VENTANA®

Roche

CINtec® p16 Histology

REF 705-4713

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IVD Σ 50

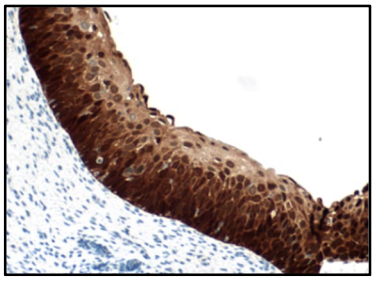

Figure 1. CINtec p16 Histology staining of cervical squamous epithelial cells.

INTENDED USE

CINtec p16 Histology is an immunohistochemistry assay for the qualitative detection of the p16^{INK4a} protein on formalin-fixed, paraffin-embedded tissue sections prepared from cervical biopsies. It is indicated to be used in conjunction with H&E stained slides prepared from the same cervical tissue specimen as an aid to increase diagnostic accuracy and inter-observer agreement in the diagnosis of high-grade cervical intraepithelial neoplasia.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

CINtec p16 Histology consists of a single component: anti-p16^{INK4a} (E6H4), a mouse monoclonal primary antibody produced against the p16^{INK4a} protein.

As a cyclin-dependent kinase inhibitor, p16^{INK4a} (p16) plays a key role in cell cycle progression and cellular differentiation.[1,2,3,4] The p16^{INK4a} protein controls the retinoblastoma protein (pRB)-mediated G1-S-phase transition and triggers cell cycle arrest in the course of the cellular differentiation process.[1,5] In normal, terminally differentiated cells, p16^{INK4a} is expressed at low levels, typically not detectable by immunohistochemistry.[1,5] Research studies have identified strong over-expression of p16^{INK4a} in pre-cancerous and cancerous tissues to be closely linked to the expression of human papillomavirus (HPV) E7 oncoprotein.[1,3,6,7,8]

Immunohistochemistry (IHC) detection of p16 overexpression may aid in the interpretation of cervical histology specimens. The p16 protein has been reported to be over-expressed in neoplastic squamous epithelial cells of the cervix uteri, whereas it has been found to be mostly absent in normal epithelium and non-neoplastic lesions.[1,2,5,6,7] Numerous studies have investigated the correlation between p16 overexpression and the presence of cervical intraepithelial neoplasia (CIN).[8,9] Overexpression of p16 has been observed in virtually all CIN3 lesions, the vast majority of CIN2 lesions, and typically within 40% to 60% of squamous cervical lesions classified as CIN1 in H&E stained tissue sections.[8-13]

CLINICAL SIGNIFICANCE

Diagnostic interpretation of cervical biopsy specimens establishes the basis for patient treatment decisions. CIN1 is the histologic manifestation of an HPV infection. In general, it is recommended that patients diagnosed with CIN1 lesions return for follow-up evaluation in one year.[14] For cervical disease, CIN2 is the most commonly used clinical threshold for treatment.[14] Excisional or ablative therapy is recommended for patients diagnosed with CIN2 or CIN3. The risk of excisional treatment to the patient of child-bearing age includes adverse effects on future pregnancies.[15,16,17] Therefore, accurate diagnosis of CIN and in particular CIN2 and CIN3 is important in patient management decisions.[18]

Morphological interpretation of cervical biopsy specimens by hematoxylin and eosin (H&E) only is subject to interobserver variability.[18-25] Several studies have evaluated the adjunctive use of p16 stained-slides and the effect on interobserver reliability in diagnostic interpretation of cervical histology specimens by pathologists. In all of these studies, the diagnostic agreement between pathologists improved significantly when p16-stained slides were interpreted along with H&E-stained slides compared to interpretation of the H&E-stained slide alone.[10,11,13,21,22,26,27,28]

Furthermore, several studies assessed the effect on diagnostic accuracy of cervical histology interpretation when p16-stained slides were used along with H&E-stained slides. Dijkstra and colleagues (2010) showed an almost perfect agreement between diagnoses established with support of p16-stained slides interpreted by a single pathologist compared to the adjudicated diagnoses made by an expert pathologist panel based on H&E staining only.[10] Bergeron and colleagues reported a significant increase in diagnostic accuracy when interpretation included both p16-stained slides and H&E-stained slides compared with H&E-stained slide interpretation alone ($p = 0.0004$) with sensitivity for ≥ CIN2 increasing from 77% to 87%.[11] In a recent prospective, population-based study in which an academic clinical center in the US analyzed more than 1450 consecutive cervical biopsy cases, staining for p16 was found "to be a useful and reliable diagnostic adjunct for distinguishing biopsies with and without CIN2+."[12] Therefore, the adjunctive interpretation of H&E-stained slides comprising cervical biopsy sections together with consecutive slides from the same tissue specimen immunostained for p16 has the potential to significantly improve diagnostic agreement in the interpretation of cervical biopsies.

PRINCIPLE OF THE PROCEDURE

CINtec p16 Histology is a mouse monoclonal primary antibody produced against the p16 protein. CINtec p16 Histology binds to p16 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits a nuclear and/or cytoplasmic staining pattern. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheets for further information.

MATERIAL PROVIDED

CINtec p16 Histology contains sufficient reagent for 50 tests.

One 5 mL dispenser of CINtec p16 Histology contains approximately 5.0 µg of a mouse monoclonal antibody.

