CINtec® Histology

REF 705-4793

06680003001

IVD Σ 50

REF 725-4793

06680011001

IVD Σ 250 **Rx Only**

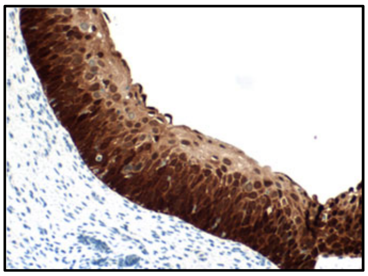

Figure 1. Diffuse CINtec Histology staining of cervical squamous epithelium

INTENDED USE

CINtec® Histology is a qualitative immunohistochemistry (IHC) test using mouse monoclonal anti-p16 antibody clone E6H4, and is intended for use in the light microscopic assessment of the p16^{INK4a} protein in formalin-fixed, paraffin-embedded (FFPE) cervical punch biopsy tissues using OptiView DAB IHC Detection Kit on a VENTANA BenchMark ULTRA instrument. The test is indicated as an adjunct to examination of hematoxylin and eosin (H&E) stained slide(s), to improve consistency in the diagnosis of cervical intraepithelial neoplasia (CIN). Diagnosis of CIN presence or level should be based on H&E stained slide(s) and other clinical and laboratory test information.

Intended for *in vitro* diagnostic (IVD) use. Prescription Use Only.

PROFESSIONAL SOCIETY RECOMMENDATIONS

The College of the American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) has recommended the use of adjunctive p16 immunohistochemistry (IHC) for the interpretation of squamous cervical lesions according to the following criteria (Lower Anogenital Squamous Terminology (LAST) Standardization Project for HPV-Associated Lesions (more commonly known as LAST recommendations or guidelines)[1]):

- When the H&E morphologic differential diagnosis is between pre-cancer (CIN2 or CIN3) and a mimic of pre-cancer (e.g., processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting);
- If the pathologist is entertaining an H&E morphologic interpretation of CIN2 (under the old terminology, which is a biologically equivocal lesion falling between the morphologic changes of HPV infection (low-grade lesion) and pre-cancer);
- As an adjudication tool for cases in which there is a professional disagreement in histologic specimen interpretation, with the caveat that the differential diagnosis includes a pre-cancerous lesion (CIN2 or CIN3);
- As an adjunct to morphologic assessment for biopsy specimens interpreted as ≤CIN1 that are at high risk for missed high grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV-16+, or AGC (NOS).

SUMMARY AND EXPLANATION

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

As a cyclin-dependent kinase inhibitor, p16^{INK4a} (p16) plays a key role in cell cycle regulation and cellular differentiation.[2,3,4,5] The p16 protein controls the retinoblastoma protein (pRB)-mediated G1-S phase transition and triggers cell cycle arrest in the course of the cellular differentiation process.[2,6] In normal, terminally differentiated cells, p16 is expressed at low levels typically not detectable by immunohistochemistry (IHC).[2,6] Research studies have identified strong overexpression of p16 in pre-cancerous and cancerous tissues to be closely linked to the expression of human papillomavirus (HPV) E7 oncoprotein.[2,4,8,9]

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 squamous neoplastic epithelial cells of the cervix uteri, whereas it has been found to be mostly absent in normal epithelium and non-neoplastic lesions.[2,3,6,9] Numerous studies have investigated the correlation between p16 overexpression and the presence of cervical intraepithelial neoplasia (CIN).[10,11] 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 Hematoxylin and Eosin (H&E) stained tissue sections.[10,11,12,13,14]

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 1 year.[15] For cervical disease, CIN2 is the most commonly used clinical threshold for treatment.[15] 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.[16-18] Therefore, accurate diagnosis of CIN and in particular CIN2 and CIN3 is important in patient management decisions.[19]

Morphological interpretation of cervical biopsy specimens by H&E only is subject to interobserver variability.[19-25] 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.[12,13,22,26,27,28]

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.[12] Bergeron and colleagues (2010) 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%.[13] In a recent prospective, population-based study in which an academic clinical center in the US analyzed more than 1,450 consecutive cervical biopsy cases, staining for p16 was found "to be a useful and reliable diagnostic adjunct for distinguishing biopsies with and without CIN2+".[14] 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.

In 2012, the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) issued the Lower Anogenital Squamous Terminology (LAST) consensus recommendations.[1] The LAST consensus recommendations provide guidance for clinical use of p16 IHC along with H&E to improve the detection of HPV-associated pre-cancerous lesions within cervical (and other lower anogenital tract) squamous tissues in specific circumstances. The use of p16 IHC is recommended in specific circumstances as listed above under Professional Society Recommendations. In 2014, the World Health Organization (WHO) adopted the LAST consensus recommendations; the adjunctive use of p16 IHC in evaluation of cervical biopsies is now considered recommended standard of care.[29]

Caution in the use of p16 IHC in the evaluation of cervical biopsies has been advocated in published literature as well. Clark, et.al., reported "cases were initially overdiagnosed as

HSIL because pathologists (1) overused p16 IHC on unequivocal LSIL, or (2) upgraded questionable lesions to HSIL based on non-block p16 staining patterns (patchy or focal).[30] The authors "advocate judicious use of p16 in the designated circumstances and careful interpretation of staining patterns in the context of morphology." Mills, et al. concluded that the data from their study "reinforces the LAST recommendations that p16 should only be used selectively for problematic scenarios, such as CIN2 because of its inherent lack of reproducibility, cases in which one is struggling between CIN1 and CIN2, and benign mimics of CIN3.[31]

PRINCIPLE OF THE PROCEDURE

CINtec Histology contains a mouse monoclonal primary antibody that binds to the p16 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The specific antibody is localized using OptiView DAB IHC Detection Kit (250), Cat. No. 760-700 / Mat. No. 06396500001. Refer to the OptiView DAB IHC Detection Kit package insert for further information.

REAGENT PROVIDED

CINtec Histology contains a mouse monoclonal antibody purified from cell culture supernatant.

CINtec Histology (705-4793) contains sufficient reagent for 50 tests. One CINtec Histology 5 mL dispenser contains approximately 5.0 µg of a mouse monoclonal antibody.

CINtec Histology (725-4793) contains sufficient reagent for 250 tests. One CINtec Histology 25 mL dispenser contains approximately 25.0 µg of a mouse monoclonal antibody.

The CINtec Histology reagent is diluted in 0.05M Tris-HCl containing 1% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10.0 mg/mL. Specific antibody concentration is approximately 1.0 µg/mL. There is no known non-specific antibody reactivity observed in this product.

This antibody is optimized for use on the VENTANA BenchMark ULTRA IHC/ISH instrument in combination with the OptiView DAB IHC Detection Kit. No reconstitution, mixing, dilution, or titration is required.

Refer to the OptiView DAB IHC Detection Kit package insert for further information.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as the OptiView DAB IHC Detection Kit and ancillary components, including negative and positive tissue control slides, are not provided.

