)	0 (0.0)
30-39	1869 (37.0)	1151 (52.0)
40-49	859 (17.0)	552 (25.0)
50-59	585 (11.6)	395 (17.9)
60-65	176 (3.5)	114 (5.2)
Ethnicity		
Hispanic or Latino	1325 (26.3)	562 (25.4)
Not Hispanic or Latino	3666 (72.7)	1629 (73.6)
Unknown	18 (0.4)	7 (0.3)
Not Reported	37 (0.7)	14 (0.6)
Race		
American Indian/Alaskan Native	30 (0.6)	16 (0.7)
Asian	95 (1.9)	42 (1.9)
Black/African-American	1308 (25.9)	601 (27.2)
Native Hawaiian/Pacific Islander	12 (0.2)	1 (0.0)
White	3387 (67.1)	1467 (66.3)
Other	144 (2.9)	65 (2.9)
Unknown/Not Reported	70 (1.4)	20 (0.9)
Highest Level of Education		
Elementary	203 (4.0)	110 (5.0)
High School (or GED)	1399 (27.7)	640 (28.9)
Vocational/Some College	1302 (25.8)	535 (24.2)
College Degree	1402 (27.8)	601 (27.2)
Some Graduate Work	87 (1.7)	32 (1.4)
Graduate Degree (Master's or Higher)	461 (9.1)	233 (10.5)
Unknown/Not Reported	192 (3.8)	61 (2.8)

D. Safety and Effectiveness Results

1. Safety Results

   The analysis of safety was based on the 5,046 patients. The key safety outcomes for this study (percent of false negative and percent of false positive) are presented in Tables below.

   Adverse effects that occurred in the PMA clinical study:

   There were no adverse events in the PMA clinical study.

2. Effectiveness Results

The analysis of effectiveness was based on the 5,046 evaluable patients. Key effectiveness outcomes (sensitivity, specificity, positive and negative likelihood ratios and positive and negative predictive values along with 95% confidence intervals) are presented in Tables 27 through 64 below.

EXPECTED RESULTS

A total of 35,263 women 25 - 65 years undergoing routine cervical cancer screening across 32 clinical sites were enrolled in the study. Of these, 5046 women had cobas 4800 positive results: 785 women had HPV 16+ results, 287 women had HPV18+ results and 3,974 women had 12 Other HR HPV+ results.

Tables 27 through 29 below show percent of positive, negative and invalid results of the CINtec *PLUS* Cytology test for women 25 - 65 years old stratified by testing site, by age group and by Pap cytology results

Table 27: Percent of CINtec *PLUS* Cytology Results for Women 25 - 65 Years Old Stratified by Testing Site

	cobas 4800 12 Other HR HPV+		
	CINtec *PLUS* Cytology Result		
Site	**Positive**	**Negative**	**Invalid**
Site 1	45.7% (315/690)	47.5% (328/690)	6.8% (47/690)
Site 2	47.4% (439/926)	47.5% (440/926)	5.1% (47/926)
Site 3	45.8% (697/1522)	49.2% (749/1522)	5.0% (76/1522)
Site 4	29.3% (245/836)	59.8% (500/836)	10.9% (91/836)
Combined	42.7% (1696/3974)	50.8% (2017/3974)	6.6% (261/3974)

	cobas 4800 HPV 16+			**cobas 4800 HPV 18+**		
	CINtec *PLUS* Cytology Result			**CINtec *PLUS* Cytology Result**		
Site	**Positive**	**Negative**	**Invalid**	**Positive**	**Negative**	**Invalid**
Site 1	61.7% (79/128)	29.7% (38/128)	8.6% (11/128)	51.2% (21/41)	43.9% (18/41)	4.9% (2/41)
Site 2	63.0% (114/181)	30.9% (56/181)	6.1% (11/181)	58.3% (35/60)	35.0% (21/60)	6.7% (4/60)
Site 3	65.7% (209/318)	30.2% (96/318)	4.1% (13/318)	57.7% (79/137)	38.7% (53/137)	3.6% (5/137)
Site 4	59.5% (94/158)	35.4% (56/158)	5.1% (8/158)	28.6% (14/49)	63.3% (31/49)	8.2% (4/49)
Combined	63.2% (496/785)	31.3% (246/785)	5.5% (43/785)	51.9% (149/287)	42.9% (123/287)	5.2% (15/287)

Table 28: Percent of CINtec *PLUS* Cytology Results for Women 25 - 65 Years Old Stratified by Age Group

	cobas 4800 12 Other HR HPV+					
Age Group (Years)	**CINtec *PLUS* Cytology Result**					
	Positive		**Negative**		**Invalid**	
25-29	46.6% (617/1324)		46.8% (620/1324)		6.6% (87/1324)	
30-39	42.5% (606/1427)		50.9% (727/1427)		6.6% (94/1427)	
40-49	36.1% (228/632)		58.1% (367/632)		5.9% (37/632)	
50-65	41.5% (245/591)		51.3% (303/591)		7.3% (43/591)	
Combined	42.7% (1696/3974)		50.8% (2017/3974)		6.6% (261/3974)	
Age Group (Years)	**cobas 4800 HPV 16+**			**cobas 4800 HPV 18+**		
	CINtec *PLUS* Cytology Result			**CINtec *PLUS* Cytology Result**		
	Positive	**Negative**	**Invalid**	**Positive**	**Negative**	**Invalid**
25-29	72.8% (131/180)	22.2% (40/180)	5.0% (9/180)	47.2% (25/53)	49.1% (26/53)	3.8% (2/53)
30-39	66.0% (217/329)	29.2% (96/329)	4.9% (16/329)	52.2% (59/113)	41.6% (47/113)	6.2% (7/113)
40-49	49.4% (81/164)	42.7% (70/164)	7.9% (13/164)	49.2% (31/63)	46.0% (29/63)	4.8% (3/63)
50-65	59.8% (67/112)	35.7% (40/112)	4.5% (5/112)	58.6% (34/58)	36.2% (21/58)	5.2% (3/58)
Combined	63.2% (496/785)	31.3% (246/785)	5.5% (43/785)	51.9% (149/287)	42.9% (123/287)	5.2% (15/287)

Table 29: Percent of CINtec *PLUS* Cytology Results for Women 25 - 65 Years Old Stratified by Pap Cytology Results

	cobas 4800 12 Other HR HPV+		
	CINtec *PLUS* Cytology Result		
Pap Cytology	**Positive**	**Negative**	**Invalid**
NILM	29.9% (775/2590)	62.3% (1614/2590)	7.8% (201/2590)
ASC-US	58.6% (372/635)	39.1% (248/635)	2.4% (15/635)
LSIL	75.0% (419/559)	22.5% (126/559)	2.5% (14/559)
ASC-H	86.2% (50/58)	13.8% (8/58)	0.0% (0/58)
HSIL	91.4% (53/58)	6.9% (4/58)	1.7% (1/58)

	cobas 4800 12 Other HR HPV+		
	CINtec *PLUS* Cytology Result		
Pap Cytology	**Positive**	**Negative**	**Invalid**
AGC or ACIS	90.9% (10/11)	9.1% (1/11)	0.0% (0/11)
UNSAT	25.9% (15/58)	22.4% (13/58)	51.7% (30/58)
Combined	42.7% (1694/3969)	50.7% (2014/3969)	6.6% (261/3969)

	cobas 4800 HPV 16+			cobas 4800 HPV 18+		
	CINtec *PLUS* Cytology Result			CINtec *PLUS* Cytology Result		
Pap Cytology	**Positive**	**Negative**	**Invalid**	**Positive**	**Negative**	**Invalid**
NILM	42.8% (167/390)	50.0% (195/390)	7.2% (28/390)	34.4% (54/157)	61.1% (96/157)	4.5% (7/157)
ASC-US	73.9% (99/134)	23.9% (32/134)	2.2% (3/134)	56.8% (25/44)	36.4% (16/44)	6.8% (3/44)
LSIL	84.9% (118/139)	11.5% (16/139)	3.6% (5/139)	78.6% (44/56)	17.9% (10/56)	3.6% (2/56)
ASC-H	93.6% (44/47)	4.3% (2/47)	2.1% (1/47)	85.7% (6/7)	0.0% (0/7)	14.3% (1/7)
HSIL	100.0% (51/51)	0.0% (0/51)	0.0% (0/51)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)
AGC or ACIS	100.0% (11/11)	0.0% (0/11)	0.0% (0/11)	100.0% (10/10)	0.0% (0/10)	0.0% (0/10)
UNSAT	41.7% (5/12)	8.3% (1/12)	50.0% (6/12)	33.3% (1/3)	0.0% (0/3)	66.7% (2/3)
Combined	63.1% (495/784)	31.4% (246/784)	5.5% (43/784)	52.1% (149/286)	42.7% (122/286)	5.2% (15/286)

Population of Women 30 - 65 Years Old with NILM Pap Cytology Results

Tables 30 and 31 below show percent of positive, negative and invalid results of the CINtec *PLUS* Cytology test for women 30 - 65 years with NILM Pap cytology results stratified by testing site and by age group.