CINtec p16 Histology is diluted in Tris-HCl with carrier protein, and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 1.0 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CINtec p16 Histology is a recombinant mouse monoclonal antibody purified from cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

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 may be required for staining but are not provided:

1. Microscope slides, positively charged
2. Negative Control (Monoclonal) (Cat. No. 760-2014 / Mat. No. 05266670001)
3. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / Mat. No. 06396500001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. Antibody Diluent (Cat. No. 251-018 / 05261899001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / Mat. No. 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / Mat. No. 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / Mat. No. 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / Mat. No. 05424569001)
12. Hematoxylin II counterstain (Cat. No. 790-2208 / Mat. No. 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / Mat. No. 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

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

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with CINtec p16 Histology when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[29] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

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. 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.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[30,31]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. 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.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. 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 product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
WARNING 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to the tables below for recommended staining protocols.

CINtec p16 Histology has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 705-4713.

Table 2. Recommended staining protocol for CINtec p16 Histology with *ultra*View Universal DAB Detection Kit on a BenchMark IHC/ISH instrument.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1 64 minutes 95°C
Antibody (Primary)	16 minutes, 37°C	20 minutes, 36 °C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

Table 3. Recommended staining protocol for CINtec p16 Histology with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 48 minutes	ULTRA CC1 48 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	8 minutes, 37°C	12 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[32]

NEGATIVE REAGENT CONTROL

In addition to staining with CINtec p16 Histology, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

Examples of positive control tissues for CINtec p16 Histology are normal pancreas, normal tonsil and cervical carcinoma.

Within normal tonsil tissue, there is nuclear and/or cytoplasmic staining of scattered squamous epithelial cells primarily in crypt epithelium and scattered follicular dendritic cells in germinal centers and absence of staining in the majority of lymphocytes (staining of rare lymphocytes may be observed).

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CINtec p16 Histology is nuclear and cytoplasmic.

Overexpression of the $p16^{INK4a}$ biomarker within cervical biopsy specimens is represented as a diffuse continuous staining of cells of the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of cells of superficial cell layers. This continuous, diffuse staining pattern represents positive CINtec p16 Histology status. Focal staining is represented by non-continuous staining of isolated cells or small cell clusters, particularly not of the basal and parabasal cells. Focal staining and no p16 staining represent negative CINtec p16 Histology status. The p16 staining pattern and CINtec p16 Histology status criteria are outlined in Table 4.

Table 4. CINtec p16 Histology status and p16 staining patterns.

CINtec p16 Histology Status	p16 Staining Pattern	Staining Description
Positive	Diffuse	Continuous staining of cells of the basal and parabasal cell layers of the squamous cervical epithelium, with or without staining of the intermediate or intermediate to superficial cell layers
Negative	Focal	A staining of isolated cells or small cell clusters; i.e., a non-continuous staining, particularly not of the basal and parabasal cells
	No p16 staining	A negative staining reaction in the squamous epithelium

SPECIFIC LIMITATIONS

CINtec p16 Histology may demonstrate fibroblast and columnar epithelial staining in cervical tissues, which does not interfere with interpretation.

OptiView detection system is generally more sensitive than *ultra*View detection system. The user must validate the results obtained with this reagent and detection systems.

Patient tissue should be stained within 24 weeks of sectioning from the tissue block. Staining performance with CINtec p16 Histology on sections that have been stored at room temperature for longer than 24 weeks has not been verified.

Samples should be fixed at least 1 hour in 10% NBF, zinc formalin or Z-fix, or at least 3 hours in AFA. Use of fixation times or fixative types other than those recommended can lead to false negative results. Alcohol formalin and PREFER fixatives are not recommended for use with this assay.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity were determined by staining multiple cases of normal and neoplastic human tissues with CINtec p16 Histology. The results are listed in Table 5 and Table 6. Many normal tissues demonstrated staining of a few cells or specific cell types as noted. This is expected due to the role of the p16 protein in cell cycle regulation.

Table 5. Sensitivity/Specificity of CINtec p16 Histology was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Positive cells in normal tissue
Cerebrum	4/4	Glial cells
Cerebellum	3/3	Purkinje cells
Adrenal gland	3/3	Adrenocortical epithelial cells
Ovary	3/3	Stromal cells
Pancreas	3/3	Islets of Langerhans, acinar cells
Lymph node	3/3	Lymphocytes, follicular dendritic cells
Parathyroid gland	2/3	Chief cells
Pituitary gland	3/3	Anterior pituitary epithelial cells
Testis	3/3	Spermatogenic cells, Leydig cells
Thyroid	3/3	Follicular cells
Breast	3/3	Myoepithelial cells, luminal epithelial cells, stromal cells
Spleen	3/3	Lymphocytes, follicular dendritic cells
Tonsil	6/6	Squamous epithelial cells, lymphocytes, follicular dendritic cells
Thymus	3/3	Epithelial reticular cells, lymphocytes, Hassall's corpuscles
Bone marrow	2/3	Myeloid cells
Lung	3/3	Pneumocytes, bronchial epithelial cells
Heart	0/3	No positive cells
Esophagus	3/3	Squamous epithelial cells
Stomach	3/3	Epithelial cells, fundic glands
Small intestine	3/3	Epithelial cells
Colon	3/3	Epithelial cells
Appendix	0/3	No positive cells
Liver	0/3	No positive cells
Salivary gland	3/3	Striated duct epithelial cells, serous acinar cells
Pharynx/Oral cavity	2/3	Respiratory epithelial cells, striated duct epithelial cells, mucous acinar cells, serous acinar cells
Kidney	3/3	Tubular epithelial cells, glomeruli mesangial cells
Prostate	3/3	Acinar cells, basal cells
Bladder	3/3	Urothelial cells
Endometrium	3/3	Endometrial glandular cells, stromal cells
Cervix[a]	1/120	Squamous epithelial cells
Skeletal muscle	0/3	No positive cells
Skin	0/3	No positive cells
Nerve	4/4	Schwann cells
Mesothelium	0/3	No positive cells
Soft tissue	3/3	Endothelial cells, fibroblasts, ductal cells

Tissue	# positive / total cases	Positive cells in normal tissue

[a] Tissues evaluated include normal cervix and chronic cervicitis. Cervix cases were interpreted based on the CINtec p16 Histology scoring algorithm which counts normal squamous (focal staining), endocervical or stromal cell staining as negative.