1. Microscope slides, positively charged
2. Drying oven capable of maintaining a temperature of 60°C ± 5°C
3. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
4. Xylene (Histological grade)
5. Ethanol or reagent alcohol (Histological grade)
    - 100% solution: Undiluted ethanol or reagent alcohol
    - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
    - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
6. Deionized or distilled water
7. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / Mat. No. 06396500001)
8. EZ Prep Concentrate (10X) (Cat. No. 950-102 / Mat. No. 05279771001)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / Mat. No. 05353955001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / Mat. No. 05424534001)
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 (Permount Fisher Cat. No. SP15-500 or equivalent)
15. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
16. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
17. Light microscope
18. Absorbent wipes
19. BenchMark ULTRA (Cat. No. 750-600 / Mat. No. 05342716001)

STORAGE

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 Histology when used with the OptiView DAB IHC Detection Kit and the BenchMark ULTRA instrument.

On the basis of xenograft models generated from the Calu-3 human cell-line, which is moderately positive for p16 expression, Ventana recommends tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours.[32] However, fixation times of 1-72 hours in 10% NBF gave equivalent CINtec Histology staining results. Acceptable CINtec Histology staining was also achieved with fixation in Zinc formalin fixative or Z-fix for at least 1 hour, while alcohol-formalin-acetic acid (AFA) was also acceptable with a fixation time of at least 3 hours.

The amount of fixative used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15°–25°C).[33]

Alcohol formalin and PREFER fixatives are not recommended for use with CINtec Histology. Xenograft tissues fixed in alcohol formalin demonstrate weaker or variable staining, and those fixed in PREFER fixative demonstrate inappropriate staining.

Delay-to-fixation studies using 10% NBF have revealed no loss in CINtec Histology staining intensity on xenograft specimens subjected to post-excision fixation delays of up to 5 hours.

Paraffin-embedded sections should be cut approximately 4 µm thick and mounted on positively charged glass slides. Because antigenicity of cut tissue sections may diminish over time, slides should be stained promptly after cutting from the paraffin block. However, unstained cervical tissue slides stored at 2-8°C or 30°C for up to 24 weeks demonstrated similar CINtec Histology staining intensity compared to the tissue specimens prepared from the same block and stained with CINtec Histology on day 1.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For Prescription Use Only.
3. ProClin 300 solution is used as a preservative in this reagent. 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.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

CINtec Histology has been developed for use on the BenchMark ULTRA instrument in combination with the OptiView DAB IHC Detection Kit, and ancillary reagents. An assay-specific staining procedure must be used with the CINtec Histology assay. Refer to Table 1 for the recommended staining protocol and required staining procedure.

Any deviation from recommended test procedures may invalidate CINtec Histology staining results using the CINtec Histology product.

Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

Use of appropriate controls is recommended.

Table 1. Recommended Staining Protocol for CINtec Histology and Negative Reagent Control with the OptiView DAB IHC Detection Kit on the BenchMark ULTRA instrument

Staining Procedure: U CINtec Histology	
Procedure Type	**Parameter Input**
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 48 minutes
Antibody (Primary)	CINtec Histology OR negative reagent control
Antibody (Primary) Incubation	12 minutes, 36°C

Variations in individual specimens, in tissue fixation and processing conditions, in general lab instrument and environmental conditions, or in individual pathologist's preferences may necessitate an increase or decrease in the primary antibody incubation time or cell conditioning pretreatment time. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[34]

QUALITY CONTROL PROCEDURES

Negative Reagent Control

Ventana strongly recommends a negative reagent control be used to stain an adjacent section of the patient specimen tissue on a separate slide from the CINtec Histology-stained slide. Negative Control (Monoclonal) (Cat. No. 760-2014 / Mat. No. 05266670001), a negative reagent control mouse monoclonal antibody, is recommended for use in place of the primary antibody to evaluate nonspecific staining. The incubation period for the negative reagent control antibody should be the same as that for the primary antibody.

Tissue Controls

Ventana strongly recommends running tissue controls when staining patient specimens with CINtec Histology. p16-positive and p16-negative control tissues fixed and processed in the same manner as the patient specimen should be run on each patient specimen slide. Staining conditions for p16-positive and p16-negative control tissues should be evaluated for every set of test conditions used. Positive control tissue is used to confirm that the antibody was applied and the instrument functioned properly; while negative control tissue is used to detect minor levels of reagent degradation or instrument out-of-specification issues.

For optimal quality control, cervical carcinoma or CIN2/3 cervical tissue positive for p16 staining is suitable for use as a positive tissue control, and normal cervical tissue negative for p16 staining is suitable for use as a negative tissue control. Criteria for evaluation are described in Table 2.

Alternatively, normal human tonsil tissue is suitable for use as a tissue control. Tonsil contains both positive and negative staining elements for p16 staining with CINtec Histology. 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).

Control tissue should be autopsy, biopsy, or surgical specimens prepared and fixed in a manner identical to the test specimen. Such tissue may be used to monitor all steps of the analysis, from tissue preparation through staining. A tissue section fixed or processed differently from the test specimen may provide a suitable control for all reagents and method steps except fixation and tissue preparation.

Known positive and known negative tissue controls should be utilized only for monitoring the performance of processed tissues and test reagents.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing on a series of tissues with known IHC performance characteristics representing p16-positive and -negative tissues. (Refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[35] or the CLSI Approved Guideline.[36]). Cervical tissues with known CINtec Histology status are suitable for assay verification, as well as normal human tonsil.

INTERPRETATION OF RESULTS

The VENTANA automated immunostaining procedure using the OptiView DAB IHC Detection Kit causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the CINtec Histology antibody. A qualified pathologist experienced in IHC procedures must evaluate system-level controls and qualify the stained product before interpreting results.

Positive/Negative System-Level Tissue Controls

The CINtec Histology-stained positive and negative tissue controls should be examined to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product on the positive control tissue within the nuclei and/or cytoplasm of the target cells is indicative of positive reactivity.

If the positive or negative tissue controls fail to demonstrate appropriate staining or demonstrate a change in clinical diagnostic interpretation, any results with the test specimens should be considered invalid.

Table 2. Evaluation Criteria for CINtec Histology-stained Cervical Control Tissues

Control Tissue	Acceptable	Unacceptable
CIN2, CIN3, or cervical carcinoma*	Diffuse continuous staining of cells of the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of cells of intermediate, or intermediate and superficial cell layers; or diffuse continuous staining of invasive carcinoma	No staining observed with CINtec Histology or staining of isolated cells or small cell clusters (i.e. non-continuous staining)
Normal cervix	Either a negative staining reaction or a staining of isolated cells or small cell clusters, (i.e. non-continuous staining)	Diffuse continuous staining of cells of the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of cells of intermediate, or intermediate and superficial cell layers; or diffuse continuous staining of invasive carcinoma

*Tissues should first be qualified as p16-positive (i.e., diffuse p16 staining) before being used as controls.

Negative Reagent Control

Nonspecific staining can be evaluated using the negative reagent control slide. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid.