Table 30: Percent of CINtec *PLUS* Cytology Results for NILM Women 30 - 65 Years Old Stratified by Testing Site

	cobas 4800 12 Other HR HPV+					
	CINtec *PLUS* Cytology Result					
Site	**Positive**		**Negative**		**Invalid**	
Site 1	32.2% (99/307)		61.2% (188/307)		6.5% (20/307)	
Site 2	34.9% (158/453)		59.6% (270/453)		5.5% (25/453)	
Site 3	29.4% (174/592)		63.7% (377/592)		6.9% (41/592)	
Site 4	17.4% (73/420)		71.0% (298/420)		11.7% (49/420)	
Combined	28.4% (504/1772)		63.9% (1133/1772)		7.6% (135/1772)	
	cobas 4800 HPV 16+			**cobas 4800 HPV 18+**		
	CINtec *PLUS* Cytology Result			**CINtec *PLUS* Cytology Result**		
Site	**Positive**	**Negative**	**Invalid**	**Positive**	**Negative**	**Invalid**
Site 1	44.3% (27/61)	41.0% (25/61)	14.8% (9/61)	23.8% (5/21)	66.7% (14/21)	9.5% (2/21)
Site 2	42.9% (30/70)	51.4% (36/70)	5.7% (4/70)	43.8% (14/32)	46.9% (15/32)	9.4% (3/32)
Site 3	38.5% (40/104)	57.7% (60/104)	3.8% (4/104)	45.1% (23/51)	52.9% (27/51)	2.0% (1/51)
Site 4	40.5% (30/74)	54.1% (40/74)	5.4% (4/74)	11.1% (3/27)	88.9% (24/27)	0.0% (0/27)
Combined	41.1% (127/309)	52.1% (161/309)	6.8% (21/309)	34.4% (45/131)	61.1% (80/131)	4.6% (6/131)

Table 31: Percent of CINtec *PLUS* Cytology Results for NILM Women 30 - 65 Years Old Stratified by Age Group

	cobas 4800 12 Other HR HPV+					
Age Group (Years)	CINtec *PLUS* Cytology Result					
	Positive		**Negative**		**Invalid**	
30-39	29.6% (276/934)		62.6% (585/934)		7.8% (73/934)	
40-49	24.4% (104/427)		69.3% (296/427)		6.3% (27/427)	
50-65	30.2% (124/411)		61.3% (252/411)		8.5% (35/411)	
Combined	28.4% (504/1772)		63.9% (1133/1772)		7.6% (135/1772)	
Age Group (Years)	**cobas 4800 HPV 16+**			**cobas 4800 HPV 18+**		
	CINtec *PLUS* Cytology Result			**CINtec *PLUS* Cytology Result**		
	Positive	**Negative**	**Invalid**	**Positive**	**Negative**	**Invalid**
30-39	45.8% (71/155)	49.0% (76/155)	5.2% (8/155)	27.4% (17/62)	67.7% (42/62)	4.8% (3/62)
40-49	32.6% (30/92)	57.6% (53/92)	9.8% (9/92)	30.3% (10/33)	60.6% (20/33)	9.1% (3/33)

	cobas 4800 12 Other HR HPV+					
Age Group (Years)	**CINtec *PLUS* Cytology Result**					
	Positive		**Negative**		**Invalid**	
50-65	41.9% (26/62)	51.6% (32/62)	6.5% (4/62)	50.0% (18/36)	50.0% (18/36)	0.0% (0/36)
Combined	41.1% (127/309)	52.1% (161/309)	6.8% (21/309)	34.4% (45/131)	61.1% (80/131)	4.6% (6/131)

CLINICAL PERFORMANCE

The IMPACT clinical study evaluated (i) the performance of the CINtec *PLUS* Cytology test as a triage test to stratify cobas 4800 HPV positive women 25 - 65 years old in primary HPV cervical cancer screening, for referral to colposcopy and (ii) the performance of the CINtec *PLUS* Cytology test as a triage test to stratify cobas 4800 HPV positive women 30–65 years with NILM Pap cytology in adjunctive cervical cytology and HPV screening, for referral to colposcopy.

Baseline Phase

The study enrolled 35,263 women 25 - 65 years undergoing routine cervical cancer screening in the US from September 2017 to October 2018 at 32 clinical sites in the Baseline Phase. A total of 34,914 women were eligible to participate in the study.

All women had one cervical sample collected in PreservCyt media at Study Visit 1 (SV1). The specimens were collected using a spatula/brush or broom-type collection device; approximately half of the specimens were collected with each device. Pap cytology and HPV testing by cobas 4800 HPV Test (Roche Molecular Systems, Inc.) were performed on the PreservCyt samples for all subjects at four testing sites. Pap cytology results were classified according to the criteria of *The Bethesda System for Reporting Cervical Cytology*. CINtec *PLUS* Cytology testing was performed on all women with positive cobas 4800 HPV Test results using the residual volume remaining in the PreservCyt cervical sample collected at SV1.

Women 25 - 65 years old with positive cobas 4800 HPV Test results were invited to undergo colposcopy. Colposcopy was performed at Study Visit 2 (SV2) with colposcopists blinded to the CINtec *PLUS* Cytology results. Colposcopy was conducted following the principles recommended by the American Society for Colposcopy and Cervical Pathology (ASCCP) as follows. Biopsies were obtained on all visible lesions, and a single random cervical biopsy was obtained from the squamocolumnar junction (SCJ) if no lesions were visible. Endocervical curettage was performed in all patients in whom the SCJ was not visualized or only partially visualized. All biopsies were examined by a Central Pathology Review (CPR) process which included up to three expert pathologists. Discordant histology results were adjudicated according to a pre-defined protocol. The slides that were prepared from the biopsies and reviewed by the CPR panel were stained with hematoxylin and eosin (H&E) and CINtec Histology test (p16 IHC assay). The expert pathologist first evaluated H&E-stained slides to establish the $CPR_{H\&E}$ reference diagnosis, then evaluated both H&E- and p16 histology-stained slides for that case to establish the $CPR_{H\&E+p16}$ reference diagnosis. Additionally, CPR results where diagnosis was based on the H&E-stained slides with adjunctive interpretation of the p16-stained slides only when a case met LAST (Lower Anogenital Squamous Terminology) criteria (excluding ASC-US/HPV16+ as a LAST criterion) were derived using results from $CPR_{H\&E}$ and $CPR_{H\&E+p16}$ diagnoses.

The clinical performance of the CINtec *PLUS* Cytology test in cobas 4800 HPV positive women was assessed at the clinical endpoints ≥CIN2 and ≥CIN3. All performance analyses presented below use CPR results derived according to LAST criteria, excluding ASC-US/HPV16+ as a criterion, as the reference diagnosis. Throughout, the following confidence intervals were calculated: Wilson score method for sensitivity and specificity; FDA recommended score method for PPV, NPV, 1-NPV, PLR, and NLR; and normal approximation method for positivity rate.

Performance Characteristics in the Population of Women 25 - 65 Years Old with cobas 4800 HPV+ Results

A total of 5,046 women 25 - 65 years with cobas 4800 HPV Test positive results were included in the study.

Women 25 - 65 Years Old with cobas 4800 12 Other HR HPV+ Results

Among 5,046 women with HPV positive results, there were 3,974 women with 12 Other HR HPV positive results. Among them, 2,931 women had valid CINtec *PLUS* Cytology results (Positive or negative) and valid CPR diagnosis. Results of CINtec *PLUS* Cytology results and CPR results are presented in the table 32 below.

Table 32: CINtec *PLUS* Cytology Results for Women 25 - 65 Years Old with cobas 4800 12 Other HR HPV+ Results

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	891	194	176	78	3	1342
Negative	1451	81	44	13	0	1589
Total	2342	275	220	91	3	2931
Note: 2 ACIS cases were classified to cancer category, of which 2 were positive for CINtec *PLUS* Cytology						

Performance of the CINtec *PLUS* Cytology test in detecting high-grade cervical disease (≥CIN2and ≥CIN3) in 12 Other HR HPV+ women is presented in Table 33 below. The sensitivity and specificity of CINtec *PLUS* Cytology for the detection of ≥CIN2 were 81.8% and 58.5%, respectively, whereas for the detection of ≥CIN3, sensitivity and specificity were 86.2% and 55.6% respectively. Positive predictive values (PPV) of the test for ≥CIN2 and ≥CIN3 were 19.2% and 6.0%, respectively. The risk (1-NPV) for ≥CIN3 in 12 Other HR HPV+ women with negative CINtec *PLUS* Cytology results was 0.8%.