Table 6. Sensitivity/Specificity of CINtec p16 Histology was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebellum)	1/1
Adenocarcinoma (Head and neck)	1/1
Squamous cell carcinoma (Head and neck)	0/1
Serous carcinoma (Ovary)	1/1
Granulosa cell tumor (Ovary)	1/1
Teratoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	1/1
Ductal adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	1/1
Embryonal carcinoma (Testis)	1/1
Follicular carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	1/1
Invasive ductal carcinoma (Breast)	1/1
Invasive lobular carcinoma (Breast)	1/1
Adenoma (Adrenal gland)	1/1
Pheochromocytoma (Adrenal gland)	1/1
Diffuse large B-cell lymphoma (Spleen)	0/1
Pleomorphic adenoma (Salivary gland)	1/1
Warthin tumor (Salivary gland)	1/1
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	1/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	1/1
Adenocarcinoma (Stomach)	1/1
Gastrointestinal stromal tumor (Stomach)	1/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (Small intestine)	1/1
Adenocarcinoma (Colon)	1/1
Adenosquamous carcinoma (Colon)	1/1
Carcinoid tumor (Appendix)	1/1
Hepatocellular carcinoma (Liver)	1/1
Cholangiocarcinoma (Liver)	0/1
Renal cell carcinoma (Kidney)	1/2
Papillary renal adenoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyoma (Uterus)	0/1
Leiomyosarcoma (Uterus)	1/1
Endometrioid carcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Cervical intraepithelial neoplasia I (CIN I) (Cervix)	12/37
CIN I-II, borderline low vs high grade (Cervix)	2/8
CIN II (Cervix)	52/60
CIN II-III, high grade (Cervix)	1/3
CIN III (Cervix)	65/67
Squamous cell carcinoma (Cervix)	73/76
Adenosquamous carcinoma (Cervix)	2/2
Adenocarcinoma (Cervix)	1/1
Neuroendocrine carcinoma (Cervix)	1/1
Alveolar rhabdomyosarcoma (Muscle)	0/1
Invasive melanoma (Skin)	1/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	0/1
Schwannoma (Peripheral nerve)	1/1
Neurofibrosarcoma (Nerve)	1/1
Anaplastic large cell lymphoma (Lymph node)	1/1
Follicular lymphoma (Lymph node)	1/1
Hodgkin lymphoma (Lymph node)	1/1
Urothelial cell carcinoma (Bladder)	1/1
Squamous cell carcinoma (Bladder)	0/1
Plasmacytoma (Extramedullary)	1/1
Mesothelioma (Mesothelium)	1/1
Pleural solitary fibrous tumor (Mesothelium)	1/1
Angiosarcoma (Soft tissue)	1/1
Liposarcoma (Soft tissue)	1/1

Between Instrument Precision

Two studies were completed to assess between instrument precision. One study was performed on BenchMark XT instrument and BenchMark ULTRA instrument using *ultra*View Universal DAB Detection Kit. A second study was performed on BenchMark ULTRA instrument with OptiView DAB IHC Detection Kit.

In the first study, the sections from two multi-tissue blocks containing cervical squamous cell carcinoma, tonsil and pancreas were stained on three BenchMark XT instruments and three BenchMark ULTRA instruments with *ultra*View Universal DAB Detection Kit (5 sections from each multi-tissue block per each instrument). The p16 stain intensities were within 0.5 points of the median score in 100% of all tissues when stained across three BenchMark XT instruments. The p16 stain intensities were within 0.5 point of the median score in 100% of cervical squamous cell carcinoma (15/15), 93% of tonsil (14/15) and

93% of pancreas (14/15) when stained on three Benchmark ULTRA instruments. All tissues stained with CINtec p16 Histology had acceptable background staining.

In the second study, the precision of the CINtec p16 Histology test was determined across three BenchMark ULTRA instruments by staining replicate slides of 28 cervical cases (eight normal cervix, six CIN1, six CIN2, four CIN3, and four cervical carcinoma cases) using OptiView DAB IHC detection kit. Each case was stained on each of three BenchMark ULTRA instruments with each of three lots of CINtec p16 Histology. Overall, nine CINtec p16 Histology-stained slides from each case were included in the study (three lots of CINtec p16 Histology, three BenchMark ULTRA instruments). Each CINtec p16 Histology-stained slide was then paired with a hematoxylin and eosin (H&E) stained slide from the same case. All slides were randomized, and then evaluated by a single pathologist blinded to the case diagnosis for p16 stain intensities, positive or negative CINtec p16 Histology status and background. The data showed that 97.6% of tissues had stain intensity scores within 0.5 points across all instruments. In addition, 100% of sections stained with CINtec p16 Histology on three BenchMark ULTRA instruments demonstrated the same CINtec p16 Histology status. All tissues stained with CINtec p16 Histology had acceptable background staining.

