CINtec® *PLUS* Cytology

REF 760-100

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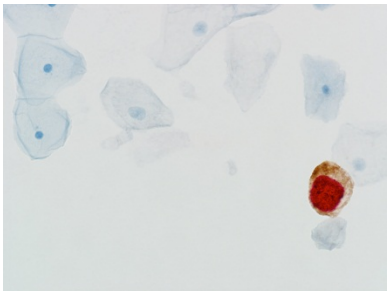

Figure 1. Cervical epithelial cell positive for p16^{INK4a} (brown cytoplasmic stain) and for Ki-67 (red nuclear stain)

INTENDED 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 p16INK4a (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.

CONTRAINDICATIONS

There are no known contraindications associated with CINtec *PLUS* Cytology.

SUMMARY AND EXPLANATION

In eukaryotic cells, control of progression of the cell division cycle is regulated by a complex pattern of controlled expression and post-translational modifications of cell-cycle regulating proteins. The p16^{INK4a} protein plays a major role in this mechanism of regulation of the eukaryotic cell cycle. It is part of the retinoblastoma protein (pRb)-mediated control of the G1/S phase transition, and it triggers cell cycle arrest in the course of cellular differentiation processes. Thus p16^{INK4a} provides an anti-proliferative effect during regular cell cycle progression.[1] In terminally differentiated epithelial cells, p16^{INK4a} expression is down-regulated to levels typically not detectable by immunocytochemistry (ICC).[2] In cervical dysplasia, overexpression of p16 is regarded as a surrogate biomarker for transforming HPV infections, reflecting the activation of HPV E6/E7 oncoprotein-driven cell proliferation.[2-5] Detection of p16 in cervical cytology preparations has been proposed as a valuable adjunctive marker to triage women with abnormal Pap cytology results as well as with positive HPV test results.[3-5] However, because p16-specific staining may be observed in individual metaplastic or endocervical cells in which p16 may be expressed to exert its physiological normal, growth-suppressive cellular function, interpretation of p16 single-stained cervical cytology preparations requires identification of p16 immunoreactive cells and further classification of these cells regarding signs of morphologic abnormalities.[2-4]

The combined simultaneous detection of p16 and the proliferation marker Ki-67 within the same cell by ICC has been shown to be a valuable tool to identify dysplastic cervical cells in cytology preparations without the need for morphologic interpretation.[3,7,8] Ki-67 is a nuclear and nucleolar protein strictly associated with cell proliferation and is undetectable by standard immunostaining methods in resting (G0) cells.[6] Under normal physiologic conditions, expression of the proliferation-associated protein Ki-67 is mutually exclusive of the anti-proliferative protein p16. In contrast, cells where the retinoblastoma protein (pRB)-mediated pathway controlling the cell-cycle progression is abrogated upstream of the tumor suppressor function of p16 (such as in epithelial cells expressing the high-risk HPV E6/E7 oncoproteins) may proliferate and thus may express Ki-67 in the presence of functional p16.[2-3]

Therefore, the detection of individual cells in cervical cytology preparations that simultaneously co-express p16 and Ki-67 may serve as a morphology-independent indicator of cells with cell cycle dysregulation and thus may be used as an indicator of the presence of transforming HPV infections and underlying cervical intraepithelial neoplasia.[2,3] In the recent past, numerous studies have been performed and published evaluating the potential value and clinical usefulness of p16/Ki-67 dual-stained cytology for the identification of women that may benefit from referral to colposcopy based on various primary cervical cancer screening results, including for the triage of women with Atypical Squamous Cells of Undetermined Significance (ASC-US) or Low grade Squamous Intraepithelial Lesion (LSIL) Pap cytology results, women who are high-risk HPV positive in primary HPV screening, or women who are Negative for Intraepithelial Lesion or Malignancy (NILM)/HPV positive in clinical settings where Pap cytology/HPV co-testing is used for primary screening.[7-25]

PRINCIPLE OF THE PROCEDURE

The CINtec *PLUS* Cytology test contains a set of reagents for the simultaneous immunocytochemical detection of the p16^{INK4a} and Ki-67 proteins in cytological specimens obtained from the uterine cervix. The proteins are detected using a ready-to-use cocktail of primary monoclonal antibodies which contains a monoclonal mouse antibody directed against human p16^{INK4a} protein (clone E6H4) and a primary recombinant rabbit antibody directed against human Ki-67 protein (clone 274-11AC3V1). 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 collected in PreservCyt Solution:

- 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, as shown in Figure 2
- 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, as shown in Figure 3.

The chromogenic reactions are based on the HRP-mediated conversion of 3,3'-diaminobenzidine tetrahydrochloride (DAB) and the AP-mediated conversion of Fast

Red with Naphthol Phosphate resulting in a brown precipitate at the p16^{INK4a} antigen site and a red precipitate at the Ki-67 antigen site, respectively.

After automated counterstaining and bluing, 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 light microscopy.

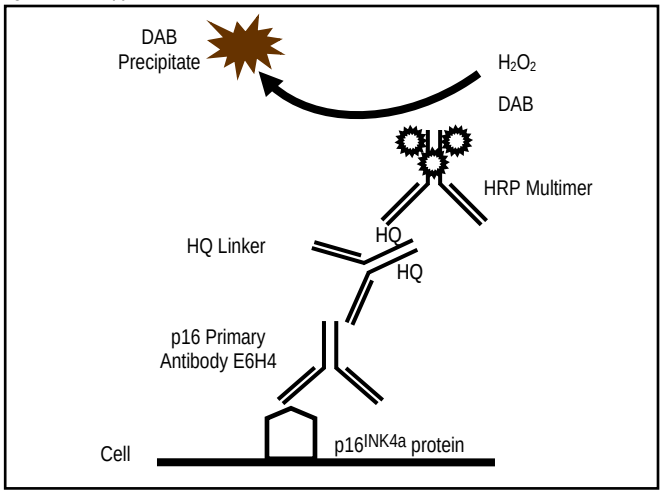

Figure 2. Detection of human p16^{INK4a} protein.

