VENTANA®

Roche

CINtec® *PLUS* Cytology Kit

REF 605-100

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IVD Σ 100

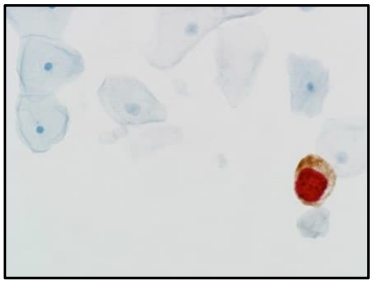

Figure 1. Cervical epithelial cell positive for p16^{INK4a} (brown cytoplasmic stain) and for Ki-67 (red nuclear stain).

INTENDED USE

The CINtec *PLUS* Cytology Kit is an immunocytochemistry assay intended for laboratory use for simultaneous qualitative detection of the p16^{INK4a} and Ki-67 proteins in cervical cytology preparations stained on a BenchMark IHC/ISH instrument. It is indicated to be used as an aid in the identification of women with high-grade cervical intraepithelial lesions in a screening population, and in the sub-groups of patients with a Pap cytology result of ASC-US (atypical squamous cells of undetermined significance) or LSIL (low-grade squamous intraepithelial lesion), or in patients with positive high-risk HPV test results.

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

The product is intended for in vitro diagnostic (IVD) use.

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.[2,3,4,5] 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.[3,4,5] 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.[2,3,4]

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. This co-expression of p16 and Ki-67 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 utility 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. These results include 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 Kit 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 recombinant 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:

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

The chromogenic reactions are based on the HRP-mediated conversion of 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 inspection.

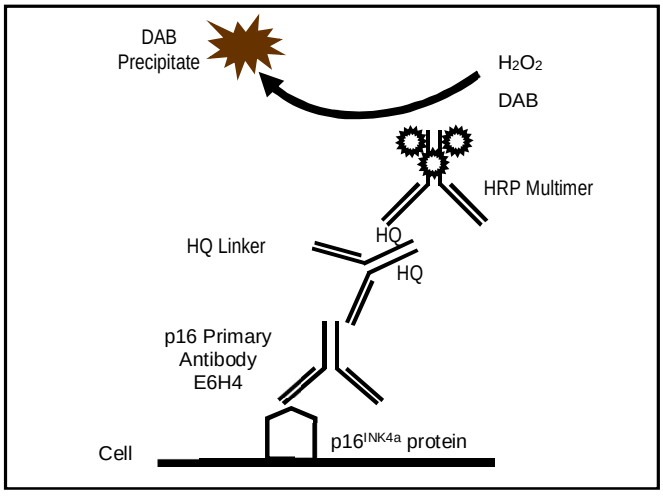

Figure 2. Detection of human p16^{INK4a} protein.

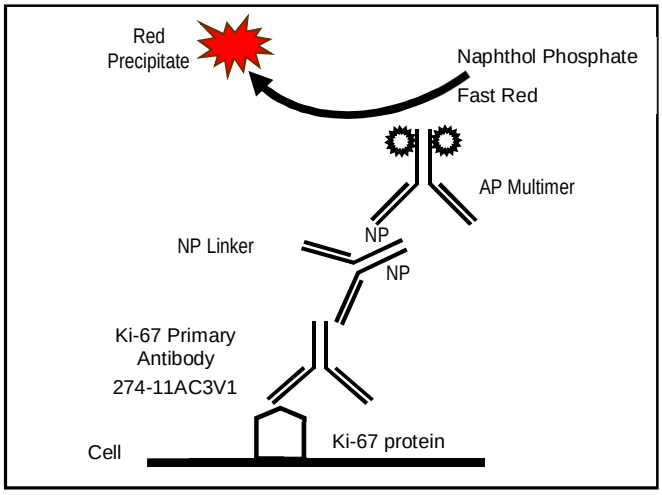

Figure 3. Detection of human Ki-67 protein.

MATERIAL PROVIDED

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

One 10 mL dispenser	CINtec *PLUS* Cytology Primary Antibody Cocktail (p16/Ki-67) contains a cocktail of recombinant 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) in a buffer containing protein with ProClin 300 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 preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Red AP Multimer contains a mouse monoclonal AP-labeled anti-NP tertiary antibody (<20 µg/mL) in a buffer containing protein with ProClin 300 preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Red Naphthol Phosphate contains Naphthol Phosphate (<1%) with ProClin 300 preservative.
One 10 mL dispenser	CINtec *PLUS* Cytology Fast Red contains Fast Red (<1%) in acetate buffer with ProClin 300 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 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 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 H_2O_2 contains hydrogen peroxide (<1%) in a phosphate buffer solution.

RECONSTITUTION, MIXING, DILUTION, TITRATION

The CINtec *PLUS* Cytology Kit is optimized for use on BenchMark IHC/ISH instruments. 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, necessitating regular performance of in-house controls.

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

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

The following reagents and materials are required for staining but not provided with the detection kit:

1. Appropriate controls (optional, please refer to Quality Control section)
2. Hematoxylin Counterstain (Cat. No. 760-2021 / 05266726001)
3. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
4. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
5. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
6. Cell Conditioning Solution (CC2) (Cat. No. 950-123 / 05279798001)
7. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
8. ULTRA Cell Conditioning Solution (ULTRA CC2) (Cat. No. 950-223 / 05424542001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Reagent Grade Ethanol denatured (purity ≥ 95%)
12. BenchMark IHC/ISH instrument
13. Superfrost Plus microscope slides (Thermo Fisher Scientific) for conventional smears are recommended
14. ThinPrep Arcless microscope slides (Hologic REF 70126-002) or Superfrost Plus microscope slides (VWR REF 48311-703)
15. Roche Cell Collection Medium (REF 07994753190)
16. PreservCyt® Solution (Hologic REF 234004)
17. BD SurePath PreCoat slides (included in the SurePath GYN kit)
18. SurePath™ Preservative Fluid (BD REF 490522)
19. CC/Mount aqueous mounting medium (Roche REF 7342098001; Diagnostic BioSystems P/N: K 002; Sigma-Aldrich P/N: C9368)
20. Optional: Drying oven capable of maintaining a temperature of 60°C ± 5°
21. General purpose laboratory equipment

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze. The user must validate any storage conditions other than those specified in the method sheet. This detection 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.

Every detection kit is expiration dated. When properly stored, the product is stable to 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 unknown 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. Do not use beyond the specified number of tests.
4. 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.
5. 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.
6. Materials of human or animal origin should be handled as potentially biohazardous and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[26,27]
7. 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.

8. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
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. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This detection kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
DANGER 	H317	May cause an allergic skin reaction.
	H350	May cause cancer.
	H412	Harmful to aquatic life with long lasting effects.
	P201	Obtain special instructions before use.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P308 + P313	IF exposed or concerned: Get medical advice/ attention.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

This product contains CAS #'s:

- 868272-85-9: 3,3'-Diaminobenzidine tetrahydrochloride hydrate
- 2682-20-4: 2-methyl-2H-isothiazol-3-one
- 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

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[28], clinicians should follow the recommended sampling techniques.