Additionally, Between-instrument intermediate precision was determined across three BenchMark ULTRA PLUS instruments by staining duplicate slides of 24 cervical tissue cases (eleven normal cervix, one CIN1, two CIN2, seven CIN3, and three squamous cell carcinoma). Test slides were randomized then evaluated by a single pathologist blinded to the case diagnosis for positive or negative CINtec p16 Histology status, morphology and non-specific staining (background). The overall percent agreement was 99.3%. All tissues stained with CINtec p16 Histology had 100% acceptable morphology and non-specific staining.

Between-Lot Precision

Lot-to-lot precision of CINtec p16 Histology was evaluated by testing three lots of the CINtec p16 Histology on a BenchMark ULTRA instrument using the OptiView DAB IHC Detection Kit. Sections from each of 26 cervical biopsy tissue specimens (six normal cervix, six CIN1, six CIN2, six CIN3, and two cervical carcinoma cases) were stained in duplicate using each of CINtec p16 Histology lots. Each tissue slide stained with CINtec p16 Histology was paired with an adjacent H&E slide and a negative reagent control slide from the same case. Slide sets were randomized, and evaluated by a single pathologist blinded to the case diagnosis and lot number. The CINtec p16 Histology status (positive = diffuse p16 staining / negative = focal or no p16 staining) was determined based on the CINtec p16 Histology slide. The CIN categories [CIN2+ (CIN2, CIN3, ACIS, or invasive carcinoma combined into a single category) / CIN1- (no CIN or CIN1 combined into a single category)] were determined based on adjunctive interpretation of the H&E and CINtec p16 Histology slides. The results of this study indicate that CINtec p16 Histology performs reproducibly across three formulated production lots of the antibody. All cases showed 100% positive/negative agreement for the CINtec p16 Histology status across three production lots. In addition, cervical cases showed 98.7% agreement for CIN category across the three production lots. A summary of the data is shown in Table 7. The background was acceptable in 100% of tissues stained.

Table 7. Primary antibody lot-to-lot reproducibility of the CINtec p16 Histology assay on cervical samples as measured by CINtec p16 Histology status (positive/negative) and CIN category (CIN2+/CIN1-).

Reproducibility	Evaluation	Average Positive Agreement (n/N)	Average Negative Agreement (n/N)	Overall Percent Agreement (n/N)
Lot-to-lot	CINtec p16 Histology Status	100.0% (352/352)	100.0% (264/264)	100.0% (308/308)
	CIN Category	98.2% (322/328)	98.0% (290/296)	98.1% (306/312)

Within-Day Repeatability and Day-to-Day Precision

Overall, three studies were performed to assess within-day repeatability and day-to-day precision. In the first and second study, the pathologist evaluated tissues based on p16 staining intensities (0-4), while in the third study the CINtec p16 Histology status (positive /negative) in cervical biopsies was evaluated.

In the first study, the sections from two multi-tissue blocks containing cervical squamous cell carcinoma, tonsil, and pancreas tissues were stained on one BenchMark XT instrument with *ultra*View Universal DAB Detection Kit. In the second study two multi-tissue blocks containing tonsil, pancreas and three cervical cases (invasive squamous cell carcinoma, CIN1- , CIN2+) were stained on one BenchMark ULTRA instrument with OptiView DAB IHC Detection Kit. Within-day repeatability of the CINtec p16 Histology assay was tested by staining 14 replicate sections from each multi-tissue block with CINtec p16 Histology. CINtec p16 Histology passed the acceptance criteria with 100% of tissues staining within 0.5 points of the median score in both studies. Day-to-day precision was tested across 5 non-consecutive days spanning a minimum of a 20 day period. In both studies, CINtec p16 Histology passed the acceptance criteria with 100% of tissues staining within 0.5 points of the median score for within-day repeatability and day-to-day Precision. All tissues stained with CINtec p16 Histology had acceptable background.

The third study evaluated 24 cervical tissue specimens (three cervical squamous cell carcinoma, six CIN3, six CIN2, six CIN1, three normal cervical cases) by CINtec p16 Histology status (positive/negative) on one BenchMark ULTRA instrument with OptiView DAB IHC Detection Kit. The testing was performed across 5 non-consecutive days spanning a minimum of 20 day period. On each testing, day two slides from each case were stained with CINtec p16 Histology (150 slides total), and one slide from each case was stained with a negative reagent control (75 slides total). For within-day repeatability analysis, CINtec p16 Histology status (positive/negative) was compared within the same case and between two evaluable replicates from the same day. Since there were 5 days considered in this study, the total number of comparisons for each case for within-day repeatability was 5. The total number of comparisons for the day-to-day precision analysis was 120 (24 cases x 5 comparisons per case).

The results indicated 100% within-day repeatability and 100% day-to-day precision when the tissues were evaluated based on CINtec p16 Histology status. All sections stained with CINtec p16 Histology had acceptable background staining.

Additionally, Within-run repeatability was determined by staining 5 slides each from 24 cervical tissue cases (eleven normal cervix, one CIN1, two CIN2, seven CIN3, and three squamous cell carcinoma) on a BenchMark ULTRA PLUS instrument. Test slides were randomized then evaluated by a single pathologist blinded to the case diagnosis for positive or negative CINtec p16 Histology status, morphology and non-specific staining (background). The overall percent agreement was 97.5%. All tissues stained with CINtec p16 Histology had 100% acceptable morphology and non-specific staining.