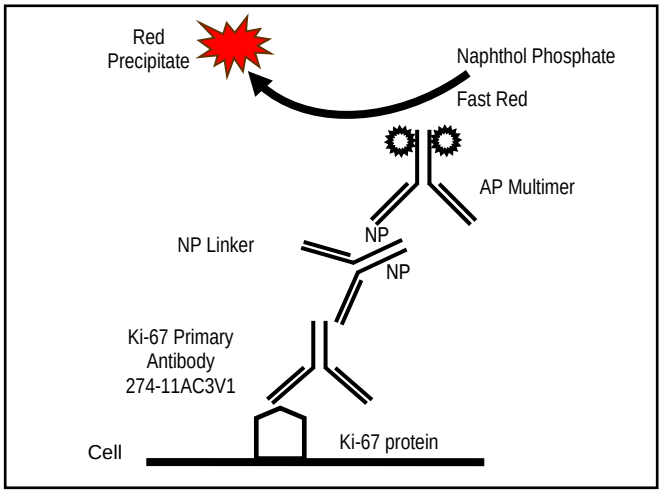

Figure 3. Detection of human Ki-67 protein.

REAGENTS PROVIDED

The CINtec *PLUS* Cytology test contains sufficient reagent for 100 tests.

One 10 mL dispenser	CINtec *PLUS* Cytology Primary Antibody Cocktail (p16/Ki-67) contains a cocktail of monoclonal mouse antibody clone E6H4 directed to human p16^{INK4a} protein and primary recombinant rabbit antibody clone 274-11AC3V1 directed against human Ki-67 protein (<5 µg/mL total antibody) in a buffer containing protein with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Red anti-Rabbit NP Linker contains NP-labeled goat anti-rabbit IgG (<10 µg/mL; NP is a proprietary hapten covalently attached to the goat antibody) in a buffer containing protein with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Red AP Multimer contains a mouse monoclonal anti-NP-labeled AP tertiary antibody (<20 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Red Naphthol phosphate contains Naphthol phosphate (<1%) with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Fast Red contains Fast Red (<1%) in acetate buffer with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology DAB Peroxidase inhibitor contains hydrogen peroxide solution (<5%).
One 10 mL dispenser	CINtec *PLUS* Cytology DAB anti-Mouse HQ Linker contains HQ- labeled goat anti-mouse IgG (<40 µg/mL; HQ is a proprietary hapten covalently attached to the goat antibody) in a buffer containing protein with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology DAB HRP Multimer contains a mouse monoclonal anti-HQ-labeled HRP tertiary antibody (<10 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology DAB contains 3,3'-diaminobenzidine tetrahydrochloride (<1%) in a proprietary stabilizer solution with a proprietary preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology DAB H2O2 contains hydrogen peroxide (<1%) in a phosphate buffer solution.

RECONSTITUTION, MIXING, DILUTION, TITRATION

The CINtec *PLUS* Cytology test is optimized for use on the BenchMark ULTRA instrument. No reconstitution, mixing, dilution, or titration of kit reagents is required.

Deviations from the recommended procedures for fixation and further processing of the cervical cytological specimens may produce substantial variability in results.

For more information about controls, see the Quality Control section.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as ancillary components, including negative and positive control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

The following reagents and materials are required for staining but not provided with CINtec *PLUS* Cytology:

1. Appropriate controls (please refer to Quality Control section)
2. Hematoxylin (P/N 760-2021 / 05266726001)
3. Bluing Reagent (P/N 760-2037 / 05266769001)
4. Reaction Buffer Concentrate (10X) (P/N 950-300 / 05353955001)
5. ULTRA Cell Conditioning Solution (ULTRA CC1) (P/N 950-224 / 05424569001)
6. ULTRA LCS (Predilute) (P/N 650-210 / 05424534001)
7. Reagent grade ethanol denatured (purity 95%)
8. BenchMark ULTRA instrument
9. ThinPrep 2000 or ThinPrep 5000 Processor
10. ThinPrep Pap Test Filter for Gynecologic Applications
11. PreservCyt Solution
12. ThinPrep Arcless microscope slides (Hologic P/N 70126-002) or Superfrost Plus microscope slides (VWR P/N: 48311-703)
13. CC/Mount™ aqueous mounting medium (Diagnostic BioSystems P/N: K 002; Sigma-Aldrich P/N: C9368)
14. Optional: Drying oven capable of maintaining a temperature of 60°C ± 5°C
15. Xylene (Histological grade)
16. Deionized water
17. Glass coverslips and xylene-based mounting medium or plastic film coverslip method sufficient to cover cytology preparations

18. Light microscope
19. Mild dishwashing detergent

STORAGE

Store at 2-8°C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert. This reagent kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and the stability of the reagents, replace the dispenser's cap after every use and immediately place the dispensers in the refrigerator in an upright position.

The CINtec *PLUS* Cytology test has an expiration date. When properly stored, the reagents are stable through the date indicated on the label. Do not use the product beyond the expiration date for the prescribed storage method. There are no definitive signs to indicate instability of this product; therefore, a positive control should be run simultaneously with patient specimens. Your local support representative should be contacted immediately if there is an indication of reagent instability.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Warning: Possible carcinogen. The International Agency for Research on Cancer (IARC) and the US National Toxicology Program (NTP) have listed benzidine, a compound closely related to 3, 3'-diaminobenzidine tetrahydrochloride (DAB), as a known human carcinogen.
5. Do not use the product if the packaging of any of its components is damaged. Should packaging be compromised or components damaged, please notify your local support representative without delay.
6. The reagents have been optimally diluted and further dilution may result in loss of antigen staining. The user must validate any such change.
7. ProClin 300 solution is used as a preservative in this solution. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
8. Materials of human or animal origin should be handled as biohazardous and disposed of with proper precautions.
9. Take reasonable precautions when handling reagents. Avoid contact of reagents with eyes, skin, and mucous membranes. Avoid inhalation of reagents. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials. If reagents come in contact with sensitive areas, wash with copious amounts of water.
10. Avoid microbial contamination of reagents as it may cause incorrect results.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. When handling and disposing of cytological specimens, including all specimens before and after fixation, as well as all materials exposed to them, adhere to the safety precautions for handling potentially infectious material as well as applicable waste disposal requirements.
13. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

SPECIMEN PREPARATION

Cytological specimens must be adequately handled to preserve the specimens for immunocytochemical procedures. All specimens should be subjected to standard methods of cell processing.