Table 33: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 12 Other HR HPV+ Results

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	81.8 (257/314) (77.2, 85.7)	86.2 (81/94) (77.8, 91.7)
Specificity (%)	58.5 (1532/2617) (56.6, 60.4)	55.6 (1576/2837) (53.7, 57.4)
Prevalence (%)	10.7 (314/2931)	3.2 (94/2931)
PPV (%)	19.2 (257/1342) (18.1, 20.2)	6.0 (81/1342) (5.4, 6.5)
NPV (%)	96.4 (1532/1589) (95.5, 97.2)	99.2 (1576/1589) (98.7, 99.5)
1-NPV (%)	3.6 (57/1589) (2.8, 4.5)	0.8 (13/1589) (0.5, 1.3)
PLR	1.97 (1.84, 2.11)	1.94 (1.74, 2.09)
NLR	0.31 (0.24, 0.39)	0.25 (0.15, 0.40)
Positivity Rate (%)	45.8 (1342/2931) (44.0, 47.5)	
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals		

Women 25 - 65 Years Old with cobas 4800 HPV16 + Results

Among 5,056 women with HPV positive results, there were 785 women with HPV16 positive results. Among them, 597 women had valid CINtec *PLUS* Cytology results (Positive or Negative) and valid CPR diagnosis. Results of CINtec *PLUS* Cytology results and CPR results are presented in the table 34 below.

Table 34: CINtec *PLUS* Cytology Results for Women 25 - 65 Years Old with cobas 4800 HPV16+ Results

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Pos	190	35	67	100	10	402
Neg	172	11	5	7	0	195
Total	362	46	72	107	10	597
Note: 6 ACIS cases were classified to cancer category, of which 6 were positive for CINtec *PLUS* Cytology						

Performance of the CINtec *PLUS* Cytology test in detecting high-grade cervical disease (≥CIN2 and ≥CIN3) in HPV16+ women is presented in Table 35 below. Sensitivity of CINtec *PLUS* Cytology for the detection of high-grade cervical disease was 93.7% for ≥CIN2 and 94.0% for ≥CIN3, whereas specificity was 44.9% for ≥CIN2 and 39.2% for ≥CIN3. PPV was 44.0% for ≥CIN2 and 27.4% for ≥CIN3 and risk for negative CINtec *PLUS* Cytology (1-NPV) was 6.2% for ≥CIN2 and 3.6% for ≥CIN3.

Table 35: Performance of CINtec *PLUS* Cytology Women 25-65 Years Old with cobas 4800 HPV16+ Results

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	93.7 (177/189) (89.2, 96.3)	94.0 (110/117) (88.2, 97.1)
Specificity (%)	44.9 (183/408) (40.1, 49.7)	39.2 (188/480) (34.9, 43.6)
Prevalence (%)	31.7 (189/597)	19.6 (117/597)
PPV (%)	44.0 (177/402) (41.7, 46.5)	27.4 (110/402) (25.6, 29.1)
NPV (%)	93.8 (183/195) (89.9, 96.4)	96.4 (188/195) (93.1, 98.2)
1-NPV (%)	6.2 (12/195) (3.6, 10.1)	3.6 (7/195) (1.8, 6.9)
PLR	1.70 (1.55, 1.87)	1.55 (1.41, 1.68)
NLR	0.14 (0.08, 0.24)	0.15 (0.07, 0.30)
Positivity Rate (%)	67.3 (402/597) (63.9, 70.8)	
PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals		

Women 25 - 65 Years Old with Cobas 4800 HPV18 + Results

Among 5,046 women with HPV positive results, there were 287 women with HPV18+ results. Among them, 224 women had valid CINtec *PLUS* Cytology results (positive or negative) and valid CPR diagnosis. The disposition of CINtec *PLUS* Cytology results by CPR results is presented in Table 36 below.

Table 36: CINtec *PLUS* Cytology Results for Women 25-65 Years Old with cobas 4800 HPV18+ Results

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Pos	68	21	15	9	4	117
Neg	92	10	3	1	1	107
Total	160	31	18	10	5	224
Note: 3 ACIS cases were classified to cancer category, of which 2 were positive for CINtec *PLUS* Cytology						

Performance of the CINtec *PLUS* Cytology test in detecting high-grade cervical disease (≥CIN2 and ≥CIN3) in HPV18+ women is presented in Table 37 below. Sensitivity and specificity of CINtec *PLUS* Cytology for the detection of ≥CIN2 were 84.8% and 53.4%, respectively. Sensitivity and specificity for ≥CIN3 were 86.7% and 50.2%, respectively. PPV was 23.9% for ≥CIN2 and 11.1% for ≥CIN3 whereas risk for negative CINtec *PLUS* Cytology results (1-NPV) was 4.7% for ≥CIN2 and 1.9% for ≥CIN3.

Table 37: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 HPV18+

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	84.8 (28/33) (69.1, 93.3)	86.7 (13/15) (62.1, 96.3)
Specificity (%)	53.4 (102/191) (46.3, 60.3)	50.2 (105/209) (43.5, 57.0)
Prevalence (%)	14.7 (33/224)	6.7 (15/224)
PPV (%)	23.9 (28/117) (19.8, 27.7)	11.1 (13/117) (8.1, 13.2)
NPV (%)	95.3 (102/107) (90.8, 97.9)	98.1 (105/107) (94.8, 99.5)
1-NPV (%)	4.7 (5/107) (2.1, 9.2)	1.9 (2/107) (0.5, 5.2)
PLR	1.82 (1.43, 2.22)	1.74 (1.22, 2.11)
NLR	0.28 (0.12, 0.59)	0.27 (0.07, 0.76)
Positivity Rate (%)	52.2 (117/224) (45.9, 58.5)	

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Performance Characteristics of CINtec *PLUS* Cytology vs Pap Cytology for Women 25 - 65 Years Old with cobas 4800 HPV Positive Results

The comparative performance of CINtec *PLUS* Cytology vs Pap cytology in cobas 4800 HPV+ women is presented in Tables 38 and 39 below for the cobas 4800 12 Other HR HPV+ population, cobas 4800 HPV16+ population in Tables 40 and 41), and cobas 4800 HPV18+ population in Tables 42 and 43. Across the three genotype groups, an increase in sensitivity and a decrease in specificity for the detection of high-grade cervical disease were observed for CINtec *PLUS* Cytology vs Pap cytology with the maximum increase in sensitivity (diff=23.1%) and the minimum decrease in specificity (diff=7.9%) observed in the cobas 4800 12 Other HR HPV+ population (Table 39). In all cases, the use of CINtec *PLUS* Cytology resulted in a substantial reduction of the risk of disease for negative CINtec *PLUS* Cytology versus NILM Pap cytology (3.4%, 9.1%, and 3.3% lower for ≥CIN2; 0.8%, 5.1%, and 0.5% lower for ≥CIN3 in cobas 4800 12 Other HR HPV+, cobas 4800 HPV16+, and cobas 4800 HPV18+ populations, respectively).