Additionally, Between-day intermediate precision was determined by staining duplicate slides of 24 cervical tissue cases (eleven normal cervix, one CIN1, two CIN2, seven CIN3, and three squamous cell carcinoma) on a BenchMark ULTRA PLUS instrument on 5 non-consecutive days over at least a 20 day period. Between-day intermediate precision overall percent agreement was 98.8%. All tissues stained with CINtec p16 Histology had 100% acceptable morphology and non-specific staining.

Between Platform and Detection Kit Accuracy

The accuracy of the assay was demonstrated across the BenchMark ULTRA and BenchMark XT platforms, using the OptiView DAB IHC Detection Kit and the *ultra*View Universal DAB Detection Kit. Overall, 186 cervical cases were stained with CINtec p16 Histology and evaluated for CINtec p16 Histology status (positive/negative) and background (acceptable/unacceptable). The overall percent agreements were 98.3-98.4% for each pairwise combination of platforms within a detection kit, and each pairwise combination of detection kits within a platform. All evaluable cases stained with CINtec p16 Histology had acceptable background staining.

Additionally, a study was conducted to compare the staining performance of CINtec p16 Histology, using the OptiView DAB IHC Detection Kit on the BenchMark ULTRA PLUS instrument versus the BenchMark ULTRA instrument. One hundred twenty (120) cervical tissue cases (60 positive for CINtec p16 Histology and 60 negative for CINtec p16 Histology) were stained, and the stained slides were evaluated by a pathologist who determined the CINtec p16 Histology status. The overall percent agreement was 99.1%. All tissues stained with CINtec p16 Histology had 100% acceptable morphology and non-specific staining.

Within-Reader Precision and Between-Reader Precision

Within-reader and reader-to-reader precision was evaluated on 50 cervical cases (16 normal cervix, 12 CIN1, 12 CIN2, 6 CIN3, and four cervical carcinoma cases) stained with CINtec p16 Histology on BenchMark ULTRA with OptiView DAB IHC Detection Kit.

All slides were randomized, and then evaluated by three pathologists for positive/negative CINtec p16 Histology status. Pathologists were blinded to the case diagnosis. The CINtec p16 Histology-stained slides were re-randomized for a second evaluation of the CINtec

p16 Histology status by each of the three pathologists following a 4-week washout period. The overall percent agreement for both within-reader and between-reader precision for CINtec p16 Histology status was 98.7%, as shown in Table 8.

In the within-reader and between-reader precision study for CIN category, each CINtec p16 Histology slide was paired with an H&E-stained slide from the same case and the paired slide sets were randomized. CIN category (CIN2+/CIN1-) was evaluated by three pathologists based on adjunctive interpretation of the H&E-stained and CINtec p16 Histology-stained slides. Following a washout period of at least 4 weeks, slide pairs were re-randomized, and a second evaluation of the CIN category by each of the three pathologists was performed. Data shown in Table 8 demonstrates that the overall percent agreement for within-reader and between-reader precision for CIN category was 98.0% and 90%, respectively.

Table 8. Within-reader and between-reader precision of CINtec p16 Histology on cervical samples as measured by CINtec p16 Histology status (positive/negative) and CIN category (CIN2+/CIN1-).

Reader Precision	Evaluation	Average Positive Agreement (95% CI)	Average Negative Agreement (95% CI)	Overall Percent Agreement (95% CI)
Within-reader	CINtec p16 Histology Status	98.7% (93.9-100.0%)	98.6% (93.0-100.0%)	98.7% (94.0-100.0%)
	CIN Category	97.4% (89.1-100.0%)	98.4% (92.6-100.0%)	98.0% (92.0-100.0%)
Between-reader	CINtec p16 Histology Status	98.7% (93.1-100.0%)	98.6% (92.3-100.0%)	98.7% (93.9-100.0%)
	CIN Category	87.0% (71.8-97.6%)	91.9% (83.0-98.5%)	90.0% (80.0-98.0%)

CI: Confidence interval

Reproducibility Study (Laboratory-to-Laboratory Precision Study)

An inter-laboratory reproducibility study for CINtec p16 Histology demonstrated reproducibility of the assay in determining CINtec p16 Histology status and CIN category, using 27 cervical cases (10 No CIN, 5 CIN1, 5 CIN2, 5 CIN3, and two cervical carcinoma cases) across three BenchMark ULTRA instruments on each of 3 non-consecutive days at three external laboratories. The specimen were randomized and evaluated by a total of six pathologists (two pathologists/site) for both CINtec p16 Histology status (positive/negative) and for CIN category (CIN2+/CIN1-) based on adjunctive interpretation of the H&E-stained and CINtec p16 Histology-stained slides. Pathologists were blinded to the case diagnosis. The CINtec p16 Histology status and CIN category results are shown in Table 9 and Table 10, respectively. The morphology and background staining acceptability rates for all six pathologists across all sites were 96.3% and 97.1%, respectively. The data indicate excellent agreement in assay reproducibility across sites, days, and pathologists.

Table 9. Inter-laboratory reproducibility: agreement for CINtec p16 Histology status (positive/negative) of cervical samples.