To avoid obscuring elements such as blood and mucus and ensure a sample that is adequate per Bethesda Guidelines[26], clinicians should follow the recommended sampling techniques.

The following slide preparation method is suitable for use with the CINtec *PLUS* Cytology test:

- Slides prepared on a ThinPrep 2000 Processor or ThinPrep 5000 Processor (Hologic Inc.) using ThinPrep Arcless microscope slides (Hologic Inc.) or Superfrost Plus slides.

It is recommended to run appropriate controls simultaneously with patient specimens (please refer to Quality Control section for further details).

PreservCyt Sample Preparation

Cytologic samples in PreservCyt Solution (PC) intended for immunocytochemistry staining using CINtec *PLUS* Cytology 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.

ThinPrep Arcless microscope slides or Superfrost Plus slides are required for immunocytochemical staining with CINtec *PLUS* Cytology on the BenchMark ULTRA instrument.

Slide Preparation from Samples Collected in PreservCyt Using the ThinPrep 2000 Processor

Slides are prepared from samples collected in PreservCyt using the ThinPrep 2000 Processor (Hologic Inc.) according to the manufacturer's instructions for the preparation of slides for Pap cytology staining. After the ThinPrep 2000 Processor sequence is finished the processed slide sits in a fixative vial containing a 95% reagent grade ethanol solution. Remove the vial from the fixative bath holder of the ThinPrep 2000 Processor and transfer the processed slide from the vial into a slide container filled with a 95% reagent grade ethanol solution. Incubate the slides in ethanol for a minimum of 15 minutes to a maximum of 60 minutes. Change the ethanol solution in the fixative vial and slide bucket after every 20 slides prepared. Upon completion of the incubation time remove the slides from the ethanol solution and dry the slides lying horizontally on a flat surface for at least 60 minutes. Dried slides can be stored at room temperature protected from light and must be stained with CINtec *PLUS* Cytology within 7 days of preparation.

Remove any labels that may have been applied to the slide before immunocytochemical staining with the CINtec *PLUS* Cytology test. Apply the corresponding label generated by the BenchMark ULTRA instrument.

Slide Preparation from Samples Collected in PreservCyt Using the ThinPrep 5000 Processor

Slides are prepared from samples collected in PreservCyt using the ThinPrep 5000 Processor (Hologic Inc.) according to the manufacturer's instructions for the preparation of slides for Pap cytology staining. After the ThinPrep 5000 Processor sequence is finished the processed slides sit in a slide rack which is immersed in a 95% reagent grade ethanol solution containing fixative bath. Remove the fixative bath or slide bucket from the ThinPrep 5000 Processor and incubate slides for an additional minimum of 15 minutes to a maximum of 60 minutes. Change the ethanol solution in the slide bucket after each run. Upon completion of the incubation time, remove the slides from the ethanol solution and dry the slides lying horizontally on a flat surface for at least 60 minutes. 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.

Before immunocytochemical staining with the CINtec *PLUS* Cytology test, remove the original slide label used by the ThinPrep 5000 Processor and apply the corresponding label generated by the BenchMark ULTRA instrument.

STAINING PROCEDURE

The CINtec *PLUS* Cytology test has been developed for use on the BenchMark ULTRA instrument in combination with VENTANA ancillary reagents and accessories. Refer to Table 1 for recommended staining protocols.

The CINtec *PLUS* Cytology test has been optimized with the parameters indicated in Table 1; nonetheless the user must validate results obtained with this kit.

The parameters for the automated protocols can be displayed, printed and edited according to the instructions in the Operator's Manuals for the BenchMark ULTRA instrument.

Table 1. Recommended Staining Protocol for Prepared Slides on the BenchMark ULTRA Instrument

Staining Procedure: CINtec PLUS Cytology	
Procedure Parameter	Selection
Cell Conditioning	CC1 Cell Conditioning 16 minutes
CINtec *PLUS* Cytology Primary Antibody Cocktail (p16/Ki-67) (Primary)	16 minutes
CINtec *PLUS* Cytology HQ Linker	12 minutes
CINtec *PLUS* Cytology HQ Multimer	8 minutes
CINtec *PLUS* Cytology NP Linker	8 minutes
CINtec *PLUS* Cytology NP Multimer	8 minutes
Counterstain (hematoxylin)	8 minutes
Post Counterstain (blueing)	4 minutes

BenchMark ULTRA Instrument Operation

1. Apply slide bar code label corresponding to the protocol to be performed.
2. Load the CINtec *PLUS* Cytology test dispensers and required ancillary reagents onto the reagent tray and place them on the instrument.
3. Check bulk fluids and empty waste.
4. Load the slides onto the instrument.
5. Start the staining run.
6. At the completion of the run, remove the slides from the instrument.

POST PROCESSING PROCEDURE - MOUNTING AND COVERSLIPPING

To maintain optimal sensitivity and to prevent fading of chromogens, a two-step mounting procedure is required.

Remove the slides from the BenchMark ULTRA instrument and gently agitate and rinse slides with distilled or deionized water and mild dishwashing detergent until the liquid coverslip is completely removed from the slides.

NOTE: Take care not to let the water directly hit the slides. Run the water at a minimal force.

The slides will be mounted following a two-step protocol and the following steps must be performed sequentially:

1. Aqueous mounting:
   - Incubate slides in distilled or deionized water for at least 1 min;
   - Slides not being coverslipped should remain in distilled or deionized water during application of the CC/Mount aqueous mounting media to the other slides;
   - Remove single slide from distilled or deionized water and carefully wipe the back side of the slide with a paper towel to remove excess water. Do not drain or wipe water off front of slide (sample side);
   - Hold slide at a slight angle and apply 4-6 drops of CC/Mount aqueous mounting medium per slide. Avoid generation of air bubbles. To prevent bubble formation, the first drop can be discarded onto a paper towel before applying CC/Mount on the specimen preparation area of the slide;
   - Gently tilt and rotate the glass slide to generate a thin layer of mounting medium to fully cover the specimen preparation area (do not yet apply a glass or film coverslip); check the distribution of the mounting medium on the slide by visual inspection;
   - Clean excess CC/Mount aqueous mounting media from the back and edges of the slide. Use wet paper towel if necessary;
   - For drying, place prepared slides in a horizontal position and incubate slides at 37-60°C for 1 hour, or alternatively overnight at ambient temperature;
2. Glass or film coverslipping:
   - After complete drying of the CC/Mount aqueous mount, allow slides to equilibrate to room temperature, if needed. Incubate slides in xylene for a minimum of 1 minute and up to a maximum of 20 minutes. Then, coverslip the slides with glass coverslips using a xylene-based mounting medium or xylene based film coverslipping method.