Table 38: CINtec *PLUS* Cytology vs Pap Cytology Results in cobas 4800 12 Other HR HPV+ Women 25 - 65 Years Old

CINtec *PLUS* Cytology Result	CPR Diagnosis of ≥CIN2			CPR Diagnosis of ≤CIN1		
	Pap Cytology Result			Pap Cytology Result		
	Abnormal	NILM	Total	Abnormal	NILM	Total
Positive	171	85	256	559	515	1074
Negative	13	43	56	309	1211	1520
Total	184	128	312	868	1726	2594
CINtec *PLUS* Cytology Result	**CPR Diagnosis of ≥CIN3**			**CPR Diagnosis of ≤CIN2**		
	Pap Cytology Result			**Pap Cytology Result**		

CINtec *PLUS* Cytology Result	CPR Diagnosis of ≥CIN2			CPR Diagnosis of ≤CIN1		
	Pap Cytology Result			Pap Cytology Result		
	Abnormal	NILM	Total	Abnormal	NILM	Total
	Abnormal	NILM	Total	Abnormal	NILM	Total
Positive	61	20	81	669	580	1249
Negative	2	11	13	320	1243	1563
Total	63	31	94	989	1823	2812

Table 39: Performance of CINtec *PLUS* Cytology vs Pap Cytology in cobas 4800 12 Other HR HPV+ Women 25 - 65 Years Old

Performance Measure	CPR Diagnosis of ≥CIN2		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	82.1 (256/312) (77.4, 85.9)	59.0 (184/312) (53.4, 64.3)	23.1 (17.3, 28.7)
Specificity (%)	58.6 (1520/2594) (56.7, 60.5)	66.5 (1726/2594) (64.7, 68.3)	-7.9 (-10.1, -5.8)
PPV (%)	19.2 (256/1330) (18.1, 20.3)	17.5 (184/1052) (15.9, 19.0)	1.8 (0.2, 3.3)
1-NPV (%)	3.6 (56/1576) (2.8, 4.4)	6.9 (128/1854) (6.0, 7.8)	-3.4 (-4.4, -2.3)
Prevalence (%)	10.7 (312/2906)		
Performance Measure	**CPR Diagnosis of ≥CIN3**		
	CINtec *PLUS* Cytology	**Pap Cytology**	**Difference**
Sensitivity (%)	86.2 (81/94) (77.8, 91.7)	67.0 (63/94) (57.0, 75.7)	19.1 (9.8, 28.4)
Specificity (%)	55.6 (1563/2812) (53.7, 57.4)	64.8 (1823/2812) (63.0, 66.6)	-9.2 (-11.3, -7.2)
PPV (%)	6.1 (81/1330) (5.5, 6.5)	6.0 (63/1052) (5.1, 6.8)	0.1 (-0.7, 0.9)
1-NPV (%)	0.8 (13/1576) (0.5, 1.3)	1.7 (31/1854) (1.2, 2.2)	-0.8 (-1.3, -0.3)
Prevalence (%)	3.2 (94/2906)		

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Table 40: CINtec *PLUS* Cytology vs Pap Cytology Results in cobas 4800 HPV16+ Women 25 - 65 Years Old

CINtec *PLUS* Cytology Result	CPR Diagnosis of ≥CIN2			CPR Diagnosis of ≤CIN1		
	Pap Cytology Result			Pap Cytology Result		
	Abnormal	NILM	Total	Abnormal	NILM	Total
Positive	140	36	176	126	98	224
Negative	4	8	12	36	146	182
Total	144	44	188	162	244	406
CINtec *PLUS* Cytology Result	**CPR Diagnosis of ≥CIN3**			**CPR Diagnosis of ≤CIN2**		
	Pap Cytology Result			**Pap Cytology Result**		
	Abnormal	**NILM**	**Total**	**Abnormal**	**NILM**	**Total**
Positive	90	20	110	176	114	290

CINtec *PLUS* Cytology Result	CPR Diagnosis of ≥CIN2			CPR Diagnosis of ≤CIN1		
	Pap Cytology Result			Pap Cytology Result		
	Abnormal	NILM	Total	Abnormal	NILM	Total
Negative	2	5	7	38	149	187
Total	92	25	117	214	263	477

Table 41: Performance of CINtec *PLUS* Cytology vs Pap Cytology in cobas 4800 HPV16+ Women 25 - 65 Years Old

Performance Measure	CPR Diagnosis of ≥CIN2		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	93.6 (176/188) (89.2, 96.3)	76.6 (144/188) (70.0, 82.1)	17.0 (10.9, 23.5)
Specificity (%)	44.8 (182/406) (40.1, 49.7)	60.1 (244/406) (55.3, 64.7)	-15.3 (-20.6, -9.8)
PPV (%)	44.0 (176/400) (41.7, 46.4)	47.1 (144/306) (43.5, 50.6)	-3.1 (-6.6, 0.5)
1-NPV (%)	6.2 (12/194) (3.6, 10.2)	15.3 (44/288) (12.0, 19.0)	-9.1 (-13.4, -4.8)
Prevalence (%)	31.6 (188/594)		
Performance Measure	**CPR Diagnosis of ≥CIN3**		
	CINtec *PLUS* Cytology	**Pap Cytology**	**Difference**
Sensitivity (%)	94.0 (110/117) (88.2, 97.1)	78.6 (92/117) (70.4, 85.1)	15.4 (7.9, 23.4)
Specificity (%)	39.2 (187/477) (34.9, 43.7)	55.1 (263/477) (50.6, 59.5)	-15.9 (-20.7, -11.0)
PPV (%)	27.5 (110/400) (25.7, 29.2)	30.1 (92/306) (27.1, 32.9)	-2.6 (-5.4, 0.2)
1-NPV (%)	3.6 (7/194) (1.8, 7.0)	8.7 (25/288) (6.2, 11.8)	-5.1 (-8.3, -1.7)
Prevalence (%)	19.7 (117/594)		

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Table 42: CINtec *PLUS* Cytology vs Pap Cytology Results in cobas 4800 HPV18+ Women 25 - 65 Years Old

CINtec *PLUS* Cytology Result	CPR Diagnosis of ≥CIN2			CPR Diagnosis of ≤CIN1		
	Pap Cytology Result			Pap Cytology Result		
	Abnormal	NILM	Total	Abnormal	NILM	Total
Positive	21	7	28	51	38	89
Negative	2	2	4	21	81	102
Total	23	9	32	72	119	191
CINtec *PLUS* Cytology Result	**CPR Diagnosis of ≥CIN3**			**CPR Diagnosis of ≤CIN2**		
	Pap Cytology Result			**Pap Cytology Result**		
	Abnormal	**NILM**	**Total**	**Abnormal**	**NILM**	**Total**
Positive	11	2	13	61	43	104
Negative	1	1	2	22	82	104
Total	12	3	15	83	125	208

Table 43: Performance of CINtec *PLUS* Cytology vs Pap Cytology in cobas 4800 HPV18+ Women 25 - 65 Years Old

Performance Measure	CPR Diagnosis of ≥CIN2		
	CINtec *PLUS* Cytology	**Pap Cytology**	**Difference**
Sensitivity (%)	87.5 (28/32) (71.9, 95.0)	71.9 (23/32) (54.6, 84.4)	15.6 (-3.6, 33.9)
Specificity (%)	53.4 (102/191) (46.3, 60.3)	62.3 (119/191) (55.3, 68.9)	-8.9 (-16.6, -1.0)
PPV (%)	23.9 (28/117) (19.9, 27.6)	24.2 (23/95) (18.8, 29.3)	-0.3 (-5.8, 5.3)
1-NPV (%)	3.8 (4/106) (1.5, 8.2)	7.0 (9/128) (4.0, 11.0)	-3.3 (-8.1, 1.3)
Prevalence (%)	14.3 (32/223)		
Performance Measure	**CPR Diagnosis of ≥CIN3**		
	CINtec *PLUS* Cytology	**Pap Cytology**	**Difference**
Sensitivity (%)	86.7 (13/15) (62.1, 96.3)	80.0 (12/15) (54.8, 93.0)	6.7 (-20.5, 33.2)
Specificity (%)	50.0 (104/208) (43.3, 56.7)	60.1 (125/208) (53.3, 66.5)	-10.1 (-17.5, -2.5)
PPV (%)	11.1 (13/117) (8.1, 13.2)	12.6 (12/95) (8.8, 15.6)	-1.5 (-5.1, 2.0)
1-NPV (%)	1.9 (2/106) (0.5, 5.2)	2.3 (3/128) (0.8, 5.2)	-0.5 (-3.4, 2.3)
Prevalence (%)	6.7 (15/223)		

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Table 44: Summary of CINtec *PLUS* Cytology test performance for Women 25 - 65 years old with different HPV genotypes

HPV Genotyping	Pre-test risk of ≥CIN2 (prevalence)	Post-test risk of ≥CIN2		Pre-test risk of ≥CIN3 (prevalence)	Post-test risk of ≥CIN3	
		CINtec *PLUS* Cytology Positive	**CINtec *PLUS* Cytology Negative**		**CINtec *PLUS* Cytology Positive**	**CINtec *PLUS* Cytology Negative**
HPV16+	31.7%	44.0%	6.2% (12/195) 95% CI: (3.6, 10.1)	19.6%	27.4%	3.6% (7/195) 95% CI: (1.8, 6.9)
HPV18+	14.7%	23.9%	4.7% (5/107) 95% CI: (2.1, 9.2)	6.7%	11.1%	1.9% (2/107) 95% CI: (0.5, 5.2)
12 Other HR HPV+	10.7%	19.2%	3.6% (57/1589) 95% CI: (2.8, 4.5)	3.2%	6.0%	0.8% (13/1589) 95% CI: (0.5, 1.3)

Performance Characteristics in the Population of cobas 4800 HPV Positive Women 30 - 65 Years Old with NILM Pap Cytology Results

A total of 2,212 cobas 4800 HPV positive women 30-65 years with NILM Pap cytology results were included in the study**.**

Women 30 - 65 Years Old with cobas 4800 12 Other HR HPV+ Results and NILM Pap Cytology

Among 2,212 women with HPV positive results and NILM Pap cytology, there were 1,772 women with 12 Other HR HPV+ women. Among them, 1,291women had valid CINtec *PLUS* Cytology results (positive or negative) and valid CPR diagnosis. The disposition of CINtec *PLUS* Cytology results by CPR results is presented in Table 45 below.