Inter-laboratory reproducibility (CINtec p16 Histology status)	Average Positive Agreement	Average Negative Agreement	Overall Percent Agreement
Between-site (3 sites)	96.2% (91.2-99.3%)	93.9% (86.3-99.0%)	95.3% (90.6-99.2%)
Between-day (3 non-consecutive days)	98.2% (95.9-99.7%)	97.1% (93.3-99.5%)	97.8% (95.5-99.5%)
Between-reader (2 pathologists/site)	95.5% (87.8-100.0%)	92.9% (82.6-100.0%)	94.4% (87.1-100.0%)

Table 10. Inter-laboratory reproducibility: agreement for CIN category (CIN2+/CIN1-) of cervical samples based on adjunctive interpretation of H&E-stained and CINtec p16 Histology-stained slides.

Inter-laboratory reproducibility (CIN Category)	Average Positive Agreement	Average Negative Agreement	Overall Percent Agreement
Between-site (3 sites)	94.4% (86.8-98.8%)	94.1% (86.7-98.6%)	94.3% (88.5-98.6%)
Between-day (3 non-consecutive days)	96.9% (93.1-99.2%)	96.6% (93.0-99.1%)	96.8% (94.0-99.1%)
Between-reader (2 pathologists/site)	95.0% (87.4-98.9%)	94.8% (88.6-98.9%)	94.9% (89.3-98.7%)

CLINICAL PERFORMANCE

Diagnostic Agreement

The CERvical Tissue AdjunctIve aNalysis (CERTAIN) study was conducted to demonstrate that the adjunctive reading of CINtec p16 Histology results in an improvement in consistency of the diagnosis of cervical intraepithelial neoplasia (CIN), levels of agreement between Community Pathologists (CP) and Expert Pathologists (XP) readings of cervical punch biopsy tissue.

The CERTAIN clinical study was performed on 1100 retrospectively collected FFPE cervical punch biopsy specimens, which represent a colposcopy referral population. An XP derived reference diagnosis was established for each study case using the H&E-stained slides only and using the H&E and CINtec p16 Histology-stained slides. Two XPs established their independent diagnoses (No CIN, CIN1, CIN2, CIN3, adenocarcinoma in situ, or invasive carcinoma) based on the H&E-stained slides for each of the 1100 cases. The pathologists were also provided with the following clinical information: patient age, Pap cytology result and HPV test result (if available). Discordant cases were evaluated by a third XP. Cases for which a two out of three majority diagnosis was not achieved were reviewed during an adjudication review meeting that included all three XPs. Majority (or consensus) results established the expert-derived reference diagnosis for each case evaluated in the study (termed XP1, or H&E reference diagnosis). After a minimum of 4-week washout period, the same XPs evaluated both the H&E and CINtec p16 Histology-stained slides to establish their diagnosis (No CIN, LSIL-histology/CIN1, HSIL-histology/CIN2, HSIL-histology/CIN3, adenocarcinoma in situ, or invasive carcinoma) (termed XP2, or H&E + CINtec p16 Histology reference diagnosis). The process of establishing the majority diagnoses was the same as that used for establishing the reference diagnosis on H&E-stained slides only. Seventy, (70), Board Certified CPs, from across the United States, participated in the study. In the first round (Round 1, CP1), the 1100 H&E-stained cases were divided into four reading sets of 275 cases with comparable distributions of individual diagnostic categories per reference diagnosis. The 70 CPs were assigned to four groups consisting of either 17 or 18 pathologists per group. For each case within their assigned reading set, the pathologists were provided with the following clinical information: patient age, Pap cytology result and HPV test result (if available). The CPs independently rendered their diagnoses on the H&E-stained slide for each of their assigned cases (No CIN, CIN1, CIN2, CIN3, adenocarcinoma in situ, or invasive carcinoma). Thus, each study case was individually read by either 17 or 18 community pathologists.

In the second round (Round 2, CP2), the CPs read the H&E-stained slides along with the paired corresponding CINtec p16 Histology-stained slides for the same set of cases within their assigned reading set. After at least a 4-week washout period between Round 1 and Round 2, each pathologist independently rendered their diagnoses (No CIN, LSIL-histology/CIN1, HSIL-histology/CIN2, HSIL-histology/CIN3, adenocarcinoma in situ, or invasive carcinoma). The CPs noted the CINtec p16 Histology status (CINtec p16 Histology positive = diffuse p16 staining; CINtec p16 Histology negative = focal or no p16 staining) along with their histological diagnosis using both the H&E-stained slide along with the CINtec p16 Histology-stained slide. The primary objective of this study was to demonstrate improvement of diagnostic agreement without compromising the positive percent agreement, i.e. the probability of a positive test result agreeing with a diagnosis of ≥ CIN2 (CIN2, CIN3, ACIS, or invasive carcinoma combined into a single category) versus ≤ CIN1 (No CIN or CIN1 combined into a single category) based on H&E-stained slides (Round 1) compared with interpretation of the H&E-stained slides along with CINtec p16 Histology-stained slides (Round 2).

Improvement of Diagnostic Accuracy of Expert Pathologists

The improvement in diagnostic accuracy of expert pathologists was determined by comparing the expert pathologists H&E reference diagnosis (XP1) versus the expert pathologists H&E + CINtec p16 Histology reference diagnosis (XP2). The analysis was conducted on the interpretation of all 1100 cervical biopsies. The improvement in diagnostic accuracy between the H&E reference diagnosis by expert pathologists versus H&E + CINtec p16 Histology reference diagnosis by expert pathologists is shown in Table 11. When using H&E + CINtec p16 Histology in the diagnostic interpretation of cervical biopsies, the XPs identified 23.7% more ≥ CIN2 cases compared with diagnostic interpretation using H&E alone.