     NOTE: Slides must not be dehydrated by ascending series of alcohol before being glass or film coverslipped, because the red chromogen is soluble in alcohol.
   - Let the xylene-based mounting medium dry at room temperature.

NOTE: To minimize fading, protect slides from light and store at room temperature.

QUALITY CONTROL

Deviations from the recommended procedures for fixation and further processing of the cervical cytological specimens may produce substantial variability in results. Malfunction of the product due to handling problems or to instability does not result in obvious signs. Therefore, appropriate controls should be run simultaneously with patient specimens.

Controls

Known positive controls are recommended for monitoring all steps of specimen processing and staining. One positive control slide should be included in each staining run. Control material is not provided with the kit and must be sourced by the customer.

Known positive controls should only be utilized for monitoring the appropriate performance of processed specimens, instruments and test reagents rather than as an aid in formulating a specific diagnosis of patient samples.

Positive controls should consist of a cervical sample(s) or pooled cervical samples that have been processed in the same manner as patient samples. The samples should contain sufficient dual-stained cells to assess the presence and sensitivity of staining. HSIL (high-grade squamous intraepithelial lesion) samples have a higher likelihood of containing dual-stained cells and thus are a good source of potential control samples. The control should also include sufficient superficial squamous cells (negative elements or reference cells) to assess non-specific background. Selected samples should yield slides with dual-stained cells, >5,000 well preserved squamous cells and be free of interfering background and obscuring elements. To maximize the number of slides from each control sample, samples may be pooled together for control purpose only.

Positive Elements

Specimens processed in the same manner as the patient sample(s) and known to contain dual-stained positive cells by the CINtec *PLUS* Cytology test should be used as positive controls. Positive controls are indicative of correctly prepared specimens and proper staining techniques.

Negative Elements

A variety of different cell types are present in cervical cytology specimens. Many cells have undergone cellular differentiation and terminal cell cycle arrest and are known to be negative for the simultaneous expression of the $p16^{INK4a}$ and Ki-67 antigens (such as superficial squamous cells). These cells may serve as an internal negative control to assess non-specific background staining.

After a CINtec *PLUS* Cytology staining run is complete and before case slides are evaluated, a cytotechnologist or pathologist will assess the control slide to determine whether it is valid, as defined in the Table 2.

Table 2. Control Slide Acceptance Criteria for Determining Validity of a Run

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.

For more information on creation and evaluation of controls refer to the Interpretation Guide for CINtec *PLUS* Cytology (PN 1018620EN).

Assay Verification

The user should verify the performance of the CINtec *PLUS* Cytology test on positive and negative specimens with known performance characteristics prior to its initial use in a diagnostic procedure.

STAINING INTERPRETATION

Before interpreting CINtec *PLUS* Cytology stained slides the Interpretation Guide for CINtec *PLUS* Cytology should be reviewed.

CINtec *PLUS* Cytology staining produces two distinct colored reaction products: a brown precipitate at the $p16^{INK4a}$ antigen sites, and a red precipitate at the Ki-67 antigen sites. Brown staining of cells (cytoplasm and/or nuclei) indicates $p16^{INK4a}$ over-expression. Red staining of cells (nuclei and nucleoli) indicates expression of Ki-67. Cells stained for both antigens exhibit brown cytoplasmic staining with typically pronounced red nuclei. A qualified pathologist/ cytotechnologist experienced in immunocytochemical procedures and trained on the interpretation of CINtec *PLUS* Cytology stained slides must evaluate controls before interpreting results.

Interpretation of the test results may only be made by a trained pathologist in conjunction with the patient´s clinical history and additional diagnostic tests that have been performed.

Positive Test Result

The presence of one or more cervical epithelial cells with co-localization of specific brown cytoplasmic immunostaining and specific red nuclear immunostaining within the same cell is regarded as a positive CINtec *PLUS* Cytology test result regardless of cytomorphologic features.

Negative Test Result

If no cervical epithelial cell shows simultaneous brown cytoplasmic immunostaining and red nuclear immunostaining, the CINtec *PLUS* Cytology test result is considered negative.

The presence of cervical epithelial cells that show immunoreactivity only for one but not both markers (such as brown staining for $p16^{INK4a}$ only or red staining for Ki-67 only) is not considered a positive test result for the CINtec *PLUS* Cytology test.

Unsatisfactory for Evaluation

In addition, similar to reporting Pap cytology results, specimens should be assessed for sample adequacy according to *The Bethesda System for Reporting Cervical Cytology*.[26]

A CINtec *PLUS* Cytology result of unsatisfactory should be reported for a case that is considered “unsatisfactory for evaluation due to inadequate cellularity” unless dual-stained cells are identified. If at least one dual-stained cell is present on a slide where there are less than 5,000 well-visualized/well-preserved squamous cells present, the case shall be evaluated and reported as positive for CINtec *PLUS* Cytology. As in *The Bethesda System for Reporting Cervical Cytology*[26], extracellular elements such as bacteria, blood, mucus and inflammation are considered obscuring elements if they obscure more than 75% of the epithelial cells in the sample preparation area of the slide. A CINtec *PLUS* Cytology result of unsatisfactory should be reported for a case that is considered “unsatisfactory for evaluation due to obscuring elements” unless dual-stained cells are identified. If at least one dual-stained cell is present on a slide where there are more than 75% of the epithelial cells obscured the case shall be evaluated and reported as positive for CINtec *PLUS* Cytology. A result of unsatisfactory should also be reported for slides in which non-specific background staining interferes with interpretation. Refer to the Interpretation Guide for the full description of assay interpretation.