Patient Tissue

Patient tissue must be evaluated according to the CINtec Histology p16 staining pattern. The stained slide specimens are evaluated according to a binary rating system ("positive" or "negative") for CINtec Histology according to the criteria outlined in Table 3.

Interpretation of the results must take into consideration the fact that p16 is a cellular protein, with nuclear and/or cytoplasmic staining within cells, that is expressed at detectable levels in some low-grade cervical lesions, the majority of high-grade cervical lesions and most cervical cancers. In addition, p16 may be expressed at detectable levels in some conditions not associated with cervical dysplasia, albeit at differing levels and with different patterns of expression.

The interpretation of slides stained using CINtec Histology should be performed in conjunction with H&E-stained slides prepared from the same cervical tissue specimen. The additional information provided by the CINtec Histology-stained slides should be combined with the preliminary morphology-based diagnosis established on the H&E-stained slides in order to establish a final diagnosis.

Non-specific background staining that does not interfere with clinical interpretation of the CINtec Histology stain should be ignored.

Table 3. CINtec Histology Status and p16 Staining Patterns

CINtec 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

GENERAL LIMITATIONS

1. IHC is a multiple-step diagnostic process that requires specialized training in the selection of the appropriate reagents and tissues, in tissue fixation and processing, in IHC slide preparation, and in interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods or from inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and system-level controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate the staining results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. This product is not intended for use in flow cytometry; flow cytometry performance characteristics for this product have not been determined.
7. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms and other pathological tissues.[37]
8. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase in the OptiView DAB IHC Detection Kit.[38]
9. False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), or endogenous peroxidase activity (example: liver, brain, breast, kidney) depending on the type of immunostain used.[39]
10. As with any IHC test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

SPECIFIC LIMITATIONS

1. CINtec Histology has been solely cleared for use on the BenchMark ULTRA instrument with the OptiView DAB IHC Detection Kit and is not cleared with any other detection methods or automated staining instruments.
2. This assay has not been validated for use with cytology smears or decalcified specimens.
3. Patient tissue should be stained within 24 weeks of sectioning from the tissue block. Staining performance with CINtec Histology on sections that have been stored at room temperature for longer than 24 weeks has not been verified.
4. Ventana recommends that samples 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.
5. CINtec Histology may demonstrate fibroblast and endocervical cell staining in cervical tissues; this staining does not interfere with interpretation.

PERFORMANCE CHARACTERISTICS

Analytical Specificity/Sensitivity

Analytical specificity and sensitivity were determined by staining multiple cases of normal and neoplastic human tissues with CINtec Histology. The results are listed in Table 4 and Table 5. Positive staining is nuclear and/or cytoplasmic unless otherwise specified. No unexpected staining was observed with CINtec Histology on the normal and neoplastic tissues. Many normal tissues demonstrated staining of a few cells or specific cell types as noted. This may be expected due to the role of the p16 protein in cell cycle regulation.

Table 4. Analytical Specificity/Sensitivity of CINtec Histology Staining in FFPE Normal Tissues

Tissue	# Positive / Total cases	Cell Type
Cerebrum	1/3[a,b]	Glial cells
Cerebellum	3/3	Purkinje cells
Adrenal gland	3/3[a]	Adrenocortical epithelial cells
Ovary	2/2[a]	Stromal cells and endothelial cells
Pancreas	3/3[c]	Acinar cells
Parathyroid gland	1/1	Chief cells
Hypophysis	3/3	Anterior pituitary epithelial cells
Testis	2/3[c]	Spermatogenic and Leydig cells

Tissue	# Positive / Total cases	Cell Type
Thyroid	2/5[c]	Follicular and parafollicular cells
Breast	3/3	Myoepithelial cells, luminal epithelial cells, and stromal cells
Spleen	3/3[a]	Lymphocytes, follicular dendritic cells
Tonsil	3/3[a]	Squamous epithelial cells, lymphocytes and follicular dendritic cells
Endometrium	2/3[a]	Stromal cells
Skeletal muscle	0/3	No specific staining
Nerve (sparse)	1/3[a]	Schwann cells
Thymus	1/3[a]	Epithelial reticular cells
Myeloid (bone marrow)	0/3	No specific staining
Lung	0/4	No specific staining
Heart (cardiac muscle)	0/3	No specific staining
Esophagus	0/3	No specific staining
Stomach	0/3	No specific staining
Small intestine	3/3[a]	Lymphocytes
Colon	3/3[a]	Lymphocytes and plasma cells
Liver	2/3[c]	Hepatocytes
Salivary gland	2/3[a]	Striated duct epithelial cells
Kidney	0/3	No specific staining
Prostate	0/3	No specific staining
Cervix	2/3[a]	Stromal cells and endocervical cells
Skin	0/2	No specific staining
Mesothelium	0/2	No specific staining

[a]: few cells staining
[b]: nuclear staining only
[c]: cytoplasmic staining only

Table 5. Analytical Specificity/Sensitivity of CINtec Histology Staining in a Variety of FFPE Neoplastic Tissues

Pathology	# Positive / Total cases
Glioblastoma	1/1
Atypical meningioma	0/1
Malignant ependymoma	1/1
Malignant oligodendroglioma	0/1[a]
Ovarian serous papillary adenocarcinoma	1/1
Ovarian adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma	0/1
Thyroid medullary carcinoma	1/1[a,b]
Thyroid papillary carcinoma	0/1
Breast intraductal carcinoma	1/1
Breast invasive ductal carcinoma	2/2
Diffuse B-cell lymphoma	1/3
Lung small cell undifferentiated carcinoma	1/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	1/1
Esophageal squamous cell carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	1/1
Gastrointestinal adenocarcinoma	3/3
GIST	3/3
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/2
Leiomyoma	1/1
Uterine endometrial adenocarcinoma	1/1
Uterine endometrial clear cell carcinoma	1/1[a]
Cervical squamous cell carcinoma	2/2
Embryonal rhabdomyosarcoma	1/1
Malignant melanoma	1/1
Basal cell carcinoma	1/1[a]
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Neuroblastoma	0/1
Epithelial malignant mesothelioma	1/1
Hodgkin lymphoma	1/1
Anaplastic large cell lymphoma	1/1
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	1/1
Osteosarcoma	1/1
Spindle cell rhabdomyosarcoma	1/1

Pathology	# Positive / Total cases
Intermediate grade leiomyosarcoma	1/1

a: few cells staining
b: nuclear staining only

Tissue Thickness

Tissue thickness was evaluated using 3 unique human cervical cases (cervical carcinoma, CIN1, and normal cervix). Tissues were sectioned and tested in duplicate at 3, 4, 5, 6, and 7 microns. All tissue thicknesses demonstrated appropriate specific staining and background levels with CINtec Histology.