Table 11. Agreement between H&E reference diagnosis and H&E + CINtec p16 Histology reference diagnosis for all cases.

		H&E Reference Diagnosis					Total
		No CIN	CIN1	CIN2	CIN3	ACIS or Cancer	
H&E + CINtec p16 Histology reference diagnosis	No CIN	693	13	4	0	0	710
	LSIL-histology	46	120	4	1	0	171
	HSIL-histology	30	31	83	69	1	214
	ACIS or cancer	0	0	0	0	5	5
Total		769	164	91	70	6	1100

ACIS: adenocarcinoma in situ

Community Pathologists Interpretations using H&E versus H&E + CINtec p16 Histology Compared with an Expert-derived H&E Reference Diagnosis

Diagnostic agreement among community pathologists was determined by comparing the results of community pathologists Round 1 H&E diagnoses (CP1) versus the expert pathologists H&E reference diagnosis (XP1) and the community pathologists Round 2 H&E + CINtec p16 Histology diagnoses (CP2) vs the expert pathologists H&E reference diagnosis (XP1). As shown in Table 12, data were analyzed by determining agreement rates averaged across case and reader and calculating confidence intervals (CI). A statistically significant increase in positive percent agreement, the measure for the detection of ≥ CIN2 lesions (+6.8% with 95% CI: 4.7% to 9.0%), was observed. Negative percent agreement for the detection of ≤ CIN1 increased by 1.3% with 95% CI: 0.5% to 2.3%.

Table 12. Agreement rates of Community Pathologist reads on H&E-stained slides versus H&E-stained slides + CINtec p16 Histology-stained slides with expert-derived H&E reference diagnosis (XP1).

Endpoint	H&E	H&E + CINtec p16 Histology	Difference	p-value
Positive percent agreement (95% CI)	83.5% (79.9, 86.8)	90.3% (87.5, 92.7)	6.8% (4.7, 9.0)	< .0001
Negative percent agreement (95% CI)	90.4% (89.4, 91.4)	91.8% (90.6, 92.9)	1.3% (0.5, 2.3)	0.0032

Note: Difference does not equal 1.4% due to rounding error: H&E = 90.44%, H&E + CINtec p16 Histology = 91.76%, Difference = 1.32%.

A summary diagram for the diagnostic accuracy of the individual community pathologist readers for diagnosing ≥ CIN2 versus ≤ CIN1 using H&E-stained slides only compared to using H&E-stained slides along with CINtec p16 Histology-stained slides compared to the expert-derived H&E reference diagnosis is shown in Figure 2. The positive percent agreement (PPA) and negative percent agreement (NPA) (negative percent agreement, i.e. the agreement of a negative test result with ≤ CIN1 by XP1) of the interpretation by each pathologist for Round 1 (H&E-stained slides only – blue circles), versus Round 2 (H&E-stained slides along with CINtec p16 Histology-stained slides – red triangles) is shown. The prediction ellipses indicate the range of positive and negative percent agreement performance expected for most pathologists, in that 80% should fall within the ellipses, and 20% should fall outside. The data demonstrate that the interpretation of cervical biopsies using H&E along with CINtec p16 Histology-stained slides improves the diagnostic agreement in the interpretation of cervical biopsies and it reduces the between reader variability.

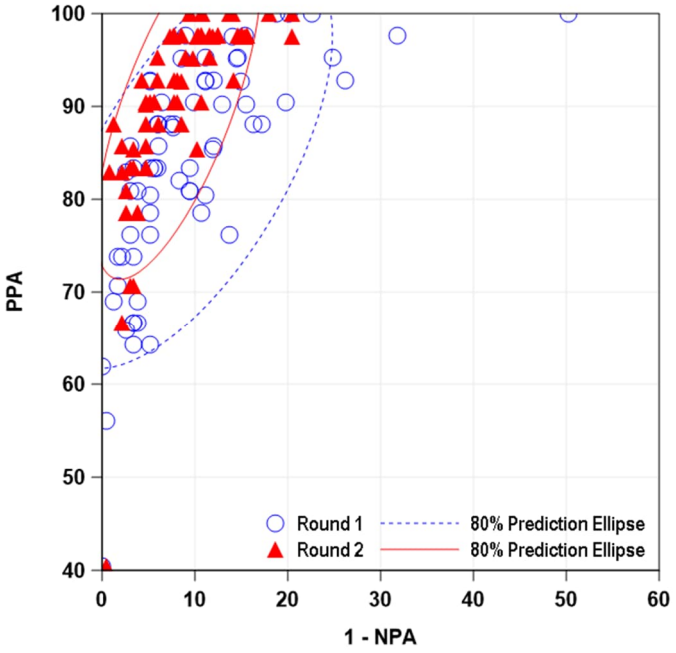

Figure 2. Summary diagram for diagnostic agreement (PPA versus 1-NPA) of community pathologists for diagnosing ≥ CIN2 versus ≤ CIN1 using H&E only (Round 1) and H&E + CINtec p16 Histology (Round 2) compared with the expert-derived H&E reference diagnosis (XP1) (80% prediction ellipses generated under assumption of bivariate normality).

Community Pathologist Interpretations using H&E versus H&E + CINtec p16 Histology Compared with an H&E + CINtec p16 Histology Expert-derived Reference Diagnosis

Next, the reading results of the community pathologists using both methods (i.e., H&E + CINtec p16 Histology versus H&E only) were compared to a reference diagnosis (XP2) established by the expert gynecopathologists using H&E plus CINtec p16 Histology-stained slides. Expert pathologists were blinded to the results of their first individual reading round and the consensus H&E reference diagnosis. The process of establishing the consensus diagnoses was the same process used for establishing the H&E reference diagnosis described previously.