LIMITATIONS

1. For professional use only. Special training is required for the performance of immunocytochemical procedures.
2. Evaluation of microscope slides stained with CINtec *PLUS* Cytology should be performed only by a certified professional who has been trained to interpret these test results.
3. The interpretation of CINtec *PLUS* Cytology staining results depends on the intensity and quality of the hematoxylin counterstaining. Deviation from the recommended incubation times (8-16 min hematoxylin incubation time) requires validation by the customer as excessive or incomplete counterstaining may interfere with proper interpretation of results.
4. The presence of vaginal douches, antifungal cream and vaginal lubricant in the sample does not affect CINtec *PLUS* Cytology test performance. However, the presence of leukocytes, mucus or vaginal deodorant in the sample may affect the on slide cellularity after the slides are stained with CINtec *PLUS* Cytology. The performance of the CINtec *PLUS* Cytology test in the presence of these substances may be adversely affected.
5. Cervical specimens often show visibly detectable levels of whole blood. If the concentration of whole blood exceeds 1.0%, the specimen should be lysed with glacial acetic acid (GAA) according to the ThinPrep protocol prior to slide preparation.
6. Performance of the CINtec *PLUS* Cytology test was established for ThinPrep 2000 and ThinPrep 5000 Processors. Use of the ThinPrep 3000 Processor is not recommended for preparation of slides as the spray fixation procedure performed by the instrument may lead to substantial cell loss when slides prepared are stained with the CINtec *PLUS* Cytology test.
7. Performance of the CINtec *PLUS* Cytology test was not established for women older than 65 years.
8. ThinPrep microscope slides, Arcless (Hologic P/N 70126-002) or Superfrost Plus microscope slides (VWR P/N: 48311-703) are required for the preparation of slides for ICC staining with the CINtec *PLUS* Cytology on BenchMark ULTRA instruments. ThinPrep microscope slides with an imprinted screening area may lead to inconsistent staining results.
9. The manufacturer provides these antibodies/reagents at optimal dilution for use according to the instructions provided herein, for immunocytochemistry testing on prepared liquid-based cytology (LBC) slides. Any deviation from the recommended test procedures may invalidate results; appropriate user-supplied controls should be employed and documented. Users who deviate from the recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

TROUBLESHOOTING

1. If a reagent dispenser does not dispense fluid, check the priming chamber or meniscus for foreign materials or particulates, such as fibers or precipitates. If the dispenser is blocked, do not use the dispenser and contact your local support representative. Otherwise, re-prime the dispenser by aiming the dispenser over a waste container, removing the nozzle cap, and pressing down on the top of the dispenser.
2. Crystallization originating from CINtec *PLUS* Cytology Red Naphthol phosphate dispenser may be observed occasionally. Investigations have shown no interference of crystals with interpretation of results. If crystals are observed on slides, clean the nozzle tip and prime the dispenser to ensure any crystalline debris is removed. If crystals persist, discontinue use and contact your local support representative for dispenser replacement.
3. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, check to ensure that all dispenser barrels are clear from debris.
4. If high background is observed, decrease incubation times in the staining protocols. In addition, check to ensure the Reaction Buffer bulk solution was formulated correctly, and increase hematoxylin incubation time.

5. If weak staining is observed, incubation time of the primary antibody can be increased. However, it should be noted that assay protocols other than the recommended protocol in Table 1 above have not been validated.
6. The red precipitate used to indicate Ki-67 protein expression is alcohol soluble. If Ki-67 staining is weak or not present, ensure that alcohol was not used and that the recommended post-processing procedure was followed according to the Post Processing Procedure - Mounting and Coverslipping instructions.
7. If sample washes off the slide, slides should be checked to ensure that the sample was prepared properly according to the Specimen Preparation section and recommended microscope slide type was used.
8. For corrective action, refer to the Staining Procedure section, the instrument Operator's Manual or contact your local support representative.
9. Inconsistent or uneven counterstaining of the reference squamous cells with brown background may be associated with poor tap water quality. If present in the majority of stained slides, it is recommended to use either deionized or distilled water with mild detergent when rinsing liquid coverslip from the stained slides prior to mounting.

PERFORMANCE CHARACTERISTICS

The analytical performance of the CINtec *PLUS* Cytology test was evaluated by conducting precision and other relevant studies. All staining was performed using the staining procedure as noted in the package insert on the BenchMark ULTRA instrument.

Analytical Sensitivity and Specificity

Analytical Sensitivity and Specificity of the p16 and Ki-67 primary antibodies were tested in Western blot and peptide inhibition assays.

Western blot assays used lysates from cell lines representing a range of staining intensities. Anti-p16^{INK4a} (E6H4) antibody was able to detect a band of approximately 15-20kD in the purified recombinant p16^{INK4a} protein preparation. Further, the antibody bound specifically to purified recombinant p16^{INK4a} protein and not to an equivalent amount of unrelated recombinant protein. The antibody was also shown to bind endogenous p16^{INK4a} protein expressed in lysates derived from cell lines HeLa, SK Mel 28 and DU145 and not in the p16^{INK4a} negative cell line MDA MB 231. The relative levels of p16^{INK4a} protein detected in lysates from all four cell lines on Western Blot corresponded to IHC staining data, demonstrating the sensitivity of anti-p16^{INK4a} (E6H4) antibody detection. The Ki-67 antibody clone (274-11AC3V1) binding was tested in a Western Blot assay using 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. The antibody was able to detect endogenous Ki-67 protein in cell lysates even at low expression levels, and the band intensity correlated with IHC staining for Ki-67 in these cell lines. The CINtec *PLUS* Cytology Ki-67 primary antibody (274-11AC3V1) bound to a purified recombinant Ki-67 protein fragment and not to an equivalent amount of the unrelated recombinant negative control protein. Binding in this assay was not detected in the Ki-67 low-expressing cell line LNCaP. The relative levels of Ki-67 protein detected in lysates from these cell lines on Western blot correlates with IHC staining data and the mRNA expression data. The Western blot results demonstrate that the Ki-67 primary antibody used in the CINtec *PLUS* Cytology test can detect endogenous Ki-67 protein in cell lysates and recombinant Ki-67 protein fragment in purified form.