The following slide preparation methods are suitable for use with the CINtec PLUS Cytology Kit:

- ThinPrep (Hologic Inc.) slides prepared on a ThinPrep 2000 or 5000 Processor (Hologic Inc.) using ThinPrep Arcless Microscope slides or Superfrost Plus slides according to the manufacturer's recommendation;
- BD SurePath (BD Diagnostics) slides prepared according to the manufacturer´s recommendation.
- Manually prepared slides (conventional smear slides)

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

Roche Cell Collection Medium Sample Preparation

Cytologic samples collected by a health care professional and resuspended in Roche Cell Collection Medium (RCCM) intended for immunocytochemistry staining using the CINtec *PLUS* Cytology Kit can be stored at 15-30°C for 6 weeks followed by 12 additional week refrigerated at 2-8°C.

ThinPrep Arcless Microscope slides or Superfrost Plus slides are required for immunocytochemical staining with the CINtec *PLUS* Cytology Kit on BenchMark IHC/ISH instruments.

Slide preparation from samples collected in Roche Cell Collection Medium using the ThinPrep 2000 Processor

Slides are prepared from samples collected in Roche Cell Collection Medium using the ThinPrep 2000 Processor. 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 slide(s) 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 slide(s) from the ethanol solution and dry the slide(s) 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 Kit within 7 days of preparation.

Slide preparation from samples collected in Roche Cell Collection Medium using the ThinPrep 5000 Processor

Slides are prepared from samples collected in Roche Cell Collection Medium using the ThinPrep 5000 Processor. 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 Kit within 7 days of preparation.

Before immunocytochemical staining with the CINtec *PLUS* Cytology Kit remove the original slide label used by the ThinPrep 5000 Processor.

PreservCyt Sample Preparation

Cytologic sample in PreservCyt Solution (PC) intended for immunocytochemistry staining using the CINtec *PLUS* Cytology Kit can be stored at 15-30°C for 6 weeks followed by 12 additional weeks refrigerated at 2-8°C.

ThinPrep Arcless Microscope slides or Superfrost Plus slides are required for immunocytochemical staining with the CINtec *PLUS* Cytology Kit on BenchMark IHC/ISH instruments.

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

Slides are prepared from cytologic samples collected by a health care professional and resuspended in PreservCyt using the ThinPrep 2000 Processor. 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 slide(s) 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 slide(s) from the ethanol solution and dry the slide(s) lying horizontally on a flat surface for at least 60 minutes. Dried slides can be stored at 15-30°C protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 7 days of preparation.

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

Slides are prepared from cytologic samples collected by a health care professional and resuspended in PreservCyt using the ThinPrep 5000 Processor. 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 Kit within 7 days of preparation.

Before immunocytochemical staining with the CINtec *PLUS* Cytology Kit remove the original slide label used by the ThinPrep 5000 Processor.

BD SurePath Sample Preparation

Cytologic samples collected by a health care professional and resuspended in SurePath Preservative Fluid intended for immunocytochemistry staining using the CINtec *PLUS* Cytology Kit can be stored for up to 4 weeks at 15-30°C, or for 6 months in a refrigerator at 2-10°C.

BD SurePath Slide Preparation Immediately Following Processing of a Pap Slide

Once an enriched cell pellet has been created for preparation of a slide for Pap staining, it can be used immediately for preparation of a second slide to be stained with the CINtec *PLUS* Cytology Kit. Follow manufacturer´s recommendation for use of the "Slide Preparation" [option 2] for GYN samples on the PrepStain™ instrument. The resuspension volume must be changed to 0 mL in the "Change Sample/Stain Parameters" menu option.

BD SurePath Slide Preparation from a Preserved Cell Pellet

Enriched cell pellets can be preserved by adding approximately 2 mL of SurePath Preservative Fluid and capping the sample tubes for storage (refer to manufacturer´s instructions for details). From the sample collection date, cell pellets that have been re-suspended in preservative fluid can be stored for up to 4 weeks at 15-30°C, or for 6 months in a refrigerator maintained at 2-10°C. To process a slide for the CINtec *PLUS* Cytology Kit, first bring the sample to room temperature for 60 minutes. Start with the second centrifugation step of the GYN enrichment process and proceed through the remainder of the pre-processing steps as outlined in the manufacturer´s instruction for reprocessing preserved cell pellets. Follow manufacturer´s recommendation for use of the "Slide Preparation" [option 2] for GYN samples on the PrepStain instrument.

For all preparation options listed above, remove the slide rack from the PrepStain instrument once the sample transfer step has been completed. Invert the rack to decant the liquid. Pipette 2 mL of a ≥ 95% reagent grade ethanol solution to each settling chamber and decant immediately. Rinse with 2 mL of a ≥ 95% reagent grade ethanol solution a second time and incubate for 10 minutes. Decant a second time by inverting the rack. Remove the settling chambers from the slides and let the slides dry 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 Kit within 7 days of preparation.

Use of Conventional Smears

Conventional smears should be fixed with cytological spray fixation reagent containing polyethylene glycol (e.g. Safetex Cytology Fixative, Andwin Scientific) immediately after sample collection. Spray-fixed conventional smear slides can be stored at room temperature protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 7 days of preparation.

No further pre-processing is required prior to loading slides on the BenchMark IHC/ISH instrument.

STAINING PROCEDURE

The CINtec *PLUS* Cytology Kit has been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA ancillary reagents and accessories.

The CINtec *PLUS* Cytology Kit has been optimized with the parameters indicated in Tables 2, 3 and 4; 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 instrument User Guide.

Table 2. Recommended staining protocol for prepared ThinPrep, SurePath and conventional smear slides on the BenchMark GX instrument.

Staining Procedure	GX CINtec *PLUS* Cytology		
Selectable Options	Slide Preparation Type		
	RCCM/ThinPrep	SurePath	Conventional
ThinPrep	Selected	Not Selected	Not Selected
SurePath	Not Selected	Selected	Not Selected
Other	Not Selected	Not Selected	Selected
Cell Conditioning Option	Not Selectable	Not Selectable	16 min
Antibody Inc Time	16 min	20 min	16 min
HQ Linker Inc Time	12 min	16 min	12 min
HRP Multimer Inc Time	6 min	8 min	8 min
NP Linker Inc Time	8 min	16 min	8 min
AP Multimer Inc Time	8 min	8 min	8 min
Hematoxylin	8 min	8 min	8 min
Bluing	4 min	4 min	4 min

Table 3. Recommended staining protocol for prepared ThinPrep, SurePath and conventional smear slides on the BenchMark XT instrument.

Staining Procedure	XT CINtec *PLUS* Cytology		
Selectable Options	Slide Preparation Type		
	RCCM/ThinPrep	SurePath	Conventional
ThinPrep	Selected	Not Selected	Not Selected
SurePath	Not Selected	Selected	Not Selected
Other	Not Selected	Not Selected	Selected
Cell Conditioning Option	Not Selectable	Not Selectable	16 min
Antibody Inc Time	16 min	20 min	16 min
HQ Linker Inc Time	12 min	16 min	12 min
HRP Multimer Inc Time	8 min	8 min	8 min
NP Linker Inc Time	8 min	16 min	8 min
AP Multimer Inc Time	8 min	8 min	8 min
Hematoxylin	8 min	8 min	8 min
Bluing	4 min	4 min	4 min

Table 4. Recommended staining protocol for prepared ThinPrep, SurePath and conventional smear slides on the BenchMark ULTRA or BenchMark ULTRA PLUS instruments.