Table 45: CINtec *PLUS* Cytology Results for Women 30 - 65 Years Old with cobas 4800 12 Other HR HPV+ and NILM Cytology

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	324	29	38	8	2	401
Negative	833	31	21	5	0	890
Total	1157	60	59	13	2	1291
Note: 2 ACIS cases were classified to cancer category; both ACIS cases were positive for CINtec *PLUS* Cytology						

Performance of the CINtec *PLUS* Cytology test in detecting ≥CIN2 and ≥CIN3 in cobas 4800 12 Other HR HPV+ women with NILM Pap cytology is presented in Table 46. Sensitivity and specificity for the detection of ≥CIN2 were 64.9% and 71.0%, respectively (66.7% and 69.4%, respectively, for ≥CIN3) The PPVs in this group were 12.0% for ≥CIN2 and 2.5% for ≥CIN3, whereas the residual risks were 2.9% for ≥CIN2 and 0.6% and ≥CIN3.

Table 46: Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 30 - 65 Years Old with NILM Pap Cytology

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	64.9 (48/74) (53.5, 74.8)	66.7 (10/15) (41.7, 84.8)
Specificity (%)	71.0 (864/1217) (68.4, 73.5)	69.4 (885/1276) (66.8, 71.8)
Prevalence (%)	5.7 (74/1291)	1.2 (15/1291)
PPV (%)	12.0 (48/401) (9.9, 13.9)	2.5 (10/401) (1.6, 3.2)
NPV (%)	97.1 (864/890) (96.2, 97.9)	99.4 (885/890) (99.0, 99.7)
1-NPV (%)	2.9 (26/890) (2.1, 3.8)	0.6 (5/890) (0.3, 1.0)
PLR	2.24 (1.81, 2.65)	2.18 (1.35, 2.82)
NLR	0.49 (0.35, 0.66)	0.48 (0.22, 0.84)
Positivity Rate (%)	31.1 (401/1291) (28.6, 33.5)	
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals		

Women 30 - 65 Years Old with cobas 4800 HPV16+ Results and NILM Pap Cytology

Among 2,212 women with HPV positive results and NILM Pap cytology, there were 309 women with HPV16+ results. Among them, 232 women had valid CINtec *PLUS* Cytology results (positive or negative) and valid CPR diagnosis. The disposition of CINtec *PLUS* Cytology results by CPR results is presented in Table 47 below.

Table 47: CINtec *PLUS* Cytology Results and CPR Results in cobas 4800 HPV16+ Women 30 - 65 Years Old with NILM Cytology

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	68	7	11	17	1	104
Negative	115	6	3	4	0	128
Total	183	13	14	21	1	232

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Note: 0 ACIS cases were classified to cancer category						

Performance of the CINtec *PLUS* Cytology test in detecting ≥CIN2 and ≥CIN3 in cobas 4800 HPV16+ women with NILM Pap cytology is presented in Table 48 below. Sensitivity and specificity in this population were 80.6% and 61.7%, respectively, for ≥CIN2 and 81.8% and 59.0%, respectively, for ≥CIN3. PPV and residual risk were 27.9% and 5.5%, respectively, for ≥CIN2, and 17.3% and 3.1%, respectively, for ≥CIN3.

Table 48: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 30-65 Years Old with NILM Pap Cytology

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	80.6 (29/36) (65.0, 90.2)	81.8 (18/22) (61.5, 92.7)
Specificity (%)	61.7 (121/196) (54.8, 68.3)	59.0 (124/210) (52.3, 65.5)
Prevalence (%)	15.5 (36/232)	9.5 (22/232)
PPV (%)	27.9 (29/104) (22.8, 32.7)	17.3 (18/104) (13.2, 20.8)
NPV (%)	94.5 (121/128) (90.5, 97.2)	96.9 (124/128) (93.5, 98.7)
1-NPV (%)	5.5 (7/128) (2.8, 9.5)	3.1 (4/128) (1.3, 6.5)
PLR	2.11 (1.61, 2.64)	2.00 (1.45, 2.50)
NLR	0.31 (0.16, 0.57)	0.31 (0.12, 0.66)
Positivity Rate (%)	44.8 (104/232) (38.7, 50.9)	
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals		

Women 30 - 65 Years Old with cobas 4800 HPV18+ Results and NILM Pap Cytology

Among 2,212 women with HPV positive results and NILM Pap cytology, there were 309 women with HPV18 positive results. Among them, 108 women had valid CINtec *PLUS* Cytology results (positive or negative) and valid CPR diagnosis. The disposition of CINtec *PLUS* Cytology results by CPR results is presented in Table 49 below.

Table 49: CINtec *PLUS* Cytology Results and CPR Results in cobas 4800 HPV18+ Women 30 - 65 Years Old with NILM Pap Cytology

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	30	2	4	1	1	38
Negative	65	4	0	0	1	70
Total	95	6	4	1	2	108
Note: 2 ACIS cases were classified to cancer category; 1 of the 2 ACIS cases was positive for CINtec *PLUS* Cytology.						

Performance of the CINtec *PLUS* Cytology test in detecting ≥CIN2 and ≥CIN3 in cobas 4800 HPV18+ women with NILM Pap cytology is presented in Table 50 below. Sensitivity and specificity in this population were 85.7% and 68.3%, respectively, for ≥CIN2, and 66.7% and 65.7% for ≥CIN3. PPVs were 15.8% and 5.3% for ≥CIN2 and for ≥CIN3, respectively, whereas residual risk was 1.4% for both disease cutoffs.

Table 50: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV18+ Women 30 - 65 Years Old with NILM Cytology

Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Sensitivity (%)	85.7 (6/7) (48.7, 97.4)	66.7 (2/3) (20.8, 93.9)
Specificity (%)	68.3 (69/101) (58.7, 76.6)	65.7 (69/105) (56.2, 74.1)
Prevalence (%)	6.5 (7/108)	2.8 (3/108)
PPV (%)	15.8 (6/38) (9.2, 21.1)	5.3 (2/38) (1.7, 8.3)
NPV (%)	98.6 (69/70) (95.0, 99.7)	98.6 (69/70) (96.6, 99.7)
1-NPV (%)	1.4 (1/70) (0.3, 5.0)	1.4 (1/70) (0.3, 3.4)
PLR	2.71 (1.46, 3.87)	1.94 (0.59, 3.18)
NLR	0.21 (0.04, 0.76)	0.51 (0.09, 1.24)
Positivity Rate (%)	35.2 (38/108) (26.5, 43.8)	

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; PLR = Positive Likelihood Ratio; NLR = Negative Likelihood Ratio; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Table 51: Summary of CINtec *PLUS* Cytology Test Performance for NILM Women 30 - 65 Years Old for Different HPV Genotypes

HPV Genotyping	Pre-test risk of ≥CIN2 (prevalence)	Post-test risk of ≥CIN2		Pre-test risk of ≥CIN3 (prevalence)	Post-test risk of ≥CIN3	
		CINtec *PLUS* Cytology Positive	CINtec *PLUS* Cytology Negative		CINtec *PLUS* Cytology Positive	CINtec *PLUS* Cytology Negative
HPV16+	15.5%	27.9%	5.5% (74/1291) 95% CI: (2.8, 9.5)	9.5%	17.3%	3.1% (4/128) 95% CI: (1.3, 6.5)
HPV18+	6.5%	15.8%	1.4% (1/70) 95% CI: (0.3, 5.0)	2.8%	5.3%	1.4% (1/70) 95% CI: (0.3, 5.0)
12 Other HR HPV+	5.7%	12.0%	2.9% (26/890) 95% CI: (2.1, 3.8)	1.2%	2.5%	0.6% (5/890) 95% CI: (0.3, 1.0)

3. Subgroup Analyses

3.1 *Performance of the CINtec PLUS Cytology test was evaluated stratified by age groups.*

Performance of the CINtec *PLUS* Cytology test for women 25-65 years old in detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Tables 52 through 54 below.