Peptide inhibition assays used solutions containing p16 or Ki-67 specific peptides. The primary antibody cocktail was diluted at a 1:1 volume:volume ratio with p16-specific or Ki-67 peptide solutions of various concentrations to span a range of molar ratios: approximately a 1-fold, 10-fold, 100-fold, 1,000-fold and 10,000-fold molar excess of peptide compared to the final concentration of antibody in the solution. Primary antibody cocktail containing p16-specific peptide served as a non-specific control for anti-Ki-67 antibody, and primary antibody cocktail containing Ki-67-specific peptide served as a non-specific control for anti-p16 antibody. One slide from each specimen (three cervical cytology specimen pools and one CaSki cells) was stained with each solution. The results of this study showed that the anti-p16 antibody specifically binds the p16 protein and that the anti-Ki-67 antibody specifically binds Ki-67 protein. As expected, the p16 and Ki-67 staining intensities were reduced in all specimens after staining with solutions containing the respective specific peptides at concentration at 1 M, complete inhibition was achieved with the solutions containing ≥ 10 M, while no reduction of the p16 staining intensity was observed after staining with solutions containing non-specific peptide or no peptide.

Repeatability and Intermediate Precision

Precision studies for CINtec *PLUS* Cytology staining of cervical specimens were completed to demonstrate:

- Overall precision – Percent of results same as majority call was calculated for each of 12 cervical cytology pools (3 HSIL/HPV+, 1 LSIL/HPV+, 1 ASCUS/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools) stained with 3 lots of CINtec *PLUS* Cytology, on 5 non-consecutive days, using 2 replicate slides from each pool and evaluated by 3 reader teams.
- Between-day precision – Percent of results same as majority call was calculated for each day (Day 1 – Day 5), aggregating data from 12 cervical cytology pools (3 HSIL/HPV+, 1 LSIL/HPV+, 1 ASCUS/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools), 3 lots of CINtec *PLUS* Cytology, 2 replicate slides from each pool, and 3 reader teams.
- Between-lot precision – Percent of results same as majority call was calculated for each CINtec *PLUS* Cytology lot (Lot 1-3) aggregating data from 12 cervical cytology pools (3 HSIL/HPV+, 1 LSIL/HPV+, 1 ASCUS/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools), 5 non-consecutive days, 2 replicates and 3 reader teams.

All slides were blinded and randomized, and then evaluated following the CINtec *PLUS* Cytology staining interpretation. Slides were evaluated by three reader teams, each one comprised of a cytotechnologist and a pathologist. The majority call, or pool-level mode result (positive or negative), was used as the reference to determine the percent of results same as the majority call. Percent of results same as majority call is equivalent to PPA when the majority call is positive, and to NPA when the majority call is negative. Results are summarized in Tables 3-5. All confidence intervals (CIs) were 2-sided 95% confidence intervals. CIs were calculated using the percentile bootstrap method except that when point estimates were 100%, the Wilson score method was used.

Table 3. Within-Laboratory Precision Study - Overall Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call	95% Confidence Interval
T24 Cell Line	270	Negative	100.0%	(98.6, 100.0)
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	100.0%	(95.9, 100.0)
HSIL/HPV+	270	Positive	98.9%	(96.7, 100.0)

Table 4. Within-Laboratory Precision Study - Between-Day Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call				
			Day 1	Day 2	Day 3	Day 4	Day 5
T-24 Cell Line	270	Negative	100.0%	100.0%	100.0%	100.0%	100.0%
NILM/HPV-	270	Negative	88.9%	90.7%	98.1%	98.1%	94.4%
NILM/HPV+	90	Positive	44.4%	50.0%	77.8%	66.7%	66.7%
ASCUS/HPV+	90	Positive	94.4%	88.9%	88.9%	94.4%	100.0%
LSIL/HPV+	90	Positive	100.0%	100.0%	100.0%	100.0%	100.0%
HSIL/HPV+	270	Positive	100.0%	94.4%	100.0%	100.0%	100.0%

Table 5. Within-Laboratory Precision Study – Between-Lot Precision

Pool Category	Number of Evaluations	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	100.0%	100.0%	100.0%
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	100.0%	100.0%	100.0%
HSIL/HPV+	270	Positive	100.0%	100.0%	96.7%

Table 6. Within-Laboratory Precision Study - Between-Reader Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call		
			Reader Team 1	Reader Team 2	Reader Team 3
T-24 Cell Line	270	Negative	100.0%	100.0%	100.0%
NILM/HPV-	270	Negative	95.6%	91.1%	95.6%
NILM/HPV+	90	Positive	70.0%	63.3%	50.0%
ASCUS/HPV+	90	Positive	100.0%	90.0%	90.0%
LSIL/HPV+	90	Positive	100.0%	100.0%	100.0%
HSIL/HPV+	270	Positive	98.9%	98.9%	98.9%

Reproducibility

A reproducibility study was conducted to evaluate the CINtec *PLUS* Cytology test in the detection of $p16^{INK4a}$/Ki-67 dual-staining of cervical epithelial cells in liquid-based cytology specimens at different sites. The study included 6 distinct cultures of T24 cells (a cell line that is negative for p16/Ki-67 dual-staining), and 21 specimen pools (7 HSIL/HPV+, 4 LSIL/HPV+, 4 ASCUS/HPV+, 3 NILM/HPV+, and 3 NILM/HPV- LBC pools). The T24 cell cultures came from 6 individual vials of a working cell bank. Each of 3 study sites were provided with aliquots of each specimen pool, T24 cell culture, and control pool in sufficient volume and number to support the testing planned at each site. Two study sites prepared a single slide from each specimen pool, T24 culture, and a control pool (28 slides in total) on a single slide processor (one site: ThinPrep 2000 Processor and second site: ThinPrep 5000 Processor) for each of 5 staining days. On each staining day, the site stained one set of 28 slides on one ULTRA instrument. The 5 staining days were non-consecutive and spanned at least 20 days. The third study site prepared 2 sets of 28 slides (ie, 1 set on a ThinPrep 2000 Processor and 1 set on a ThinPrep 5000 Processor) for each of 5 staining days and stained each set on separate staining runs on the 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 unsatisfactory. The reader-teams were blinded to any prior determination of HPV status, Pap cytology status, CINtec *PLUS* Cytology test results, or other clinical information.