Within-Day Repeatability and Day-to-Day Precision

Within-Day (repeatability) and Day-to-Day Precision were evaluated in a study of 24 cervical tissue specimens (3 cervical carcinoma, 6 CIN3, 6 CIN2, 6 CIN1, and 3 normal cervix cases). Two replicate slides from each of the cervical specimens were stained with CINtec Histology on a single BenchMark ULTRA instrument on each of 5 non-consecutive days. Appropriate control tissue slides were also stained in each run. Each CINtec Histology slide was paired with an H&E-stained slide from an adjacent section for evaluation. All paired slides were randomized, and then evaluated by a single pathologist blinded to the case diagnosis. CINtec Histology status (positive or negative) was determined based on the CINtec Histology-stained slide, and CIN categories (No CIN, LSIL-histology, HSIL-histology, cancer) were determined based on adjunctive interpretation of the H&E-stained and CINtec Histology-stained slides.

For Within-Day Repeatability, slides for each specimen were compared between duplicates on a single run, with data pooled over the 5 days. For Day-to-Day precision, slides from each specimen were compared across all days, using pooled data of all possible pairings. The estimate of Within-Day and Day-to-Day precision was 100%. Results are shown in Table 6.

Table 6. Within-Day Repeatability and Day-to-Day Precision of the CINtec Histology Assay on Cervical Samples: Number of Slides Agreeing with Modal CINtec Histology Status and Modal CIN Category:

		Modal CINtec Histology Status		
Modal CIN Category		**Positive**	**Negative**	**Total**
No CIN	# of cases	N = 0	N = 3	N = 3
	CINtec Histology Status		29/29 (100.0%)	29/29 (100.0%)
	CIN Category		29/29 (100.0%)	29/29 (100.0%)
LSIL-Histology	# of cases	N = 2	N = 4	N = 6
	CINtec Histology Status	20/20 (100.0%)	40/40 (100.0%)	60/60 (100.0%)
	CIN Category	20/20 (100.0%)	40/40 (100.0%)	60/60 (100.0%)
HSIL-Histology	# of cases	N = 12	N = 0	N = 12
	CINtec Histology Status	120/120 (100.0%)		120/120 (100.0%)
	CIN Category	120/120 (100.0%)		120/120 (100.0%)
Cancer	# of cases	N = 3	N = 0	N = 3
	CINtec Histology Status	30/30 (100.0%)		30/30 (100.0%)
	CIN Category	30/30 (100.0%)		30/30 (100.0%)
Total	# of cases	N = 17	N = 7	N = 24
	CINtec Histology Status	170/170 (100.0%)	69/69 (100.0%)	239/239 (100.0%)
	CIN Category	170/170 (100.0%)	69/69 (100.0%)	239/239 (100.0%)

Note: A single observation with unevaluable CINtec Histology status due to a slide on Day 1 with unacceptable background was excluded.

Instrument to Instrument Precision

Precision of the CINtec Histology test across three BenchMark ULTRA instruments was determined by staining replicate slides of 28 cervical cases (8 normal cervix, 6 CIN1, 6 CIN2, 4 CIN3, and 4 cervical carcinoma cases).

All slides were randomized, and then evaluated by a single pathologist blinded to the case diagnosis for positive or negative CINtec Histology status. Each CINtec Histology slide was then paired with an H&E-stained slide from the same case. After randomization of the paired slides, a single pathologist evaluated the CIN category (No CIN, LSIL-histology, HSIL-histology, cancer).

For Instrument-to-Instrument precision, CINtec Histology status of slides for each specimen was compared between instruments by pairwise comparisons. The estimate of Instrument-to-Instrument precision was 100%, demonstrating that CINtec Histology staining is reproducible across BenchMark ULTRA instruments.

A summary of the results for BenchMark ULTRA Instrument-to-Instrument precision of the CINtec Histology assay is shown in Table 7.

Table 7. BenchMark ULTRA Instrument-to-Instrument Precision of the CINtec Histology Assay on Cervical Samples: Number of Slides Agreeing with Modal CINtec Histology Status and Modal CIN Category

		Modal CINtec Histology Status		
Modal CIN Category		**Positive**	**Negative**	**Total**
No CIN	# of cases	N = 0	N = 8	N = 8
	CINtec Histology Status		72/72 (100.0%)	72/72 (100.0%)
	CIN Category		72/72 (100.0%)	72/72 (100.0%)
LSIL-Histology	# of cases	N = 4	N = 3	N = 7
	CINtec Histology Status	36/36 (100.0%)	27/27 (100.0%)	63/63 (100.0%)

Modal CIN Category		Modal CINtec Histology Status Positive	Negative	Total
	CIN Category	36/36 (100.0%)	27/27 (100.0%)	63/63 (100.0%)
HSIL-Histology	# of cases	N = 9	N = 0	N = 9
	CINtec Histology Status	81/81 (100.0%)		81/81 (100.0%)
	CIN Category	81/81 (100.0%)		81/81 (100.0%)
Cancer	# of cases	N = 4	N = 0	N = 4
	CINtec Histology Status	36/36 (100.0%)		36/36 (100.0%)
	CIN Category	36/36 (100.0%)		36/36 (100.0%)
Total	# of cases	N = 17	N = 11	N = 28
	CINtec Histology Status	153/153 (100.0%)	99/99 (100.0%)	252/252 (100.0%)
	CIN Category	153/153 (100.0%)	99/99 (100.0%)	252/252 (100.0%)

Lot-to-Lot Precision

Lot-to-Lot Precision of CINtec Histology was evaluated by testing 3 lots of the CINtec Histology primary antibody on duplicate slides of 24 cervical punch biopsy tissue specimens (4 normal cervix, 5 CIN1, 7 CIN2, 6 CIN3, and 2 cervical carcinoma cases) on a BenchMark ULTRA instrument using the OptiView DAB IHC Detection kit. Each CINtec Histology slide 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. CINtec Histology status (positive or negative) was determined based on the CINtec Histology slide, and CIN categories (No CIN, LSIL-histology, HSIL-histology, Cancer) were determined based on adjunctive interpretation of the H&E and CINtec Histology slides.

A summary of the results of the Lot-to-Lot precision study is shown in Table 8.

Table 8. Lot-to-Lot Precision of the CINtec Histology Assay on Cervical Samples: Number of Slides Agreeing with Modal CINtec Histology Status and Modal CIN Category

Modal CIN Category		Modal CINtec Histology Status Positive	Negative	Total
No CIN	# of cases	N = 0	N = 6	N = 6
	CINtec Histology Status		36/36 (100.0%)	36/36 (100.0%)
	CIN Category		34/36 (94.4%)	34/36 (94.4%)
LSIL-Histology	# of cases	N = 3	N = 2	N = 5
	CINtec Histology Status	18/18 (100.0%)	12/12 (100.0%)	30/30 (100.0%)
	CIN Category	18/18 (100.0%)	12/12 (100.0%)	30/30 (100.0%)
HSIL-Histology	# of cases	N = 10	N = 0	N = 10
	CINtec Histology Status	60/60 (100.0%)		60/60 (100.0%)
	CIN Category	60/60 (100.0%)		60/60 (100.0%)
Cancer	# of cases	N = 3	N = 0	N = 3
	CINtec Histology Status	18/18 (100.0%)		18/18 (100.0%)
	CIN Category	18/18 (100.0%)		18/18 (100.0%)
Total	# of cases	N = 16	N = 8	N = 24
	CINtec Histology Status	96/96 (100.0%)	48/48 (100.0%)	144/144 (100.0%)
	CIN Category	96/96 (100.0%)	46/48 (95.8%)	142/144 (98.6%)

For Lot-to-Lot precision, CINtec Histology status of slides for each specimen was compared between lots and slide replicates by pairwise comparisons. The estimate of Lot-to-Lot precision was 100%.