Table 52: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 12 Other HR HPV+ Stratified by Age Group

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
25-29 (N=928)	Sensitivity (%)	83.3 (105/126) (75.9, 88.8)	85.7 (36/42) (72.2, 93.3)
	Specificity (%)	56.4 (452/802) (52.9, 59.8)	52.7 (467/886) (49.4, 56.0)
	PPV (%)	23.1 (105/455) (21.0, 25.0)	7.9 (36/455) (6.7, 8.8)
	1-NPV (%)	4.4 (21/473) (3.0, 6.3)	1.3 (6/473) (0.6, 2.5)
	Prevalence (%)	13.6 (126/928)	4.5 (42/928)
30-39 (N=1062)	Sensitivity (%)	81.9 (95/116) (73.9, 87.8)	85.7 (30/35) (70.6, 93.7)
	Specificity (%)	58.9 (557/946) (55.7, 62.0)	55.8 (573/1027) (52.7, 58.8)
	PPV (%)	19.6 (95/484) (17.7, 21.4)	6.2 (30/484) (5.1, 6.9)
	1-NPV (%)	3.6 (21/578) (2.5, 5.2)	0.9 (5/578) (0.4, 1.8)
	Prevalence (%)	10.9 (116/1062)	3.3 (35/1062)
40-49 (N=477)	Sensitivity (%)	74.4 (32/43) (59.8, 85.1)	71.4 (5/7) (35.9, 91.8)
	Specificity (%)	63.8 (277/434) (59.2, 68.2)	60.9 (286/470) (56.4, 65.2)
	PPV (%)	16.9 (32/189) (13.7, 19.8)	2.6 (5/189) (1.3, 3.5)
	1-NPV (%)	3.8 (11/288) (2.3, 5.9)	0.7 (2/288) (0.2, 1.6)
	Prevalence (%)	9.0 (43/477)	1.5 (7/477)
50-65 (N=464)	Sensitivity (%)	86.2 (25/29) (69.4, 94.5)	100.0 (10/10) (72.2, 100.0)
	Specificity (%)	56.6 (246/435) (51.9, 61.1)	55.1 (250/454) (50.5, 59.6)
	PPV (%)	11.7 (25/214) (9.5, 13.4)	4.7 (10/214) (4.6, 5.2)
	1-NPV (%)	1.6 (4/250) (0.6, 3.5)	0.0 (0/250) (0.0, 1.1)
	Prevalence (%)	6.3 (29/464)	2.2 (10/464)

Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals

Table 53: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 HPV16+ Stratified by Age Group

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
25-29 (N=135)	Sensitivity (%)	96.0 (48/50) (86.5, 98.9)	93.3 (28/30) (78.7, 98.2)
	Specificity (%)	32.9 (28/85) (23.9, 43.5)	26.7 (28/105) (19.1, 35.8)
	PPV (%)	45.7 (48/105) (41.9, 50.1)	26.7 (28/105) (23.1, 29.7)
	1-NPV (%)	6.7 (2/30) (1.9, 20.0)	6.7 (2/30) (1.9, 19.3)
	Prevalence (%)	37.0 (50/135)	22.2 (30/135)
30-39 (N=248)	Sensitivity (%)	95.7 (90/94) (89.6, 98.3)	96.8 (60/62) (89.0, 99.1)

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
	Specificity (%)	48.1 (74/154) (40.3, 55.9)	40.9 (76/186) (34.1, 48.0)
	PPV (%)	52.9 (90/170) (49.2, 57.1)	35.3 (60/170) (32.4, 38.4)
	1-NPV (%)	5.1 (4/78) (2.0, 11.9)	2.6 (2/78) (0.7, 8.3)
	Prevalence (%)	37.9 (94/248)	25.0 (62/248)
40-49 (N=120)	Sensitivity (%)	82.4 (28/34) (66.5, 91.7)	84.2 (16/19) (62.4, 94.5)
	Specificity (%)	55.8 (48/86) (45.3, 65.8)	50.5 (51/101) (40.9, 60.0)
	PPV (%)	42.4 (28/66) (35.4, 49.6)	24.2 (16/66) (18.5, 29.3)
	1-NPV (%)	11.1 (6/54) (5.5, 19.7)	5.6 (3/54) (2.0, 12.6)
	Prevalence (%)	28.3 (34/120)	15.8 (19/120)
50-65 (N=94)	Sensitivity (%)	100.0 (11/11) (74.1, 100.0)	100.0 (6/6) (61.0, 100.0)
	Specificity (%)	39.8 (33/83) (29.9, 50.5)	37.5 (33/88) (28.1, 47.9)
	PPV (%)	18.0 (11/61) (17.0, 21.1)	9.8 (6/61) (9.5, 11.6)
	1-NPV (%)	0.0 (0/33) (0.0, 8.1)	0.0 (0/33) (0.0, 6.8)
	Prevalence (%)	11.7 (11/94)	6.4 (6/94)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Table 54: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 HPV18+ Stratified by Age Group

Age Group (Years)	Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
25-29 (N=40)	Sensitivity (%)	60.0 (3/5) (23.1, 88.2)	0.0 (0/1) (0.0, 79.3)
	Specificity (%)	54.3 (19/35) (38.2, 69.5)	51.3 (20/39) (36.2, 66.1)
	PPV (%)	15.8 (3/19) (6.4, 25.7)	0.0 (0/19) (0.0, 4.3)
	1-NPV (%)	9.5 (2/21) (2.9, 18.7)	4.8 (1/21) (4.7, 6.6)
	Prevalence (%)	12.5 (5/40)	2.5 (1/40)
30-39 (N=86)	Sensitivity (%)	85.7 (12/14) (60.1, 96.0)	87.5 (7/8) (52.9, 97.8)
	Specificity (%)	54.2 (39/72) (42.7, 65.2)	51.3 (40/78) (40.4, 62.1)
	PPV (%)	26.7 (12/45) (19.4, 33.3)	15.6 (7/45) (9.7, 19.9)
	1-NPV (%)	4.9 (2/41) (1.4, 12.9)	2.4 (1/41) (0.4, 8.9)
	Prevalence (%)	16.3 (14/86)	9.3 (8/86)
40-49 (N=50)	Sensitivity (%)	83.3 (5/6) (43.6, 97.0)	100.0 (3/3) (43.9, 100.0)
	Specificity (%)	54.5 (24/44) (40.1, 68.3)	53.2 (25/47) (39.2, 66.7)
	PPV (%)	20.0 (5/25) (11.0, 27.7)	12.0 (3/25) (11.3, 16.1)
	1-NPV (%)	4.0 (1/25) (0.7, 13.0)	0.0 (0/25) (0.0, 6.6)

Age Group (Years)	Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
	Prevalence (%)	12.0 (6/50)	6.0 (3/50)
50-65 (N=48)	Sensitivity (%)	100.0 (8/8) (67.6, 100.0)	100.0 (3/3) (43.9, 100.0)
	Specificity (%)	50.0 (20/40) (35.2, 64.8)	44.4 (20/45) (30.9, 58.8)
	PPV (%)	28.6 (8/28) (25.5, 36.2)	10.7 (3/28) (10.3, 13.9)
	1-NPV (%)	0.0 (0/20) (0.0, 11.8)	0.0 (0/20) (0.0, 8.2)
	Prevalence (%)	16.7 (8/48)	6.3 (3/48)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Performance of the CINtec *PLUS* Cytology test for women 30 - 65 years old with NILM Pap cytology in detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Tables 55 through 57 below.