Data were directly entered into a clinical database and analyzed to determine reproducibility of the assay across multiple sites, days, and reader-teams. Results are summarized in Tables 7 and 8.

Table 7. Between-Site Reproducibility Result

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call		
			Site 1	Site 2	Site 3
T24 Cell Line	240	Negative	100.0%	100.0%	100.0%
NILM/HPV-	120	Negative	100.0%	86.7%	95.0%
NILM/HPV+	120	Positive	60.0%	60.0%	71.7%
ASCUS/HPV+	158	Positive	72.5%	82.5%	89.7%
LSIL/HPV+	159	Positive	85.0%	77.5%	94.8%
HSIL/HPV+	280	Positive	100.0%	100.0%	100.0%

Table 8. Reproducibility Study – Overall Precision

Pool Category	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same Result as Majority Call		
		%	(n/N)	95% CI
T24 Cell Line	Negative	100%	240/240	(98.4, 100.0)
NILM/HPV-	Negative	94.2%	113/120*	(90.0, 100.0
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+	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. 4 out of 60 slides prepared from the 3 NILM/HPV negative pools had dual stained cells; 3 slides had one dual-stained cell and 1 slide had 2 dual-stained cells.

EXPECTED RESULTS

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 old 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.

A total of 35,263 women 25–65 years old undergoing routine cervical screening across 32 clinical studies were enrolled in the study. Of these, 5046 women had cobas 4800 positive results: 785 women had HPV16+ results, 287 women had HPV18+ results, and 3,974 women had 12 Other HR HPV+ results.

Population of cobas 4800 HPV Positive Women 25-65 Years Old

Tables below show percent of positive, negative, and invalid CINtec *PLUS* Cytology results for women 25-65 years old stratified by testing site (Table 9), by age group (Table 10), and by Pap cytology result (Table 11).

Table 9. Percent of CINtec *PLUS* Cytology Results for Women 25-65 Years Old Stratified by Testing Site and HPV Genotype

	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 HPV16+			cobas 4800 HPV18+		
	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 10. Percent of CINtec *PLUS* Cytology Results for Women 25-65 Years Old Stratified by Age Group and HPV Genotype

Age Group (Years)	cobas 4800 12 Other HR HPV+		
	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 HPV16+			cobas 4800 HPV18+		
	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 11. Percent of CINtec *PLUS* Cytology Results for Women 25-65 Years Old Stratified by Pap Cytology Result and HPV Genotype

Pap Cytology	cobas 4800 12 Other HR HPV+		
	CINtec *PLUS* Cytology Result		
	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)
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)

Pap Cytology	cobas 4800 HPV16+			cobas 4800 HPV18+		
	CINtec *PLUS* Cytology Result			CINtec *PLUS* Cytology Result		
	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 cobas 4800 HPV Positive Women 30-65 Years Old with NILM Pap Cytology Results

Tables below show percent of positive, negative and invalid results for women 30-65 years old with NILM Pap cytology results stratified by testing site (Table 12) and by age group (Table 13).

Table 12. Percent of CINtec *PLUS* Cytology Results for Women 30-65 Years Old with NILM Cytology Stratified by Testing Site and HPV Genotype

Site	cobas 4800 12 Other HR HPV+		
	CINtec *PLUS* Cytology Result		
	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)

Site	cobas 4800 HPV16+			cobas 4800 HPV18+		
	CINtec *PLUS* Cytology Result			CINtec *PLUS* Cytology Result		
	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 13. Percent of CINtec *PLUS* Cytology Results for Women 30-65 Years Old with NILM Cytology Stratified by Age Group and HPV Genotype

Age Group (Years)	cobas 4800 12 Other HR HPV+		
	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 HPV16+			cobas 4800 HPV18+		
	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)
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 trial 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 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*[26]. 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 p16 immunohistochemistry (IHC) assay (CINtec Histology, Ventana Medical Systems, Inc.). 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[27] (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 cobas 4800 HPV Positive Women 25-65 Years Old

A total of 5,046 women 25-65 years old with cobas 4800 HPV 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. The disposition of CINtec *PLUS* Cytology results by CPR result is presented in Table 14.

Table 14. CINtec *PLUS* Cytology Results and CPR Results in 12 Other HR HPV+ Women 25-65 Years Old

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Pos	891	194	176	78	3	1342
Neg	1451	81	44	13	0	1589
Total	2342	275	220	91	3	2931
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 high-grade cervical disease (≥CIN2and ≥CIN3) in 12 Other HR HPV+ women is presented in Table 15. 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 15. Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 25-65 Years Old

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.		

Performance of the CINtec *PLUS* Cytology test for women 25-65 years old with 12 Other HR HPV positive results in detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Table 16.

Table 16. Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 25-65 Years Old 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.			

Performance of the CINtec *PLUS* Cytology test in detecting ≥CIN2 and ≥CIN3 in 12 Other HR HPV+ women, stratified by HPV vaccination status, is presented in Table 17.

Table 17. Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 25-65 Years Old 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.			

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

Among 5,046 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. The disposition of CINtec *PLUS* Cytology results by CPR results is presented in Table 18.

Table 18. CINtec *PLUS* Cytology Results and CPR Results in cobas 4800 HPV16+ Women 25-65 Years Old

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; all 6 ACIS cases 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 19. 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 19. Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 25-65 Years Old

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)	
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 of the CINtec *PLUS* Cytology test for women 25-65 years old with HPV16+ results in detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Table 20.

Table 20. Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+Women 25-65 Years Old 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)
	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.			

Performance of the CINtec *PLUS* Cytology test in detecting ≥CIN2 and ≥CIN3 stratified by HPV vaccination status is presented in Table 21.

Table 21. 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 (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)

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

Among 5,046 women with HPV positive results, there were 287 women with HPV18 positive 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 22.

Table 22. CINtec *PLUS* Cytology Results and CPR results in cobas 4800 HPV18+ Women 25-65 Years Old

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 y; 2 of the 3 ACIS cases 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 23. 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 (1-NPV) was 4.7% for ≥CIN2 and 1.9% for ≥CIN3.