The community pathologists reading results using H&E-stained slides only versus H&E-stained slides along with CINtec p16 Histology-stained slides were analyzed and compared against the expert-derived H&E + CINtec p16 Histology reference diagnosis (Table 13). The data demonstrate a statistically significant increase in PPA (+11.5% with 95% CI: 9.3% to 13.5%) and NPA (+3.0% with 95% CI: 2.2% to 3.7%).

Table 13. Agreement rates of Community Pathologists for reads on H&E-stained slides versus H&E-stained slides + CINtec p16 Histology-stained slides with expert-derived H&E + CINtec p16 Histology reference diagnosis (XP2).

Endpoint	H&E	H&E + CINtec p16 Histology	Difference	p-value
PPA % (95% CI)	73.3% (69.6, 76.9)	84.8% (82.1, 87.1)	11.5% (9.3, 13.5)	< .0001
NPA % (95% CI)	92.2% (91.3, 93.1)	95.2% (94.4, 96.0)	3.0% (2.2, 3.7)	< .0001

CI: Confidence interval

A summary diagram for the diagnostic accuracy of the individual community pathologist readers for diagnosing ≥ CIN2 versus ≤ CIN1 using H&E-stained slides only versus using H&E-stained slides together with CINtec p16 Histology-stained slides compared to the expert-derived H&E + CINtec p16 Histology reference diagnosis is shown in Figure 3. The PPA and NPA of the interpretation by each pathologist for Round 1 (H&E-only – blue circles) versus Round 2 (H&E + CINtec p16 Histology – red triangles) is shown. The prediction ellipses indicate the range of PPA and NPA performance expected for most pathologists, in that 80% should fall within the ellipses, and 20% should fall outside. The data demonstrate that the interpretation of cervical biopsies using H&E along with CINtec p16 Histology-stained slides improves the diagnostic consistency in the interpretation of cervical biopsies and it reduces the between reader variability.

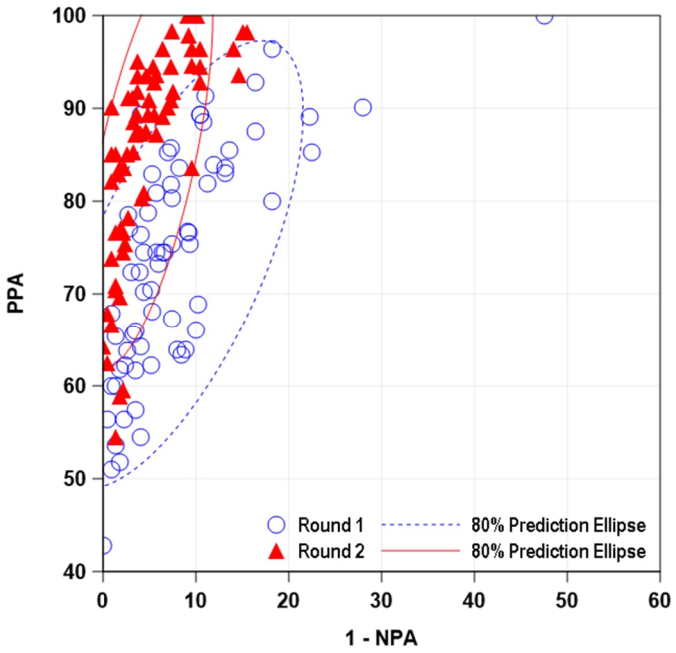

Figure 3. Summary diagram for diagnostic agreement (PPA versus 1 NPA) of community pathologists for diagnosing ≥ CIN2 versus ≤ CIN1 using H&E only (Round 1) and H&E + CINtec p16 Histology (Round 2) compared with the expert-derived H&E + CINtec p16 Histology reference diagnosis (XP2) (80% prediction ellipses generated under assumption of bivariate normality).

CINtec p16 Histology Staining Performance

The secondary objective of this study was to assess the staining performance of the CINtec p16 Histology assay as determined by the community pathologists during review of the study slides. A total of 19250 CINtec p16 Histology status interpretations were rendered during the study by the 70 community pathologists. The staining performance criteria assessed included overall staining acceptability, background staining acceptability, and morphology acceptability. The study data demonstrated > 99% acceptability rates for all three staining criteria (Table 14).

Table 14. CINtec p16 Histology staining performance.

Endpoint	Number of Interpretations n/N	Rate
Staining acceptability	19074 / 19250	99.09%
Morphology acceptability	19249 / 19250	99.99%
Background acceptability	19249 / 19250	99.99%

Conclusions

The use of CINtec p16 Histology-stained slides as an adjunct to the interpretation of H&E-stained slides increases the diagnostic agreement in the detection of high-grade CIN (≥ CIN2) lesions on cervical punch biopsies. This improved agreement is driven both by increases in PPA (the agreement of a positive test result with ≥ CIN2 diagnosis) and NPA (the agreement of a negative test results with CIN1 or No CIN diagnosis). Furthermore, the consistency of diagnoses improves between community pathologists with each other and with an expert panel.

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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:

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Symbols

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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
G	Updates to Warnings and Precautions section. Updated to current template.

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CONTACT INFORMATION

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606

www.roche.com

CE 0123