Table 55: Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 30 - 65 Years Old with NILM Cytology Stratified by Age Group

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
30-39 (N=668)	Sensitivity (%)	69.0 (29/42) (54.0, 80.9)	72.7 (8/11) (43.4, 90.3)
	Specificity (%)	70.3 (440/626) (66.6, 73.7)	68.5 (450/657) (64.8, 71.9)
	PPV (%)	13.5 (29/215) (10.6, 16.1)	3.7 (8/215) (2.2, 4.8)
	1-NPV (%)	2.9 (13/453) (1.8, 4.2)	0.7 (3/453) (0.2, 1.4)
	Prevalence (%)	6.3 (42/668)	1.6 (11/668)
40-49 (N=309)	Sensitivity (%)	60.9 (14/23) (40.8, 77.8)	33.3 (1/3) (6.1, 79.2)
	Specificity (%)	76.6 (219/286) (71.3, 81.1)	73.9 (226/306) (68.7, 78.5)
	PPV (%)	17.3 (14/81) (11.8, 22.7)	1.2 (1/81) (0.2, 3.0)
	1-NPV (%)	3.9 (9/228) (2.3, 5.9)	0.9 (2/228) (0.3, 1.2)
	Prevalence (%)	7.4 (23/309)	1.0 (3/309)
50-65 (N=314)	Sensitivity (%)	55.6 (5/9) (26.7, 81.1)	100.0 (1/1) (20.7, 100.0)
	Specificity (%)	67.2 (205/305) (61.8, 72.2)	66.8 (209/313) (61.4, 71.8)
	PPV (%)	4.8 (5/105) (2.3, 7.1)	1.0 (1/105) (0.9, 1.1)
	1-NPV (%)	1.9 (4/209) (0.8, 3.1)	0.0 (0/209) (0.0, 0.4)
	Prevalence (%)	2.9 (9/314)	0.3 (1/314)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Table 56: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 30 - 65 Years Old with NILM Cytology

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
30-39 (N=119)	Sensitivity (%)	87.5 (21/24) (69.0, 95.7)	85.7 (12/14) (60.1, 96.0)
	Specificity (%)	61.1 (58/95) (51.0, 70.2)	56.2 (59/105) (46.6, 65.3)
	PPV (%)	36.2 (21/58) (29.2, 43.3)	20.7 (12/58) (14.9, 25.6)
	1-NPV (%)	4.9 (3/61) (1.7, 11.6)	3.3 (2/61) (0.9, 8.8)
	Prevalence (%)	20.2 (24/119)	11.8 (14/119)
40-49 (N=63)	Sensitivity (%)	55.6 (5/9) (26.7, 81.1)	66.7 (4/6) (30.0, 90.3)
	Specificity (%)	66.7 (36/54) (53.4, 77.8)	66.7 (38/57) (53.7, 77.5)
	PPV (%)	21.7 (5/23) (11.0, 33.4)	17.4 (4/23) (8.2, 26.6)
	1-NPV (%)	10.0 (4/40) (4.4, 16.3)	5.0 (2/40) (1.5, 10.3)
	Prevalence (%)	14.3 (9/63)	9.5 (6/63)
50-65 (N=50)	Sensitivity (%)	100.0 (3/3) (43.9, 100.0)	100.0 (2/2) (34.2, 100.0)
	Specificity (%)	57.4 (27/47) (43.3, 70.5)	56.3 (27/48) (42.3, 69.3)
	PPV (%)	13.0 (3/23) (12.2, 17.8)	8.7 (2/23) (8.3, 11.9)
	1-NPV (%)	0.0 (0/27) (0.0, 6.1)	0.0 (0/27) (0.0, 4.8)
	Prevalence (%)	6.0 (3/50)	4.0 (2/50)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Table 57: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV18+ Women 30 - 65 Years Old with NILM Cytology Stratified by Age Group

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
30-39 (N=52)	Sensitivity (%)	50.0 (1/2) (9.5, 90.5)	0.0 (0/1) (0.0, 79.3)
	Specificity (%)	72.0 (36/50) (58.3, 82.5)	70.6 (36/51) (57.0, 81.3)
	PPV (%)	6.7 (1/15) (1.3, 14.4)	0.0 (0/15) (0.0, 5.7)
	1-NPV (%)	2.7 (1/37) (0.5, 5.1)	2.7 (1/37) (2.7, 3.3)
	Prevalence (%)	3.8 (2/52)	1.9 (1/52)
40-49 (N=24)	Sensitivity (%)	100.0 (2/2) (34.2, 100.0)	100.0 (1/1) (20.7, 100.0)
	Specificity (%)	72.7 (16/22) (51.8, 86.8)	69.6 (16/23) (49.1, 84.4)
	PPV (%)	25.0 (2/8) (21.4, 40.9)	12.5 (1/8) (11.5, 21.8)
	1-NPV (%)	0.0 (0/16) (0.0, 7.9)	0.0 (0/16) (0.0, 5.0)
	Prevalence (%)	8.3 (2/24)	4.2 (1/24)
50-65 (N=32)	Sensitivity (%)	100.0 (3/3) (43.9, 100.0)	100.0 (1/1) (20.7, 100.0)

Age Group (Years)	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
	Specificity (%)	58.6 (17/29) (40.7, 74.5)	54.8 (17/31) (37.8, 70.8)
	PPV (%)	20.0 (3/15) (18.1, 28.8)	6.7 (1/15) (6.4, 10.0)
	1-NPV (%)	0.0 (0/17) (0.0, 9.5)	0.0 (0/17) (0.0, 4.8)
	Prevalence (%)	9.4 (3/32)	3.1 (1/32)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

3.2 *Performance of the CINtec PLUS Cytology test was evaluated stratified by HPV vaccination status.*

Performance of the CINtec *PLUS* Cytology test for women 25 - 65 years old in detecting ≥CIN2 and ≥CIN3 stratified by HPV vaccination status is presented in Tables 58 through 60 below.

Table 58: Performance of CINtec *PLUS* Cytology for Women 25 - 65 Years Old with cobas 4800 12 Other HR HPV+ Stratified by Vaccination Status

Vaccinated Status	Performance Measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=2371)	Sensitivity (%)	82.9 (209/252) (77.8, 87.1)	86.3 (63/73) (76.6, 92.4)
	Specificity (%)	58.9 (1249/2119) (56.8, 61.0)	55.8 (1282/2298) (53.7, 57.8)
	PPV (%)	19.4 (209/1079) (18.2, 20.5)	5.8 (63/1079) (5.2, 6.3)
	1-NPV (%)	3.3 (43/1292) (2.5, 4.3)	0.8 (10/1292) (0.4, 1.3)
	Prevalence (%)	10.6 (252/2371)	3.1 (73/2371))
Vaccinated (N=559)	Sensitivity (%)	77.0 (47/61) (65.1, 85.8)	85.7 (18/21) (65.4, 95.0)
	Specificity (%)	56.8 (283/498) (52.4, 61.1)	54.6 (294/538) (50.4, 58.8)
	PPV (%)	17.9 (47/262) (15.3, 20.3)	6.9 (18/262) (5.3, 7.9)
	1-NPV (%)	4.7 (14/297) (3.0, 7.1)	1.0 (3/297) (0.4, 2.4)
	Prevalence (%)	10.9 (61/559)	3.8 (21/559)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals.			

Table 59: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 25 - 65 Years Old Stratified by Vaccination Status

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=550)	Sensitivity (%)	93.6 (160/171) (88.8, 96.4)	94.1 (96/102) (87.8, 97.3)
	Specificity (%)	44.3 (168/379) (39.4, 49.4)	38.6 (173/448) (34.2, 43.2)
	PPV (%)	43.1 (160/371) (40.8, 45.6)	25.9 (96/371) (24.1, 27.6)
	1-NPV (%)	6.1 (11/179) (3.5, 10.3)	3.4 (6/179) (1.6, 6.8)
	Prevalence (%)	31.1 (171/550)	18.5 (102/550)

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Vaccinated (N=47)	Sensitivity (%)	94.4 (17/18) (74.2, 99.0)	93.3 (14/15) (70.2, 98.8)
	Specificity (%)	51.7 (15/29) (34.4, 68.6)	46.9 (15/32) (30.9, 63.6)
	PPV (%)	54.8 (17/31) (45.7, 65.3)	45.2 (14/31) (36.2, 54.9)
	1-NPV (%)	6.3 (1/16) (1.1, 24.6)	6.3 (1/16) (1.1, 24.1)
	Prevalence (%)	38.3 (18/47)	31.9 (15/47)

Table 60: Performance of CINtec *PLUS* Cytology for cobas 4800 HPV18+ Women 25 - 65 Years Old Stratified by Vaccination Status

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=209)	Sensitivity (%)	84.8 (28/33) (69.1, 93.3)	86.7 (13/15) (62.1, 96.3)
	Specificity (%)	53.4 (94/176) (46.0, 60.6)	50.0 (97/194) (43.0, 57.0)
	PPV (%)	25.5 (28/110) (21.1, 29.5)	11.8 (13/110) (8.6, 14.0)
	1-NPV (%)	5.1 (5/99) (2.3, 9.9)	2.0 (2/99) (0.6, 5.6)
	Prevalence (%)	15.8 (33/209)	7.2 (15/209)
Vaccinated (N=15)	Sensitivity (%)	NA	NA
	Specificity (%)	53.3 (8/15) (30.1, 75.2)	53.3 (8/15) (30.1, 75.2)
	PPV (%)	0.0 (0/7) (0, NA)	0.0 (0/7) (0, NA)
	1-NPV (%)	0.0 (0/8) (0, NA)	0.0 (0/8) (0, NA)
	Prevalence (%)	0.0 (0/15)	0.0 (0/15).

Performance of the CINtec *PLUS* Cytology test for NILM women 30 - 65 years old in detecting ≥CIN2 and ≥CIN3 stratified by HPV vaccination status is presented in Tables 61 through 63 below.