Staining Procedure	U CINtec *PLUS* Cytology		
Selectable Options	Slide Preparation Type		
	RCCM/ThinPrep	SurePath	Conventional
ThinPrep	Selected	Not Selected	Not Selected
SurePath	Not Selected	Selected	Not Selected
Other	Not Selected	Not Selected	Selected
Cell Conditioning Option	Not Selectable	Not Selectable	16 min
Antibody Inc Time	16 min	16 min	16 min
HQ Linker Inc Time	12 min	16 min	12 min
HRP Multimer Inc Time	8 min	8 min	8 min
NP Linker Inc Time	8 min	16 min	8 min
AP Multimer Inc Time	8 min	8 min	8 min
Hematoxylin	8 min	8 min	8 min
Bluing	4 min	4 min	4 min

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 IHC/ISH instrument and gently agitate and rinse slides with tap, deionized or distilled water and mild dishwashing detergent until the LCS is completely removed from the slides.

NOTE: Do not allow the water to run directly on top of 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:

Aqueous mounting:

1. Incubate slides in distilled or deionized water for at least 1 min;
2. Slides not being coverslipped should remain in distilled or deionized water during application of the CC/Mount aqueous mounting media to the other slides;
3. 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);
4. Hold slide at a slight angle and apply 4-6 drops of CC/Mount aqueous mounting medium per ThinPrep or SurePath slide, and 8 drops per conventional smear 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;
5. 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;
6. Clean excess CC/Mount aqueous mounting media from the back and edges of the slide. Use wet paper towel if necessary;
7. For drying, place prepared slides in a horizontal position
   - Incubate prepared ThinPrep or SurePath slides at 37-60°C for 1 hour, or alternatively overnight at room temperature;
   - Incubate conventional smear slides at 37°C for 4 hours, or at 60°C for 1 hour, or alternatively overnight at room temperature

Glass or film coverslipping:

1. 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. Next, 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.

2. 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.

Positive Control

Specimens processed in the same manner as the patient sample(s) should be used as positive controls. Positive controls are indicative of correctly prepared specimens and proper staining techniques. One positive control should be included in each staining run.

Known positive controls should only be utilized for monitoring the correct performance of processed specimens and test reagents rather than as an aid in formulating a specific diagnosis of patient samples. If the positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid.

Negative Control

A variety of different cell types present in representative cervical cytology specimens and that are known to be negative for the expression of the $p16^{INK4a}$ and Ki-67 antigens (such as superficial cells) may serve as an internal negative control to assess background staining.

Assay Verification

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

STAINING INTERPRETATION / EXPECTED RESULTS

The CINtec *PLUS* Cytology Kit 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) 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 Kit stained slides must evaluate controls before interpreting results.

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

For the interpretation of cervical cytology slides stained with the CINtec *PLUS* Cytology Kit, the slides should be evaluated with regard to the presence of cervical epithelial cells showing both cytoplasmic brown and nuclear red staining indicative of simultaneous $p16^{INK4a}$ and Ki-67 expression. In addition, similar to reporting Pap Cytology results, specimens should be assessed for sample adequacy according to Bethesda Guidelines 2015 (or TBS)[28] when reporting CINtec *PLUS* Cytology Kit test result.

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 Kit 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 Kit 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 Kit.

SPECIFIC LIMITATIONS

1. For professional use only. Special training is required for the performance of immunocytochemical procedures.
2. Evaluation of microscope slides stained with the CINtec *PLUS* Cytology Kit should be performed only by a certified professional who has been trained to interpret these test results.
3. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation and cytological criteria.
4. The interpretation of CINtec *PLUS* Cytology Kit staining results depends on the intensity and quality of the hematoxylin counterstaining. Deviation from the recommended reagents and incubation times requires validation by the customer as excessive or incomplete counterstaining may interfere with proper interpretation of results.
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. Conventional smear slides intended to be used for staining with the CINtec *PLUS* Cytology Kit shall be prepared by using Superfrost Plus microscope glass slides and Safetex Cytology Fixative (Andwin Scientific), a cytological spray fixation reagent containing polyethylene glycol. Deviation from this recommendation requires validation by the customer.
7. The use of the ThinPrep 3000 Processor is not recommended for preparation of ThinPrep samples 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 Kit.
8. ThinPrep Arcless Microscope slides or ThinPrep Microscope Slides for special processing or Superfrost Plus microscope slides are required for the preparation of ThinPrep samples for immunocytochemistry staining with the CINtec *PLUS* Cytology Kit on BenchMark IHC/ISH 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 declared expected results; appropriate 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.
10. All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

The performance of the CINtec *PLUS* Cytology Kit was evaluated though analytical reproducibility and other relevant studies.

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 Kit 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 Kit 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 an unrelated Alk-a 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 Kit staining of ThinPrep 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 ASC-US/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools) stained with 3 lots of CINtec *PLUS* Cytology Kit, 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 ASC-US/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools), 3 lots of CINtec *PLUS* Cytology Kit, 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 Kit lot (Lot 1-3) aggregating data from 12 cervical cytology pools (3 HSIL/HPV+, 1 LSIL/HPV+, 1 ASC-US/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 Kit 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 Table 5, Table 6 and Table 7. 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 5. Within-Laboratory Precision Study - Overall Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Kit 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)
ASC-US/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 6. Within-Laboratory Precision Study - Between-Day Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Kit 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%
ASC-US/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 7. Within-Laboratory Precision Study – Between-Lot Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Kit 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%
ASC-US/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%

Reader Precision

To assess reader precision, three reader teams, each comprised of a cytotechnologist and a pathologist, evaluated all slides stained for the precision studies, including 2 replicates from 12 cervical cytology pools (3 HSIL/HPV+, 1 LSIL/HPV+, 1 ASC-US/HPV+, 1 NILM/HPV+, 3 NILM/HPV- LBC pools, and 3 T24 cell line negative pools), 3 CINtec *PLUS* Cytology Kit lots, on 5 non-consecutive days. The percent of results same as majority call were calculated from the aggregated results for between the reader teams and are summarized in Table 8.

Table 8. Within-Laboratory Precision Study - Between-Reader Precision

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Kit 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%
ASC-US/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

Reproducibility studies for the CINtec *PLUS* Cytology Kit were performed to demonstrate:

- Inter-lot reproducibility of the CINtec *PLUS* Cytology Kit (Table 9),
- Intra-run (Table 10) and Inter-run (Table 11) reproducibility on the BenchMark GX, XT and ULTRA instruments
- Intra-platform reproducibility on the BenchMark GX, XT and ULTRA instruments (Table 12),
- Inter-platform reproducibility between the BenchMark GX, XT and ULTRA instruments (Table 13).

Table 9. Inter-lot reproducibility of the CINtec *PLUS* Cytology Kit.

Sample Type	Overall Percent Agreement
ThinPrep	100.0
SurePath	100.0

Table 10. Intra-run reproducibility of the CINtec *PLUS* Cytology Kit.