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 4 cervical carcinoma cases) stained with CINtec Histology. All slides were randomized, and subsequently evaluated by 3 pathologists for positive/negative CINtec Histology status. Pathologists were blinded to the case diagnosis. The CINtec Histology-stained slides were re-randomized for a second evaluation of the CINtec Histology status by each of the 3 pathologists following a 4 week washout period. Additionally, each CINtec Histology slide was paired with an H&E slide from the same case and the paired slide sets were randomized. CIN category (No CIN, LSIL-histology, HSIL-histology, Cancer) was evaluated by 3 pathologists based on adjunctive interpretation of the H&E + CINtec Histology 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 3 pathologists was performed. A summary of the results of the reader precision study is provided in Table 9.

Table 9. Within-Reader and Reader-to-Reader Precision of the CINtec Histology Assay on Cervical Samples: Number of Observations Agreeing with Modal CINtec Histology Status and Modal CIN Category

Modal CIN Category		Modal CINtec Histology Status Positive	Negative	Total
No CIN	# of cases	N = 0	N = 19	N = 19
	CINtec Histology Status		112/113 (99.1%)	112/113 (99.1%)
	CIN Category		107/113 (94.7%)	107/113 (94.7%)
LSIL-Histology	# of cases	N = 5	N = 5	N = 10
	CINtec Histology Status	29/30 (96.7%)	30/30 (100.0%)	59/60 (98.3%)
	CIN Category	27/30 (90.0%)	18/30 (60.0%)	45/60 (75.0%)
HSIL-Histology	# of cases	N = 17	N = 0	N = 17
	CINtec Histology Status	102/102 (100.0%)		102/102 (100.0%)
	CIN Category	88/102 (86.3%)		88/102 (86.3%)
Cancer	# of cases	N = 4	N = 0	N = 4
	CINtec Histology Status	24/24 (100.0%)		24/24 (100.0%)
	CIN Category	23/24 (95.8%)		23/24 (95.8%)
Total	# of cases	N = 26	N = 24	N = 50
	CINtec Histology Status	155/156 (99.4%)	142/143 (99.3%)	297/299 (99.3%)
	CIN Category	138/156 (88.5%)	125/143 (87.4%)	263/299 (88.0%)

Note: A single observation with unevaluable CINtec Histology status by Reader 2 was excluded.

For Within-Reader precision, CINtec Histology status of 2 slides for each specimen was compared between duplicates from the same reader. The estimate of Within-Reader agreement was 98.7%. For Reader-to-Reader precision, CINtec Histology status of slides from each specimen was compared across three pathologists, using pooled data of all possible pairings. The estimate of Reader-to-Reader agreement was 98.7%.

Reproducibility

A Reproducibility study (Laboratory-to-Laboratory precision study) for CINtec Histology was conducted using 27 cervical cases (10 No CIN, 5 CIN1, 5 CIN2, 5 CIN3, and 2 cervical carcinoma cases) run across 3 BenchMark ULTRA instruments on each of 3 non-consecutive days at 3 external laboratories. The specimens were randomized and evaluated by a total of 6 pathologists (2 pathologists per site) for both CINtec Histology status (positive/negative) and for CIN category (No CIN, LSIL-histology, HSIL-histology, Cancer) based on adjunctive interpretation of the H&E + CINtec Histology slides. Pathologists were blinded to the case diagnosis. A summary of the study results is provided in Table 10.

Table 10. Reproducibility of the CINtec Histology Assay on Cervical Samples: Number of Observations Agreeing with Modal CINtec Histology Status and Modal CIN Category

Modal CIN Category		Modal CINtec Histology Status Positive	Negative	Total
No CIN	# of cases	N = 0	N = 10	N = 10
	CINtec Histology Status		153/155 (98.7%)	153/155 (98.7%)
	CIN Category		134/155 (86.5%)	134/155 (86.5%)
LSIL-Histology	# of cases	N = 2	N = 2	N = 4
	CINtec Histology Status	34/34 (100.0%)	22/32 (68.8%)	56/66 (84.8%)
	CIN Category	28/34 (82.4%)	29/32 (90.6%)	57/66 (86.4%)
HSIL-Histology	# of cases	N = 11	N = 0	N = 11
	CINtec Histology Status	184/186 (98.9%)		184/186 (98.9%)
	CIN Category	176/186 (94.6%)		176/186 (94.6%)
Cancer	# of cases	N = 2	N = 0	N = 2
	CINtec Histology Status	36/36 (100.0%)		36/36 (100.0%)
	CIN Category	31/36 (86.1%)		31/36 (86.1%)
Total	# of cases	N = 15	N = 12	N = 27
	CINtec Histology Status	254/256 (99.2%)	175/187 (93.6%)	429/443 (96.8%)
	CIN Category	235/256 (91.8%)	163/187 (87.2%)	398/443 (89.8%)

Note: 43 observations with unevaluable CINtec Histology status were excluded. Missing data were distributed across all sites and days: 16 from site A, including 2 on day 1, 4 on day 2 and 10 on day 3; 17 from site B, including 3 on day 1, 8 on day 2, and 6 on day 3; and 10 from site C, including 2 on day 1, 5 on day 2, and 3 on day 3.

For Reader-to-Reader precision, CINtec Histology status of 2 slides corresponding to 2 pathologists at each site from each specimen was compared across 3 days and 3 sites and combined for all specimens. The estimates of Reader-to-Reader agreement of CINtec Histology results were 95.5% for positive CINtec Histology results and 92.9% for negative CINtec Histology results.

For Day-to-Day precision, CINtec Histology status of 2 slides corresponding to two different days from each specimen was compared across 3 days and 3 sites using pooled data of all possible pairings. The estimate of Day-to-Day agreement of CINtec Histology results were 98.2% for positive CINtec Histology results and 97.1% for negative CINtec Histology results.

For Site-to-Site precision, CINtec Histology status of 2 slides corresponding to 2 different sites from each specimen was compared across 3 sites using pooled data of all possible pairings. The estimate of Site-to-Site agreement of CINtec Histology results were 96.2% for positive CINtec Histology results and 93.9% for negative CINtec Histology results.

CLINICAL PERFORMANCE

Diagnostic Agreement

To demonstrate that the adjunctive reading of CINtec Histology will result 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 were evaluated in a clinical study.