Table 61:Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 30 - 65 Years Old with NILM Cytology Stratified by Vaccination Status

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=1183)	Sensitivity (%)	66.2 (45/68) (54.3, 76.3)	64.3 (9/14) (38.8, 83.7)
	Specificity (%)	70.8 (789/1115) (68.0, 73.4)	69.0 (807/1169) (66.3, 71.6)
	PPV (%)	12.1 (45/371) (10.0, 14.1)	2.4 (9/371) (1.5, 3.2)
	1-NPV (%)	2.8 (23/812) (2.0, 3.8)	0.6 (5/812) (0.3, 1.1)
	Prevalence (%)	5.7 (68/1183)	1.2 (14/1183)
Vaccinated (N=108)	Sensitivity (%)	50.0 (3/6) (18.8, 81.2)	100.0 (1/1) (20.7, 100.0)
	Specificity (%)	73.5 (75/102) (64.2, 81.1)	72.9 (78/107) (63.8, 80.4)
	PPV (%)	10.0 (3/30) (3.8, 17.3)	3.3 (1/30) (3.3, 4.6)
	1-NPV (%)	3.8 (3/78) (1.5, 6.3)	0.0 (0/78) (0.0, 1.0)

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
	Prevalence (%)	5.6 (6/108)	0.9 (1/108)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Table 62: Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 30 - 65 Years Old with NILM Cytology Stratified by Vaccination Status

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=219)	Sensitivity (%)	78.8 (26/33) (62.2, 89.3)	81.0 (17/21) (60.0, 92.3)
	Specificity (%)	62.4 (116/186) (55.2, 69.0)	60.1 (119/198) (53.2, 66.7)
	PPV (%)	27.1 (26/96) (21.8, 32.1)	17.7 (17/96) (13.3, 21.4)
	1-NPV (%)	5.7 (7/123) (2.9, 9.8)	3.3 (4/123) (1.3, 6.7)
	Prevalence (%)	15.1 (33/219)	9.6 (21/219)
Vaccinated (N=13)	Sensitivity (%)	100.0 (3/3) (43.9, 100.0)	100.0 (1/1) (20.7, 100.0)
	Specificity (%)	50.0 (5/10) (23.7, 76.3)	41.7 (5/12) (19.3, 68.0)
	PPV (%)	37.5 (3/8) (32.8, 55.9)	12.5 (1/8) (11.9, 20.7)
	1-NPV (%)	0.0 (0/5) (0.0, 29.4)	0.0 (0/5) (0.0, 18.2)
	Prevalence (%)	23.1 (3/13)	7.7 (1/13)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

Table 63: Performance of CINtec *PLUS* Cytology for Women 30 - 65 Years Old with cobas 4800 HPV18+ and NILM Cytology Stratified by Vaccination Status

Vaccinated status	Performance measure	CPR Diagnosis of ≥CIN2	CPR Diagnosis of ≥CIN3
Non-vaccinated (N=102)	Sensitivity (%)	85.7 (6/7) (48.7, 97.4)	66.7 (2/3) (20.8, 93.9)
	Specificity (%)	67.4 (64/95) (57.4, 76.0)	64.6 (64/99) (54.8, 73.4)
	PPV (%)	16.2 (6/37) (9.5, 21.7)	5.4 (2/37) (1.7, 8.6)
	1-NPV (%)	1.5 (1/65) (0.3, 5.4)	1.5 (1/65) (0.3, 3.7)
	Prevalence (%)	6.9 (7/102)	2.9 (3/102)
Vaccinated (N=6)	Sensitivity (%)	NA (0/0) (NA, NA)	NA (0/0) (NA, NA)
	Specificity (%)	83.3 (5/6) (43.6, 97.0)	83.3 (5/6) (43.6, 97.0)
	PPV (%)	0.0 (0/1) (0.0, NA)	0.0 (0/1) (0.0, NA)
	1-NPV (%)	0.0 (0/5) (0.0, NA)	0.0 (0/5) (0.0, NA)
	Prevalence (%)	0.0 (0/6)	0.0 (0/6)
Note: PPV = Positive Predictive Value; NPV = Negative Predictive Value; NA = Not Applicable; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals			

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 5 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. 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 Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA did not raise any new safety and effectiveness questions compared with information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The data provided demonstrate that the use of CINtec *PLUS* Cytology as a triage tool for referral of women 25 - 65 years old who are 12 Other HR HPV positive using the cobas® 4800 HPV Test in primary HPV screening to colposcopy, or referral of women 30 - 65 years old who are NILM/12 Other HR HPV positive using the cobas® 4800 HPV Test positive cotesting results to colposcopy, is acceptable.

In women 25 - 65 years old with HPV16/18 positive test results using the cobas® 4800 HPV Test in primary HPV screening or women 30 - 65 years old with NILM and HPV16/18 positive test results using the cobas® 4800 HPV Test, the CINtec *PLUS* Cytology test results can be used as additional information and in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

B. Safety Conclusions

The risks of the device are based on analytical studies and data collected in a clinical study conducted to support PMA approval as described above. With respect to the use of the CINtec *PLUS* Cytology test, a false positive may result in inappropriate referral to colposcopy. A false negative may result in a delay as noted above. An uninterpretable or invalid result is generally not harmful (unless the patient is lost to follow-up for a prolonged period).

C. Benefit-Risk Determination

The probable benefits of the device are also based on data collected in the clinical study conducted to support PMA approval as described above. The key elements of cervical (pre)cancer screening are the identification and referral of the appropriate population of at-risk individuals to colposcopy, at which time further evaluation by a physician will determine management and follow-up. In standard practice in the US, this is done by evaluation of the cervical cytology specimens (Pap test) by a qualified pathologist or cytotechnologist. Screening for cervical precancers and cancers by this system has over several decades resulted in a marked reduction in mortality rates for cervical cancer in the US. The CINtec *PLUS* Cytology test will be used to contribute to the evaluation of the slides prepared from cervical cytology specimens. The cobas HPV tests cited

in the proposed intended use assist in defining the risk level of the patient, and based on the data submitted, use of the CINtec *PLUS* Cytology test on cervical cytology specimens in conjunction with such FDA-approved HPV tests further add to the ability of the screening process to correctly define the patient's risk and accordingly assist in the appropriate decision for referral to colposcopy. This ultimately contributes to the benefit of the cervical cancer screening paradigm in maintaining the low mortality rate from cervical cancer by prevention of invasive disease (via identification and treatment of precursor lesions) in the US.

The probable risks of the device are also based on data collected in the clinical study conducted to support PMA approval as described above. The relevant risks to the patient in the cervical cancer screening process are: inappropriate referral to colposcopy and missed identification of a precancerous lesion which could lead to delayed diagnosis and/or treatment of the lesion. A substantial delay in lesion identification could result in more aggressive treatment (i.e. cervical conization) with the attendant risks of complications of that procedure, and a very prolonged delay could result in the development of invasive cancer.

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 that for the following indications for use the probable benefits outweigh the probable risks:

(a) To be used in women 25 - 65 years old with 12 Other High Risk (HR) HPV positive test results using the cobas® 4800 HPV Test in primary HPV screening, to determine the need for referral to colposcopy.

To be used in women 25 - 65 years old with HPV16/18 positive test results using the cobas® 4800 HPV Test in primary HPV screening where the CINtec *PLUS* Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

(b) To be used in women 30 - 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and 12 Other HR HPV positive test results using the cobas 4800 HPV Test in adjunctive cervical cytology and HR HPV screening, to determine the need for referral to colposcopy.

To be used in women 30 - 65 years old with NILM (Negative for Intraepithelial Lesion or Malignancy) and HPV16/18 positive test results using the cobas®4800 HPV Test in adjunctive cervical cytology and HR HPV screening where the CINtec *PLUS* Cytology test results will be used in conjunction with the physician's assessment of patient screening history, other risk factors, and professional guidelines to guide patient management.

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 benefits of use of the CINtec *PLUS* Cytology test, as part of the overall cervical cancer screening process, are aligned with those of this screening paradigm. Based on the data reviewed, the added benefit of this device to the process, for the intended use populations specified, is consistent with a clinically beneficial role of the device in triaging women with positive HR HPV test results. This has been demonstrated by acceptable performance characteristics (PPV and NPV) in the two IU populations: age 25 - 65 years old with HR HPV by cobas 4800 HPV test; and age 30 - 65 years old with NILM and HR HPV by cobas 4800 HPV tests.

XIII. CDRH DECISION

CDRH issued an approval order on March 10, 2020.

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

XIV. 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.