Sample Type	Overall Percent Agreement		
	GX	XT	ULTRA
ThinPrep	100.0	100.0	100.0
SurePath	93.8	96.3	92.6

Table 11. Inter-run reproducibility of the CINtec *PLUS* Cytology Kit.

Sample Type	Overall Percent Agreement		
	GX	XT	ULTRA
ThinPrep	100.0	96.2	100.0
SurePath	100.0	92.6	100

Table 12. Intra-platform reproducibility of the CINtec *PLUS* Cytology Kit.

Sample Type	Overall Percent Agreement		
	GX	XT	ULTRA
ThinPrep	100.0	100.0	100.0
SurePath	100.0	100.0	96.3

Table 13. Inter-platform reproducibility of the CINtec *PLUS* Cytology Kit.

Sample Type	Overall Percent Agreement
ThinPrep	100.0
SurePath	96.3

These studies were performed on both ThinPrep and SurePath cervical specimens. All studies met their pre-determined acceptance criteria.

Concordance Studies

The performance of the CINtec *PLUS* Cytology Kit for use on BenchMark GX, XT and ULTRA instruments was evaluated by comparison to the CINtec *PLUS* Kit (predicate device, Roche mtm laboratories AG, Mannheim) which has previously demonstrated high sensitivity and specificity for the presence of pre-cancerous and cancerous disease in cervical cytology specimens with respect to the presence of individual cervical epithelial cells showing a positive dual stain result for $p16^{INK4a}$ and Ki-67.[7-15,18,20,23,25-28]

Concordance studies have been performed using patient samples from the intended use population with a Pap cytology result of NILM (negative for intraepithelial lesion or malignancy), ASC-US, LSIL and HSIL for each of the individual cervical cytology preparation methods: ThinPrep, BD SurePath and conventional smear slides. For LBC specimens, two slides were prepared from each patient sample. For conventional smears, two slides were prepared from the same patient at the same visit, splitting the sampled material between two slides ("split sample technique"). One slide was tested with the CINtec *PLUS* Cytology Kit on a BenchMark IHC/ISH instrument and one slide was stained with the predicate device.

Reading algorithm for LBC specimens:

Each slide was read by two qualified readers. If they agreed on the result, it was considered the final result for that slide. If they disagreed on the result, an adjudication read was performed by a third reader, and the majority result became the final result for that slide.

Reading algorithm for conventional smear slides:

Each slide was read by two qualified readers. If both readers scored the slide as positive, the final result for that slide was positive. If they disagreed on the result, the slide was reviewed by three readers as a panel for a consensus review, and the outcome became the final result for that slide. If both readers scored the slide as negative, the slide was read by a third reader to confirm the negative result. If the third reader confirmed the negative result, then the final result for the slide was negative. If the third reader scored the slide as positive, the slide went to a panel review for consensus, which became the final result for that slide.

The agreement of test results is reported as positive percent agreement (PPA) and negative percent agreement (NPA) ± the 95% confidence interval (CI) between the CINtec *PLUS* Cytology Kit for use on a BenchMark IHC/ISH instrument and the predicate device.

Table 14. Agreement of test results between the CINtec *PLUS* Cytology Kit and predicate device on ThinPrep cervical cytology slide preparations.

		Predicate Device	
		+	-
CINtec *PLUS* Cytology Kit	+	140	25
	-	17	147
Total		157	172
PPA (n/N) (95% CI) = 140/157 x 100% = 89.2% (83.3-93.1%)			
NPA (n/N) (95% CI) = 147/172 x 100% = 85.5% (79.4-90.0%)			

Table 15. Agreement of test results between the CINtec *PLUS* Cytology Kit and the predicate device on SurePath cervical cytology slide preparations.

		Predicate Device	
		+	-
CINtec *PLUS* Cytology Kit	+	56	3
	-	9	108
Total		65	111
PPA (n/N) (95% CI) = 56/65 x 100% = 86.2% (75.7-92.5%)			
NPA (n/N) (95% CI) = 108/111 x 100% = 97.3% (92.4-99.1%)			

Table 16. Agreement of test results between the CINtec *PLUS* Cytology Kit and the predicate device on conventional smear cervical cytology slide preparations.

		Predicate Device	
		+	-
CINtec *PLUS* Cytology Kit	+	98	17
	-	20	72
Total		118	89
PPA (n/N) (95% CI) = 98/118 x 100% = 83.1% (75.3-88.8%)			
NPA (n/N) (95% CI) = 72/89 x 100% = 80.9% (71.5-87.7%)			

Roche Cell Collection Medium Study

The performance of Roche Cell Collection Medium compared to PreservCyt solution was assessed by staining of 616 pairs of cervical specimens (cases) using the CINtec *PLUS* Cytology Kit. For each case, one slide each was prepared from the respective PC or RCCM vials. All slides were read by a cytotechnologist/pathologist team, who were blinded to the identity of the slides. The CINtec *PLUS* Cytology Kit test results comparing between media (PC vs. RCCM) are summarized in Table 17. Positivity rates were calculated by dividing the number of positive cases by the total number of adequate cases for each medium. Results show a difference between the two positivity rates of 2.4% (1.5, 6.3).

Table 17. Equivalence for CINtec *PLUS* Cytology Kit Positivity Rates of Roche Cell Collection Medium (RCCM) and PreservCyt (PC).

	CINtec *PLUS* Cytology Kit Result for PC		
CINtec *PLUS* Cytology Kit Result for RCCM	Positive	Negative	Total
Positive	170	48	218
Negative	37	204	241
Total	207	252	459
RCCM positivity rate, n/N (%):	218/459 (47.5%)		
PC positivity rate, n/N (%):	207/459 (45.1%)		
Difference of positivity rates, n/N (%):	11/459 (2.4%; 95% CI: -1.5, 6.3)		

The CINtec *PLUS* Cytology Kit results for cellularity adequacy comparing PreservCyt and Roche Cell Collection Medium are summarized in Table 18. Adequate cellularity rates were calculated by dividing the number of cases with adequate cellularity by the total number of cases for each medium. Results show the difference between the two adequate cellularity rates as 3.2% (-0.0, 6.6).

Table 18. Comparison of CINtec *PLUS* Cytology Kit Cellularity Adequacy for Roche Cell Collection Medium (RCCM) and PreservCyt (PC).

	CINtec *PLUS* Cytology Kit Cellularity Adequacy for PC		
CINtec *PLUS* Cytology Kit Cellularity Adequacy for RCCM	Yes	No	Total
Yes	468	63	531
No	43	42	85
Total	511	105	616
RCCM adequacy rate, n/N (%):	531/616 (86.2%)		
PC adequacy rate, n/N (%):	511/616 (83.0%)		
Difference of adequacy rates, n/N (%):	20/616 (3.2%; 95% CI: -0.0, 6.6)		

Interlaboratory Reproducibility Study on BenchMark ULTRA PLUS Staining Platform

A reproducibility study was conducted to evaluate the CINtec *PLUS* Cytology test on the BenchMark ULTRA PLUS instrument for dual immunocytochemical detection of $p16^{INK4a}$ and Ki-67 in cytology specimens. The study included 2 distinct cultures of T24 cells, and 10 patient-derived specimen pools (2 HSIL/HPV+, 2 LSIL/HPV+, 2 ASC-US/HPV+, 2 NILM/HPV+, and 2 NILM/HPV- LBC pools). The T24 cell cultures were prepared from a single working cell bank. Each of 3 study sites were provided with aliquots of each specimen pool, T24 cell culture, and a control pool containing sufficient volume to support the testing planned at each site. On each of the five staining days, the sites prepared a test slide from each of the aliquots provided by RTD using a ThinPrep 2000 or ThinPrep 5000 slide processor. After slide preparation, each site stained one set of 13 slides on a BenchMark ULTRA PLUS 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. Background and cellularity acceptability rates for all cases were 100% for all instruments. Results are summarized in Table 19 and Table 20.