The clinical study was performed on 1,100 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 hematoxylin & eosin (H&E) stained slides only and using the H&E and CINtec Histology stained slides. Two XPs established their independent diagnoses (No CIN, CIN1, CIN2, CIN3, adenocarcinoma *in situ* (ACIS), or invasive carcinoma) based on the H&E-stained slides for each of the 1,100 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 2 out of 3 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 Histology stained slides to establish their diagnosis (No CIN, LSIL-histology/CIN1, HSIL-histology/CIN2, HSIL-histology/CIN3, ACIS, or invasive carcinoma) (termed XP2, or H&E + CINtec 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 1,100 H&E-stained cases were divided into 4 reading sets of 275 cases with comparable distributions of individual diagnostic categories per Reference Diagnosis. The 70 CPs were assigned to 4 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* (ACIS), or invasive carcinoma). Thus, each study case was individually read by either 17 or 18 community pathologists. In addition, CPs were asked during Round 1 reading whether they would request an adjunctive p16 IHC stain (CINtec Histology) in alignment with the following criteria from the LAST recommendations[1]: 1) the H&E morphologic differential diagnosis is between pre-cancer (CIN2 or CIN3) and a mimic of pre-cancer; 2) the H&E morphologic diagnosis is CIN2; or 3) the H&E morphologic diagnosis is ≤ CIN1 and the biopsy specimen is at high risk for missed high-grade disease, which is defined as prior cytologic interpretation of HSIL, ASC-H (atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion), ASC-US/HPV16+ (atypical squamous cells of undetermined significance/HPV16+), or AGC-(NOS) (atypical glandular cells- not otherwise specified).

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

Community Pathologists Reading Results using H&E versus H&E + CINtec Histology Compared with Expert-derived H&E Reference Diagnosis

Percent of CINtec Histology positive results by CIN Diagnosis

The association between majority/consensus CINtec Histology status (Positive or Negative) by expert panel and the majority/consensus diagnosis by expert panel using H&E only is illustrated in Table 11. The frequency and percent of CINtec Histology positive results were calculated for each CIN diagnosis category. CINtec Histology positive results showed an increasing trend with increasing severity of CIN diagnosis.

Table 11. Frequency and Percent of CINtec Histology positive results by CIN Diagnosis

	Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel with H&E (XP1)				
	No CIN	CIN1	CIN2	CIN3	Cancer
Percent CINtec Histology positive results	7.5% (57/755)	58.3% (95/163)	94.5% (86/91)	98.6% (69/70)	100% (1/1)

Note: 15 Observations with unevaluable CINtec Histology status were excluded; 14 were No CIN and 1 was CIN1 by expert panel using H&E only.

Improvement of Consistency among Challenging Cases

The improvement in consistency between readers was determined by comparing agreement between community pathologists and the expert pathologists' H&E reference diagnosis (i.e, CP1 vs XP1) versus the same comparison using H&E + CINtec Histology (i.e., CP2 vs XP2). Analyses were first conducted for particularly challenging cases, defined as those cases where a majority of community pathologists requested p16. There were 436 cases where a majority of CPs requested p16 per Round 1 questionnaire. For these cases, the agreement between the H&E Reference Diagnosis by expert pathologists vs H&E + CINtec Histology Reference Diagnosis is shown in Table 12.

Table 12. Agreement between H&E Reference Diagnosis and H&E + CINtec Histology Reference Diagnosis for Challenging Cases (i.e., majority of community pathologists requested p16)

		H&E Reference Diagnosis					Total
		No CIN	CIN1	CIN2	CIN3	ACIS or Cancer	
H&E + CINtec Histology Reference Diagnosis	No CIN	175	4	4	0	0	183
	LSIL-histology	15	61	4	1	0	81
	HSIL-histology	24	29	79	37	0	169
	ACIS or cancer	0	0	0	0	3	3
Total		214	94	87	38	3	436

Table 13 shows the percentage of cases where the CP majority diagnosis is the same as the H&E Reference Diagnosis or H&E + CINtec Histology Reference Diagnosis, as well as the average number of community pathologists whose diagnoses are the same as the majority CP diagnosis. The left side of the table compares CP1 to XP1, and the right side of the table compares CP2 to XP2. The bottom half of the table collapses the data into ≤CIN1 vs ≥CIN2 (when H&E is used) or ≤LSIL vs ≥HSIL (when H&E + CINtec Histology is used). For all diagnoses, agreement increases from the use of H&E by community pathologists compared to the H&E Reference Diagnosis vs the use of H&E + CINtec Histology by community pathologists compared to the H&E + CINtec Histology Reference Diagnosis.

Table 13. Community Pathologist Agreement Using H&E with H&E Reference Diagnosis, and Community Pathologist Agreement Using H&E + CINtec Histology with H&E + CINtec Histology Reference Diagnosis for Challenging Cases

	H&E Only				H&E + CINtec Histology			
	Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel – XP1				Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel – XP2			
	No CIN	CIN1	CIN2	≥CIN3	No CIN	LSIL-histology	HSIL-histology	ACIS or Cancer
Number of cases	214	94	87	41	183	81	169	3
Percent of cases with CP majority diagnosis the same as Reference Diagnosis	29.0% (62/214)	73.4% (69/94)	72.4% (63/87)	53.7% (22/41)	39.3% (72/183)	72.8% (59/81)	96.4% (163/169)	66.7% (2/3)
Number of CP with diagnosis the same as CP majority averaged over all cases	10.5	11.8	10.6	10.1	11.3	12.6	14.8	16.5
	≤CIN1		≥CIN2		≤LSIL-histology		≥HSIL-histology	
Number of cases	308		128		264		172	
Percent of cases with CP majority diagnosis the same as Reference Diagnosis	42.5% (131/308)		66.4% (85/128)		49.6% (131/264)		95.9% (165/172)	
Number of CP with diagnosis the same as CP majority averaged over all cases	11.2		10.5		11.9		14.8	

Estimates of PPA were constructed based on the comparison of the agreement between community pathologists using H&E (CP1) vs H&E Reference Diagnosis by expert pathologists (XP1) for cases with reference diagnoses of CIN2 or higher and community pathologists using H&E + CINtec Histology (CP2) vs H&E + CINtec Histology Reference Diagnosis by expert pathologists (XP2) for cases with reference diagnoses of HSIL-histology or higher. Estimates of NPA were constructed based on the comparison of the agreement between community pathologists using H&E (CP1) vs H&E Reference Diagnosis by expert pathologists (XP1) for cases with reference diagnoses of CIN1 or No CIN and community pathologists using H&E + CINtec Histology (CP2) vs H&E + CINtec Histology Reference Diagnosis by expert pathologists (XP2) for cases with reference diagnoses of LSIL-histology or No CIN. The clinical study data demonstrated a statistically significant improvement in consistency of the diagnoses by CPs when using CINtec Histology staining. Improvement in PPA was 29.5% with 95% confidence interval (CI): 21.2% to 37.7% and the improvement in NPA was 7.1% with 95% CI: 1.3% to 13.1%, as summarized in Table 14 below.