Table 23. Performance of CINtec *PLUS* Cytology in cobas 4800 HPV18+ Women 25-65 Years Old

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 of the CINtec *PLUS* Cytology test for women 25-65 years old with HPV18+ results in detecting ≥CIN2 and ≥CIN3 evaluated by age group is presented in Table 24.

Table 24. Performance of CINtec *PLUS* Cytology in cobas 4800 HPV18+ Women 25-65 Years Old 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)
	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.			

Table 25. 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 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 the following tables for the cobas 4800 12 Other HR HPV+ population (Tables 26 and 27), cobas 4800 HPV16+ population (Tables 28 and 29), and cobas 4800 HPV18+ population (Tables 30 and 31). 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 (Tables 27, 29, and 31), with the maximum increase in sensitivity (diff=23.1%) and the minimum decrease in specificity (diff=7.0%) observed in the cobas 4800 12 Other HR HPV+ population (Table 24). In all cases, the use of CINtec *PLUS* Cytology resulted in a substantial reduction of the risk of disease for negative CINtec *PLUS* Cytology test 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 26. 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
Pos	171	85	256	559	515	1074
Neg	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		
	Abnormal	NILM	Total	Abnormal	NILM	Total
Pos	61	20	81	669	580	1249
Neg	2	11	13	320	1243	1563
Total	63	31	94	989	1823	2812

Table 27. 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 28. 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
Pos	140	36	176	126	98	224
Neg	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
Pos	90	20	110	176	114	290
Neg	2	5	7	38	149	187
Total	92	25	117	214	263	477

Table 29. 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 30. 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
Pos	21	7	28	51	38	89
Neg	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
Pos	11	2	13	61	43	104
Neg	1	1	2	22	82	104
Total	12	3	15	83	125	208

Table 31. 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.

In women with 4800 12 Other HR HPV+ results, the use of the CINtec *PLUS* Cytology test resulted in an overall better risk stratification compared to the Pap cytology test; CINtec *PLUS* positive women showed a higher risk for the presence of ≥CIN2 (PPV) than women with abnormal Pap cytology and CINtec *PLUS* negative women showed a lower risk for the presence of ≥CIN2 (1-NPV) than women with NILM Pap cytology results (Table 32).

Women with HPV16+ and HPV18+ and CINtec *PLUS* Cytology negative results showed a lower risk for the presence of ≥CIN2 than HPV16+ and HPV18+ women with NILM Pap cytology results (Table 32).

Table 32. Summary of CINtec *PLUS* Cytology Test Performance for Women 25-65 Years Old with Different HPV Genotypes vs Pap Cytology Test

HPV Genotyping	Pre-test Risk of ≥CIN2 (Prevalence)	Post-test Risk of ≥CIN2 (PPV)		Post-test Risk of ≥CIN2 (1-NPV)	
		CINtec *PLUS* Cytology Positive % (95% CI)	Pap Cytology Abnormal % (95% CI)	CINtec *PLUS* Cytology Negative % (95% CI)	NILM Pap Cytology % (95% CI)
HPV16+	31.6%	44.0 (41.7, 46.4)	47.1 (43.5, 50.6)	6.2 (3.6, 10.2)	15.3 (12.0, 19.0)
HPV18+	14.3%	23.9 (19.9, 27.6)	24.2 (18.8, 29.3)	3.8 (1.5, 8.2)	7.0 (4.0, 11.0)
12 Other HR HPV+	10.7%	19.2 (18.1, 20.3)	17.5 (15.9, 19.0)	3.6 (2.8, 4.4)	6.9 (6.0, 7.8)
HPV Genotyping	Pre-test risk of ≥CIN3 (Prevalence)	Post-test risk of ≥CIN3 (PPV)		Post-test risk of ≥CIN3 (1-NPV)	
		CINtec *PLUS* Cytology Positive % (95% CI)	Pap Cytology Abnormal % (95% CI)	CINtec *PLUS* Cytology Negative % (95% CI)	NILM Pap Cytology % (95% CI)
HPV16+	19.7%	27.5 (25.7, 29.2)	30.1 (27.1, 32.9)	3.6 (1.8, 7.0)	8.7 (6.2, 11.8)
HPV18+	6.7%	11.1 (8.1, 13.2)	12.6 (8.8, 15.6)	1.9 (0.5, 5.2)	2.3 (0.8, 5.2)
12 Other HR HPV+	3.2%	6.1 (5.5, 6.5)	6.0 (5.1, 6.8)	0.8 (0.5, 1.3)	1.7 (1.2, 2.2)

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 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 33.

Table 33. 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
Pos	324	29	38	8	2	401
Neg	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 34. 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 34. Performance of CINtec *PLUS* Cytology in cobas 4800 12 Other HR HPV+ Women 30-65 Years Old with NILM 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.

Table 35. 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 36. 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)
	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.			

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 positive 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 37.

Table 37. 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
Pos	68	7	11	17	1	104
Neg	115	6	3	4	0	128
Total	183	13	14	21	1	232
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 38. 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 38. Performance of CINtec *PLUS* Cytology in cobas 4800 HPV16+ Women 30-65 Years Old with NILM 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.		

Table 39. 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 40. 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.			

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 41.

Table 41. CINtec *PLUS* Cytology Results and CPR Results in cobas 4800 HPV18+ Women 30-65 Years Old with NILM Cytology

CINtec *PLUS* Cytology Result	CPR Result					
	Normal	CIN1	CIN2	CIN3	Cancer	Total
Pos	30	2	4	1	1	38
Neg	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 42. 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 42. 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 43. 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)
	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.			

Table 44. 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.			

Table 45. 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 (22.8, 32.7)	5.5 (2.8, 9.5)	9.5%	17.3 (13.2, 20.8)	3.1 (1.3, 6.5)
HPV18+	6.5%	15.8 (9.2, 21.1)	1.4 (0.3, 5.0)	2.8%	5.3 (1.7, 8.3)	1.4 (0.3, 3.4)
12 Other HR HPV+	5.7%	12.0 (9.9, 13.9)	2.9 (2.1, 3.8)	1.2%	2.5 (1.6, 3.2)	0.6 (0.3, 1.0)

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CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)
www.ventana.com