Table 19. BenchMark ULTRA PLUS Instrument Inter-laboratory Reproducibility Study - Between-site Reproducibility

Pool Category	Number of Evaluations	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Same Results as Majority Call		
			Site A	Site B	Site C
T24 Cell Line	60	Negative	95.0%	100.0%	100.0%
NILM/HPV-	60	Negative	100.0%	95.0%	100.0%
NILM/HPV+	60	Positive	90.0%	80.0%	95.0%
ASC-US/HPV+	60	Positive	100.0%	95.0%	100.0%
LSIL/HPV+	60	Positive	100.0%	95.0%	100.0%
HSIL/HPV+	60	Positive	100.0%	100.0%	100.0%

Table 20. BenchMark ULTRA PLUS Instrument Inter-laboratory Reproducibility Study - Overall Precision

Pool Category	Mode of CINtec *PLUS* Cytology Results (Majority Call)	Percent of Results Same as Majority Call	n/N	95% Confidence Intervals
T24 Cell Line	Negative	98.3%	59/60	(96.7, 100.0)
NILM/HPV-	Negative	98.3%	59/60	(96.7, 100.0)
NILM/HPV+	Positive	88.3%	53/60	(86.7, 90.0)
ASC-US/HPV+	Positive	98.3%	59/60	(96.7, 100.0)
LSIL/HPV+	Positive	98.3%	59/60	(96.7, 100.0)
HSIL/HPV+	Positive	100.0%	60/60	(94.0, 100.0)

Concordance between BenchMark ULTRA PLUS and BenchMark ULTRA Instruments (ThinPrep Samples)

Three laboratories participated in a concordance study between the BenchMark ULTRA PLUS instrument and the BenchMark ULTRA instrument. This study utilized 220 pools from de-identified ThinPrep LBC cervical specimens prepared at Roche Tissue Diagnostics (RTD) in the following categories: 88 positive pools, 22 borderline positive pools, 22 negative intermediate pools, and 88 negative T24 cell culture pools. A set of 4 slides from each specimen were prepared at RTD using the ThinPrep 2000 or ThinPrep 5000 slide processor. The first prepared slide from each set was stained on a BenchMark ULTRA instrument at RTD. One of the 3 remaining slides were provided to each study site for staining with the CINtec *PLUS* Cytology test on the BenchMark ULTRA PLUS instrument. The BenchMark ULTRA slide stained at RTD was evaluated for the presence or absence of dual-staining and assigned result of positive, negative, or unsatisfactory by a RTD reader team, consisting of a cytotechnologist and a pathologist, to establish a reference score. At each site, a study reader-team independently evaluated the slides stained on the BenchMark ULTRA instrument for a CINtec *PLUS* Cytology test result. The slides stained on the BenchMark ULTRA PLUS instrument at each site were evaluated by that site's reader team for a CINtec *PLUS* Cytology test result. Therefore, each site reader-team evaluated one BenchMark ULTRA and one BenchMark ULTRA PLUS stained slide for each specimen. The reads of the BenchMark ULTRA and BenchMark ULTRA PLUS stained slides were separated by a two week wash-out period. All reader-teams were blinded to any prior determination of HPV status, Pap cytology status, CINtec *PLUS* Cytology test results, or other clinical information.

The performance equivalence of the CINtec *PLUS* Cytology assay was considered acceptable on the BenchMark ULTRA PLUS instrument if the PPA and NPA rates had a 2-sided 95% confidence interval lower bound of at least 85%. The acceptability rate for background and cellularity of test slides stained with CINtec *PLUS* Cytology on BenchMark ULTRA PLUS instrument was required to be acceptable on ≥90% of all test slides in order for the study to pass. The acceptability rates for background and cellularity on all test slides were 99.7% and 99.4%, respectively. Table 21 below summarizes the agreement rate of CINtec *PLUS* Cytology status between BenchMark ULTRA and BenchMark ULTRA PLUS instruments.

Table 21. BenchMark ULTRA to BenchMark ULTRA PLUS Instrument Concordance Study - Pooled Agreement of CINtec *PLUS* Cytology Status Between Platforms (ThinPrep)

CINtec *PLUS* Cytology Status						
BenchMark ULTRA PLUS	BenchMark ULTRA			Agreement		
	Positive	Negative	Total	Rate	% (n/N)	95% CI
Positive	314	13	327	PPA	95.7 (314/328)	(92.8, 98.1)
Negative	14	317	331	NPA	96.1 (317/330)	(93.5, 98.1)

CINtec *PLUS* Cytology Status						
BenchMark ULTRA PLUS	BenchMark ULTRA			Agreement		
	Positive	Negative	Total	Rate	% (n/N)	95% CI
Total	328	330	658	OPA	95.9 (631/658)	(94.1, 97.4)

Note: The pooled agreement rates pool all specimens and reader teams for ULTRA PLUS.

Note: PPA = Positive Percent Agreement; NPA = Negative Percent Agreement; OPA = Overall Percent Agreement

Within Laboratory Reproducibility Study on BenchMark ULTRA PLUS Staining Platform

A within laboratory reproducibility study was conducted to evaluate the Within-Run Repeatability, Between-Day Intermediate Precision, and Between-Instrument Intermediate Precision of the CINtec *PLUS* Cytology test in SurePath specimens. The study included 2 distinct cultures of T24 cells, and 9 patient-derived SurePath specimen pools (3 CINtec Positive/HPV+, 3 Borderline, and 3 CINtec negative/HPV-). Test slides were prepared on the BD Totalys slide preparation instrument on each of the five staining days, which were non-consecutive and spanned at least 20 days. Three BenchMark ULTRA PLUS were utilized in order to evaluate Between-Instrument Reproducibility. The test slides were evaluated by a reader team consisting of a cytotechnologist and pathologist trained to evaluate CINtec *PLUS* Cytology. Test slide concordance was determined by comparing the CINtec *PLUS* Cytology status of the test slides to the Pool-Level Mode (PLM), which is defined as the most frequent status result among all evaluations of a pool aggregating data from all instruments, all days, and all replicates for that pool. The background and cellularity acceptability rates for all test slides were 99.4% and 99.7%, respectively. Results are summarized in Table 22, Table 23, Table 24, and Table 25 below.