Table 14. Positive and Negative Agreements for CP1-XP1 Compared to CP2-XP2 for Challenging Cases

Agreement	H&E + CINtec Histology	H&E Only	Difference	95% CI
PPA	95.9% (165/172)	66.4% (85/128)	29.5%	(21.2%, 37.7%)
NPA	49.6% (131/264)	42.5% (131/308)	7.1%	(1.3%, 13.1%)

Improvement of Consistency among All Cases

The analyses that generated the results seen in Tables 12-14 for the 436 challenging cases were repeated for all 1,100 cases in the study, and the results are reported in Tables 15-17.

Table 15 shows the agreement between the H&E Reference Diagnosis by expert pathologists vs the H&E + CINtec Histology Reference Diagnosis by the same expert pathologists.

Table 15. Agreement between H&E Reference Diagnosis and H&E + CINtec Histology Reference Diagnosis for All Cases

		H&E Reference Diagnosis					Total
		No CIN	CIN1	CIN2	CIN3	ACIS or Cancer	
H&E + CINtec 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

Table 16 shows the percentage of cases where the CP majority diagnosis is the same as the H&E Reference Diagnosis or H&E + CINtec Histology Reference Diagnosis, as well as the average number of community pathologists whose diagnoses are the same as the majority CP diagnosis. The left side of the table compares CP1 to XP1, and the right side of the table compares CP2 to XP2. The bottom half of the table collapses the data into ≤CIN1 vs ≥CIN2 (when H&E is used) or ≤LSIL vs ≥HSIL (when H&E + CINtec Histology is used). For all diagnoses, agreement increases from the use of H&E by community pathologists compared to the H&E Reference Diagnosis vs the use of H&E + CINtec Histology by community pathologists compared to the H&E + CINtec Histology Reference Diagnosis.

Table 16. Community Pathologist Agreement Using H&E with H&E Reference Diagnosis and Community Pathologist Agreement Using H&E + CINtec Histology with H&E + CINtec Histology Reference Diagnosis for All Cases

	H&E Only					H&E + CINtec Histology			
	Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel – XP1					Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel – XP2			
	No CIN	CIN1	CIN2	≥CIN3		No CIN	LSIL-histology	HSIL-histology	ACIS or Cancer
Number of cases	769	164	91	76		710	171	214	5
Percent of cases with CP majority diagnosis the same as Reference Diagnosis	50.8% (391/769)	82.9% (136/164)	69.2% (63/91)	73.7% (56/76)		60.1% (427/710)	81.9% (140/171)	94.4% (202/214)	80.0% (4/5)
Number of CP with diagnosis the same as CP majority averaged over all cases	12.6	13.2	10.6	12.9		12.8	13.6	15.2	16.0
	≤CIN1		≥CIN2			≤LSIL-histology		≥HSIL-histology	
Number of cases	933		167			881		219	
Percent of cases with CP majority diagnosis the same as Reference Diagnosis	56.5% (527/933)		71.3% (119/167)			64.4% (567/881)		94.1% (206/219)	
Number of CP with diagnosis the same as CP majority averaged over all cases	12.7		11.7			13.0		15.2	

Table 17 shows PPA and NPA for CP1-XP1 vs CP2-XP2 for all 1100 cases. As with the challenging cases, statistically significant increases (22.8% for PPA and 7.9% for NPA) are seen.

Table 17. Positive and Negative Agreements for CP1-XP1 Compared to CP2-XP2 for All Cases

Agreement	H&E and CINtec Histology	H&E Only	Difference	95% CI
PPA	94.1% (206/219)	71.3% (119/167)	22.8%	(15.5%, 30.1%)
NPA	64.4% (567/881)	56.5% (527/933)	7.9%	(4.9%, 10.8%)

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

The previous tables assessed increased consistency by comparing CP1-XP1 vs CP2-XP2, Additionally, changes in agreement of community pathologists vs a fixed reference diagnosis were assessed. First, the change from CP1 to CP2 relative to XP1 was analyzed.

Data (results shown in Table 18) were analyzed by determining agreement rates averaged across case and reader and calculating confidence intervals. A statistically significant increase in PPA, the measure for the detection of ≥CIN2 lesions (+6.8% with 95% CI: 4.7% to 9.0%), was observed. Additionally, NPA for the detection of ≤CIN1 increased by 1.3% with 95% CI: 0.5% to 2.3%.

Table 18. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists Reads on H&E-Stained Slides versus H&E-Stained Slides plus CINtec Histology-Stained Slides with Expert-derived H&E Reference Diagnosis (XP1)

Endpoint	H&E	H&E + CINtec Histology	Difference	p-value
PPA % (95% CI)	83.5% (79.9, 86.8)	90.3% (87.5, 92.7)	6.8% (4.7, 9.0)	<.0001
NPA % (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 Histology=91.76%, Difference = 1.32%.

Note that CP1 vs XP1 comparison in Table 18 differs from that is shown in Table 16 because the underlying calculations differ. In Table 16, the analysis occurs at the level of the case, with the CP majority diagnosis being used for comparison to XP1. In Table 18, an observational level analysis is conducted, so that each observation is counted uniquely (N=approximately 19,250 observations in Table 18, vs N=1,100 cases in Table 16). This approach focuses on the individual reader's diagnosis rather than the consensus CP opinion of the case diagnosis. This observational level analysis is maintained in Tables 19-23 as well.

A summary diagram for the diagnostic agreement of the individual community pathologist readers for diagnosing ≥CIN2 versus ≤CIN1 using H&E-stained slides only versus using H&E-stained slides along with CINtec Histology-stained slides compared to the Expert-derived H&E Reference Diagnosis is shown in Figure 2. The PPA and 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 Histology-stained slides – 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. These data demonstrate that the interpretation of cervical biopsies using H&E along with CINtec Histology-stained slides improves the diagnostic agreement in the interpretation of cervical biopsies and it reduces the between reader variability.

VENTANA®

Roche

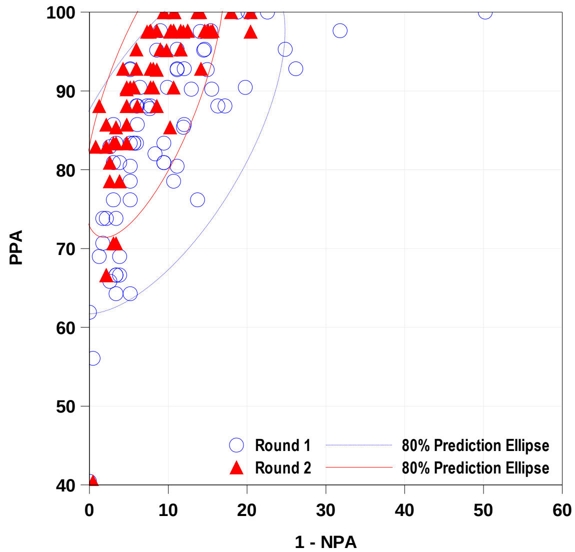

Figure 2. Summary diagram for diagnostic agreement (PPA versus 1-NPA) of community pathologists for diagnosing ≥CIN2 versus ≤CIN1 using H&E only (Round 1) and H&E + CINtec Histology (Round 2) compared with the Expert-derived H&E Reference Diagnosis (XP1) (80% prediction ellipses generated under assumption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

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

Next, the reading results of the community pathologists using both methods (i.e., H&E + CINtec Histology versus H&E only) were compared to an H&E + CINtec Histology Reference Diagnosis where also the expert gynecopathologists used H&E plus CINtec Histology-stained slides to establish the Reference Diagnosis (XP2). 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 as used for establishing the H&E Reference Diagnosis described above.