Table 22. Within Laboratory Precision Study - Overall Precision of True Positive and True Negative SurePath Samples

	Pool-level Modal Status			Agreement		
CINtec *PLUS* Status	Positive	Negative	Total	Measure	% (n/N)	95% CI
Positive	89	0	89	PPA	100.0 (89/89)	(95.9, 100.0)
Negative	0	90	90	NPA	100.0 (90/90)	(95.9, 100.0)
Total	89	90	179	OPA	100.0 (179/179)	(97.9, 100.0)

Table 23. Within Laboratory Precision Study - Between-Day Intermediate Precision

Day	Same Result With PLM, n (%)	Different Result From PLM, n (%)	Total, n (%)
Day 1	36 (100.0)	0 (0.0)	36 (100.0)
Day 2	35 (100.0)	0 (0.0)	35 (100.0)
Day 3	36 (100.0)	0 (0.0)	36 (100.0)
Day 4	36 (100.0)	0 (0.0)	36 (100.0)
Day 5	36 (100.0)	0 (0.0)	36 (100.0)

Table 24. Within Laboratory Precision Study - Within-Run Repeatability

Replicate	Same Result With PLM, n (%)	Different Result From PLM, n (%)	Total, n (%)
Replicate 1	90 (100.0)	0 (0.0)	90 (100.0)
Replicate 2	89 (100.0)	0 (0.0)	89 (100.0)

Table 25. Within Laboratory Precision Study - Between-Instrument Intermediate Precision

Instrument	Same Result With PLM, n (%)	Different Result From PLM, n (%)	Total, n (%)
Instrument 1	60 (100.0)	0 (0.0)	60 (100.0)
Instrument 2	60 (100.0)	0 (0.0)	60 (100.0)
Instrument 3	59 (100.0)	0 (0.0)	59 (100.0)

Concordance between BenchMark ULTRA PLUS and BenchMark ULTRA Staining Platforms (SurePath Samples)

An internal concordance study was conducted in order to verify equivalent staining performance of the CINtec *PLUS* Cytology kit between the BenchMark ULTRA and ULTRA PLUS staining platforms on SurePath samples. The study included 88 True Positive, 26 Borderline Positive, 26 Negative Intermediate, and 88 True Negative T24 cell line cultures. Two test slides were prepared from each sample on the BD Totalys slide preparation instrument, which were then stained with CINtec *PLUS* Cytology on either the BenchMark ULTRA or BenchMark ULTRA PLUS. The test slides were evaluated by a reader team consisting of a cytotechnologist and pathologist trained to evaluate CINtec *PLUS* Cytology. The performance equivalence of the CINtec *PLUS* Cytology assay was considered acceptable on the BenchMark ULTRA PLUS instrument if the PPA and NPA rates had a 2-sided 95% confidence interval lower bound of at least 85%. The acceptability rate for background and cellularity of test slides stained with CINtec *PLUS* Cytology on BenchMark ULTRA PLUS instrument was required to be acceptable on ≥90% of all test slides in order for the study to pass. The background and cellularity acceptability rates for all test slides were 99.6% and 99.1%, respectively. Results are summarized in Table 26 below.

Table 26. BenchMark ULTRA to BenchMark ULTRA PLUS Instrument Concordance Study (SurePath)

	ULTRA Status			Agreement		
ULTRA *PLUS* Status	Positive	Negative	Total	Measure	% (n/N)	95% CI
Positive	94	2	96	PPA	92.2 (94/102)	(85.3, 96.0)
Negative	8	119	127	NPA	98.3 (119/121)	(94.2, 99.5)
Total	102	121	223	OPA	95.5 (213/223)	(91.9, 97.5)

CLINICAL PERFORMANCE

A prospective, multi-center trial (IMPACT, IMproved Primary screening And Colposcopy Triage) was designed to evaluate the clinical performance of CINtec *PLUS* Cytology Kit on the BenchMark ULTRA instrument, using clinically collected cervical samples collected in PreservCyt® solution, for identification of high-grade cervical disease. The study was designed to evaluate the performance of the CINtec *PLUS* Cytology Kit as a reflex test in women ≥ 25 years who were HPV-positive by the cobas® 4800 HPV Test or the cobas® 6800/8800 HPV Test. Data from this study support the use of CINtec *PLUS* Cytology Kit as an aid in the identification of women with high-grade cervical intraepithelial lesions in patients with positive HR-HPV test results.

Performance Characteristics of CINtec *PLUS* Cytology Kit vs Pap Cytology for Women 25-65 Years Old with cobas® 6800/8800 HPV Test or cobas® 4800 HPV Test, Positive Results

The comparative performance of CINtec *PLUS* Cytology Kit vs Pap cytology in cobas® 6800/8800 HPV Test and cobas® 4800 HPV Test positive women is presented in the following tables for the 12 Other HR HPV+ population (Table 27), HPV16+ population (Table 28), and HPV18+ population (Table 29). 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 Kit vs Pap cytology (Table 27, Table 28, and Table 29).

Both among cobas® 6800/8800 HPV Test and cobas® 4800 HPV Test positive women, the maximum increase in sensitivity (difference = 23.1% and 24.2%, respectively) and the minimum decrease in specificity (difference = 7.9% and 8.7%, respectively) with respect to Pap cytology was observed in the 12 Other HR HPV+ populations (Table 27).

In all cases, the use of CINtec *PLUS* Cytology Kit resulted in a substantial reduction of the risk of disease for negative CINtec *PLUS* Cytology Kit results versus NILM Pap cytology. Among cobas® 6800/8800 HPV Test, positive women, 1-NPV was 3.7%, 6.1%, and 1.2% lower for ≥ CIN2 and 0.9%, 3.3%, and 0.3% lower for ≥ CIN3 in the 12 Other HR HPV+, HPV16+, and HPV18+ populations, respectively. Among cobas® 4800 HPV Test, positive women, 1-NPV was 3.4%, 9.1%, and 3.3% lower for ≥ CIN2, and 0.8%, 5.1%, and 0.5% lower for ≥ CIN3 in the 12 Other HR HPV+, HPV16+, and HPV18+ populations, respectively.

Table 27. Performance of CINtec *PLUS* Cytology Kit vs Pap Cytology in 12 other HR HPV+ Women 25-65 years old.

cobas® 6800/8800 system, 12 Other HR HPV+			
Performance Measure	CPR Diagnosis of ≥ CIN2		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	83.0 (254/306) (78.4, 86.8)	58.8 (180/306) (53.2, 64.2)	24.2 (18.3, 29.9)
Specificity (%)	56.8 (1373/2416) (54.8, 58.8)	65.5 (1583/2416) (63.6, 67.4)	-8.7 (-10.9, -6.4)
PPV (%)	19.6 (254/1297) (18.5, 20.6)	17.8 (180/1013) (16.2, 19.3)	1.8 (0.0, 3.3)
1-NPV (%)	3.6 (52/1425) (2.9, 4.6)	7.4 (126/1709) (6.5, 8.3)	-3.7 (-4.5, -2.4)
Prevalence (%)	11.2 (306/2722)		
Performance Measure	CPR Diagnosis of ≥ CIN3		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	86.0 (80/93) (77.5, 91.6)	66.7 (62/93) (56.6, 75.4)	19.4 (10.0, 28.7)
Specificity (%)	53.7 (1412/2629) (51.8, 55.6)	63.8 (1678/2629) (62.0, 65.6)	-10.1 (-12.3, -8.0)
PPV (%)	6.2 (80/1297) (5.6, 6.6)	6.1 (62/1013) (5.2, 6.9)	0.0 (-0.8, 0.8)
1-NPV (%)	0.9 (13/1425) (0.5, 1.5)	1.8 (31/1709) (1.3, 2.4)	-0.9 (-1.4, -0.3)
Prevalence (%)	3.4 (93/2722)		
cobas® 4800 system, 12 Other HR HPV+			
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: CPR = Central Pathology Review; PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals.			

Table 28. Performance of CINtec *PLUS* Cytology Kit vs Pap Cytology in HPV16+ Women 25-65 years old.

cobas® 6800/8800 system, HPV16+			
Performance Measure	CPR Diagnosis of ≥ CIN2		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	92.6 (176/190) (88.0, 95.6)	75.8 (144/190) (69.2, 81.3)	16.8 (10.7, 23.2)
Specificity (%)	57.2 (349/610) (53.3, 61.1)	68.5 (418/610) (64.7, 72.1)	-11.3 (-15.4, -7.2)
PPV (%)	40.3 (176/437) (37.9, 42.7)	42.9 (144/336) (39.4, 46.3)	-2.6 (-5.7, 0.8)
1-NPV (%)	3.9 (14/363) (2.3, 6.2)	9.9 (46/464) (7.8, 12.3)	-6.1 (-7.1, -2.6)
Prevalence (%)	23.8 (190/800)		
Performance Measure	CPR Diagnosis of ≥ CIN3		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	92.5 (111/120) (86.4, 96.0)	77.5 (93/120) (69.2, 84.1)	15.0 (7.7, 22.8)
Specificity (%)	52.1 (354/680) (48.3, 55.8)	64.3 (437/680) (60.6, 67.8)	-12.2 (-16.0, -8.3)
PPV (%)	25.4 (111/437) (23.6, 27.2)	27.7 (93/336) (24.8, 30.5)	-2.3 (-4.7, 0.3)
1-NPV (%)	2.5 (9/363) (1.3, 4.4)	5.8 (27/464) (4.2, 7.8)	-3.3 (-4.7, -1.1)
Prevalence (%)	15.0 (120/800)		
cobas® 4800 system, HPV16+			
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: CPR = Central Pathology Review; PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals.			

Table 29. Performance of CINtec *PLUS* Cytology Kit vs Pap Cytology in HPV18+ Women 25-65 years old.

cobas® 6800/8800 system, HPV18+			
Performance Measure	CPR Diagnosis of ≥ CIN2		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	83.8 (31/37) (68.9, 92.3)	73.0 (27/37) (57.0, 84.6)	10.8 (-6.7, 27.8)
Specificity (%)	62.7 (205/327) (57.3, 67.8)	73.1 (239/327) (68.0, 77.6)	-10.4 (-16.1, -4.6)
PPV (%)	20.3 (31/153) (16.8, 23.4)	23.5 (27/115) (18.6, 28.2)	-3.2 (-8.2, 1.8)
1-NPV (%)	2.8 (6/211) (1.4, 5.4)	4.0 (10/249) (2.3, 6.3)	-1.2 (-3.6, 1.3)
Prevalence (%)	10.2 (37/364)		
Performance Measure	CPR Diagnosis of ≥ CIN3		
	CINtec *PLUS* Cytology	Pap Cytology	Difference
Sensitivity (%)	87.5 (14/16) (64.0, 96.5)	81.3 (13/16) (57.0, 93.4)	6.3 (-19.6, 31.6)
Specificity (%)	60.1 (209/348) (54.8, 65.1)	70.7 (246/348) (65.7, 75.2)	-10.6 (-16.1, -5.0)
PPV (%)	9.2 (14/153) (6.7, 10.8)	11.3 (13/115) (8.0, 13.9)	-2.2 (-5.2, 0.8)
1-NPV (%)	0.9 (2/211) (0.3, 2.7)	1.2 (3/249) (0.4, 2.7)	-0.3 (-1.7, 1.1)
Prevalence (%)	4.4 (16/364)		
cobas® 4800 system, HPV18+			
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: CPR = Central Pathology Review; PPV = Positive Predictive Value; NPV = Negative Predictive Value; numbers in parentheses are (n/N) and 2-sided 95% confidence intervals.			

Performance Characteristics in the Population of cobas® 6800/8800 HPV Test or cobas® 4800 HPV Test, Positive Results, Women 30-65 Years Old with NILM Pap Cytology Results

Performance of CINtec *PLUS* Cytology Kit for detecting ≥ CIN2 and ≥ CIN3 in 12 Other HR HPV+ women with NILM Pap cytology is presented in Table 30.

In the population of cobas® 6800/8800 system, 12 Other HR HPV+ women with NILM Pap cytology, sensitivity and specificity for the detection of ≥ CIN2 were 66.7% and 69.7%, respectively (62.5% and 67.8%, respectively, for ≥ CIN3). The PPVs in this group were 13.0% for ≥ CIN2 and 2.6% for ≥ CIN3, whereas the disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 3.1% for ≥ CIN2 and 0.8% for ≥ CIN3.

In the population of cobas® 4800 system, 12 Other HR HPV+ women with NILM Pap cytology, 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 disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 2.9% for ≥ CIN2 and 0.6% for ≥ CIN3.

Table 30. Performance of CINtec *PLUS* Cytology Kit in 12 other HR HPV+ Women 30-65 years old with NILM Cytology.

	cobas® 6800/8800 system, 12 Other HR HPV+/NILM		cobas® 4800 system, 12 Other HR HPV+/NILM	
Performance Measure	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3
Sensitivity (%)	66.7 (50/75) (55.4, 76.3)	62.5 (10/16) (38.6, 81.5)	64.9 (48/74) (53.5, 74.8)	66.7 (10/15) (41.7, 84.8)

Performance Measure	cobas® 6800/8800 system, 12 Other HR HPV+/NILM		cobas® 4800 system, 12 Other HR HPV+/NILM	
	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3
Specificity (%)	69.7 (772/1108) (66.9, 72.3)	67.8 (791/1167) (65.0, 70.4)	71.0 (864/1217) (68.4, 73.5)	69.4 (885/1276) (66.8, 71.8)
Prevalence (%)	6.3 (75/1183)	1.4 (16/1183)	5.7 (74/1291)	1.2 (15/1291)
PPV (%)	13.0 (50/386) (10.8, 14.9)	2.6 (10/386) (1.6, 3.4)	12.0 (48/401) (9.9, 13.9)	2.5 (10/401) (1.6, 3.2)
NPV (%)	96.9 (772/797) (95.8, 97.8)	99.2 (791/797) (98.8, 99.6)	97.1 (864/890) (96.2, 97.9)	99.4 (885/890) (99.0, 99.7)
1-NPV (%)	3.1 (25/797) (2.2, 4.2)	0.8 (6/797) (0.4, 1.2)	2.9 (26/890) (2.1, 3.8)	0.6 (5/890) (0.3, 1.0)
PLR	2.20 (1.79, 2.59)	1.94 (1.19, 2.58)	2.24 (1.81, 2.65)	2.18 (1.35, 2.82)
NLR	0.48 (0.34, 0.64)	0.55 (0.27, 0.91)	0.49 (0.35, 0.66)	0.48 (0.22, 0.84)
Positivity Rate (%)	32.6 (386/1183) (30.0, 35.3)		31.1 (401/1291) (28.6, 33.5)	
Note: CPR = Central Pathology Review; 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 CINtec *PLUS* Cytology Kit for detecting ≥ CIN2 and ≥ CIN3 in HPV16+ women with NILM Pap cytology is presented in Table 31.

In the population of cobas® 6800/8800 system, HPV16+ women with NILM Pap cytology, sensitivity and specificity were 76.3% and 72.5%, respectively, for ≥ CIN2 and 75.0% and 70.5%, respectively, for ≥ CIN3. PPVs in this group were 23.8% for ≥ CIN2 and 14.8% for ≥ CIN3, whereas disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 3.5% for ≥ CIN2 and 2.4% for ≥ CIN3.

In the population of cobas® 4800 system, HPV16+ women with NILM Pap cytology, sensitivity and specificity were 80.6% and 61.7%, respectively, for ≥ CIN2 and 81.8% and 59.0%, respectively, for ≥ CIN3. PPVs in this group were 27.9% for ≥ CIN2 and 17.3% for ≥CIN3, whereas disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 5.5%, for ≥ CIN2, and 3.1% for ≥ CIN3.

Table 31. Performance of CINtec *PLUS* Cytology Kit in HPV16+ Women 30-65 years old with NILM Cytology.

Performance Measure	cobas® 6800/8800 system, HPV16+/NILM		cobas® 4800 system, HPV16+/NILM	
	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3
Sensitivity (%)	76.3 (29/38) (60.8, 87.0)	75.0 (18/24) (55.1, 88.0)	80.6 (29/36) (65.0, 90.2)	81.8 (18/22) (61.5, 92.7)
Specificity (%)	72.5 (245/338) (67.5, 77.0)	70.5 (248/352) (65.5, 75.0)	61.7 (121/196) (54.8, 68.3)	59.0 (124/210) (52.3, 65.5)
Prevalence (%)	10.1 (38/376)	6.4 (24/376)	15.5 (36/232)	9.5 (22/232)
PPV (%)	23.8 (29/122) (19.1, 28.2)	14.8 (18/122) (11.0, 18.1)	27.9 (29/104) (22.8, 32.7)	17.3 (18/104) (13.2, 20.8)
NPV (%)	96.5 (245/254) (94.2, 98.0)	97.6 (248/254) (95.8, 98.9)	94.5 (121/128) (90.5, 97.2)	96.9 (124/128) (93.5, 98.7)
1-NPV (%)	3.5 (9/254) (2.0, 5.8)	2.4 (6/254) (1.1, 4.2)	5.5 (7/128) (2.8, 9.5)	3.1 (4/128) (1.3, 6.5)
PLR	2.77 (2.10, 3.49)	2.54 (1.81, 3.24)	2.11 (1.61, 2.64)	2.00 (1.45, 2.50)
NLR	0.33 (0.18, 0.54)	0.35 (0.17, 0.64)	0.31 (0.16, 0.57)	0.31 (0.12, 0.66)
Positivity Rate (%)	32.4 (122/376) (28.0, 36.9)		44.8 (104/232) (38.7, 50.9)	
Note: CPR = Central Pathology Review; 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 CINtec *PLUS* Cytology Kit for detecting ≥ CIN2 and ≥ CIN3 in HPV18+ women with NILM Pap cytology is presented in Table 32.

In the population of cobas® 6800/8800 system, HPV18+ women with NILM Pap cytology, sensitivity and specificity were 75.0% and 73.3%, respectively, for ≥ CIN2, and 66.7% and 72.1% for ≥ CIN3. PPVs were 9.7% and 3.2% for ≥ CIN2 and for ≥ CIN3, respectively, whereas disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 1.3% and 0.6% for ≥ CIN2 and for ≥ CIN3, respectively.

In the population of cobas® 4800 system, HPV18+ women with NILM Pap cytology, sensitivity and specificity 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 disease risk in women with negative CINtec *PLUS* Cytology Kit results (1-NPV) was 1.4% for both disease cutoffs.

Table 32. Performance of CINtec *PLUS* Cytology Kit in HPV18+ Women 30-65 years old with NILM Cytology.

Performance Measure	cobas® 6800/8800 system, HPV18+/NILM		cobas® 4800 system, HPV18+/NILM	
	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3	CPR Diagnosis of ≥ CIN2	CPR Diagnosis of ≥ CIN3
Sensitivity (%)	75.0 (6/8) (40.9, 92.9)	66.7 (2/3) (20.8, 93.9)	85.7 (6/7) (48.7, 97.4)	66.7 (2/3) (20.8, 93.9)
Specificity (%)	73.3 (154/210) (67.0, 78.9)	72.1 (155/215) (65.7, 77.7)	68.3 (69/101) (58.7, 76.6)	65.7 (69/105) (56.2, 74.1)
Prevalence (%)	3.7 (8/218)	1.4 (3/218)	6.5 (7/108)	2.8 (3/108)
PPV (%)	9.7 (6/62) (5.4, 13.1)	3.2 (2/62) (1.0, 5.0)	15.8 (6/38) (9.2, 21.1)	5.3 (2/38) (1.7, 8.3)
NPV (%)	98.7 (154/156) (97.0, 99.6)	99.4 (155/156) (98.5, 99.9)	98.6 (69/70) (95.0, 99.7)	98.6 (69/70) (96.6, 99.7)
1-NPV (%)	1.3 (2/156) (0.4, 3.0)	0.6 (1/156) (0.1, 1.5)	1.4 (1/70) (0.3, 5.0)	1.4 (1/70) (0.3, 3.4)
PLR	2.81 (1.49, 3.97)	2.39 (0.73, 3.74)	2.71 (1.46, 3.87)	1.94 (0.59, 3.18)
NLR	0.34 (0.10, 0.81)	0.46 (0.09, 1.11)	0.21 (0.04, 0.76)	0.51 (0.09, 1.24)
Positivity Rate (%)	28.4 (62/218) (22.6, 34.3)		35.2 (38/108) (26.5, 43.8)	

Note: CPR = Central Pathology Review; 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.

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 the CINtec *PLUS* 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 exhibits weaker staining than expected, check if the selected protocol matches the specific specimen type; for example, SurePath cytology preparations require longer cell conditioning time than ThinPrep slides or conventional smear preparations. In addition, check to ensure that all dispenser barrels are clear from debris.
4. 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.
5. 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.
6. If weak staining is observed, increase incubation times in the staining protocols. On a conventional smear slide, cell conditioning incubation time can also be adjusted.
7. 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-containing hematoxylin was not used and that the recommended post-processing procedure was followed according to the Post Processing Procedure - Mounting and Coverslipping instructions.
8. 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.
9. For corrective action, refer to the Staining Procedure section, the instrument User Guide or contact your local support representative.
10. If an RCCM sample cap is misplaced or if the sample vial is leaking, replacement caps can be ordered (loose, 250/bag, REF 08037230190 or 8 trays of 48/box, REF 06913512001).

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

http://ec.europa.edu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
J	Template and Labeling Update. Addition of BenchMark ULTRA PLUS.

INTELLECTUAL PROPERTY

For USA: Rx only

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