The community pathologists' reading results using H&E-stained slides only versus H&E-stained slides along with CINtec Histology-stained slides were analyzed and compared against the Expert-derived H&E + CINtec Histology Reference Diagnosis (Table 19). These 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 19. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists for Reads on H&E-Stained Slides versus H&E-Stained slides + CINtec Histology-Stained Slides with Expert-derived H&E + CINtec Histology Reference Diagnosis (XP2)

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

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 Histology-stained slides compared to the Expert-derived H&E + CINtec 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 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. These data demonstrate that the interpretation of cervical biopsies using H&E along with CINtec Histology-stained slides improves the diagnostic consistency in the interpretation of cervical biopsies and it reduces the between reader variability.

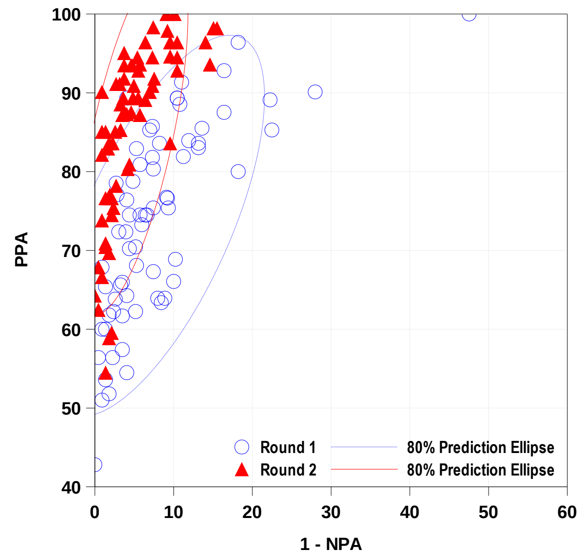

Figure 3. Summary diagram for diagnostic agreement (PPA versus 1-NPA) of community pathologists for diagnosing ≥CIN2 versus ≤CIN1 using H&E only (Round 1) and H&E + CINtec Histology (Round 2) compared with the Expert-derived H&E + CINtec Histology Reference Diagnosis (XP2) (80% prediction ellipses generated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

CINtec Histology Performance When Used According to LAST Recommendations

In 2012, the LAST recommendations resulting from a project co-sponsored by CAP and ASCCP were published in an attempt to standardize diagnostic terminology and to align it with cervical squamous lesion biology.[1] As part of this approach, recommendations for the use of biomarkers were made. The p16 biomarker was the only biomarker recommended at this point in time, and specific criteria were defined indicating when p16 IHC stain staining should be used as an adjunctive aid to the interpretation of H&E-stained slides. The potential impact of the implementation of the LAST recommendations on community pathologists' diagnostic results and their agreement with Expert-derived Reference Diagnoses was evaluated in this study. Pathologist readers were asked during Round 1 reading on H&E-stained slides only whether they would request an adjunctive p16 IHC stain in alignment with the LAST criteria. The following LAST recommendation criteria were taken into account: 1) the H&E morphologic differential diagnosis is between pre-cancer (CIN2 or CIN3) and a mimic of pre-cancer; 2) the H&E morphologic diagnosis is CIN2; 3) the H&E morphologic diagnosis is ≤CIN1 and the biopsy specimen is at high risk for missed high-grade disease, which is defined as prior cytologic interpretation of HSIL (high-grade squamous intraepithelial lesion), ASC-H (atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion), ASC-US/HPV16+ (atypical squamous cells of undetermined significance/HPV16+), or AGC-(NOS) (atypical glandular cells- not otherwise specified). Since each community pathologist participating in the study was interpreting slides independently, an additional criterion per LAST recommending the use of p16 IHC in cases of professional disagreement did not apply in the study setting.

The 70 community pathologists participating as readers in the study requested the p16 stain based on the initial review of H&E stained slides in Round 1 in 42.3% of their readings. Data from the community pathologists' diagnoses on the H&E-stained slide alone (Round 1) were compared to diagnoses established on the H&E-stained slides along with CINtec Histology-stained slides (Round 2) limited to the group of cases for which the respective pathologist requested an adjunctive p16 stain based on LAST recommendations (i.e., LAST cases). Results for the community pathologists' readings of LAST cases without or with CINtec Histology compared to the Expert-derived H&E Reference Diagnosis are shown in Table 20, and those when compared to the H&E + CINtec Histology Expert-derived Reference Diagnosis are shown in Table 21.

The data analysis for the LAST cases (i.e., cases for which the community pathologist readers requested adjunctive p16 staining) revealed that both PPA (+7.6% for H&E Reference Diagnosis; 11.8% for H&E + CINtec Histology Reference Diagnosis) and NPA (+6.0% for H&E Reference Diagnosis; 9.7% for H&E + CINtec Histology Reference Diagnosis) increased in similar and statistically significant ways with the adjunctive use of CINtec Histology (Table 20 and Table 21).

Table 20. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists Reads for Interpretation of LAST cases on H&E versus H&E + CINtec Histology with Expert-derived H&E Reference Diagnosis (XP1)

Endpoint	H&E	H&E + CINtec Histology	Difference	p-value
PPA % (95% CI)	81.4% (78.0, 84.7)	89.0% (86.1, 91.5)	7.6% (5.1, 10.1)	<.0001
NPA % (95% CI)	76.4% (74.5, 78.1)	82.4% (79.9, 84.7)	6.0% (4.1, 8.0)	<.0001

Table 21. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists Reads for Interpretation of LAST cases on H&E versus H&E + CINtec Histology with Expert-derived H&E + CINtec Histology Reference Diagnosis (XP2)

Endpoint	H&E	H&E + CINtec Histology	Difference	p-value
PPA % (95% CI)	73.4% (70.2, 76.6)	85.2% (82.8, 87.3)	11.8% (9.5, 14.0)	<.0001
NPA % (95% CI)	79.6% (77.8, 81.3)	89.3% (87.5, 91.0)	9.7% (7.8, 11.5)	<.0001

A similar positive effect of the adjunctive use of CINtec Histology-stained slides on PPA (+5.2% for H&E Reference Diagnosis; +11.0% for H&E + CINtec Histology Reference Diagnosis) was observed for non-LAST cases, i.e., cases for which the respective community pathologist did not request an adjunctive p16 IHC stain at the cost of slightly lower NPA rates (NPA difference: -1.5% for H&E Reference Diagnosis; -0.8% for H&E + CINtec Histology Reference Diagnosis) (Table 22 and Table 23).

Table 22. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists Reads for Interpretation of non-LAST cases on H&E versus H&E + CINtec Histology with Expert-derived H&E Reference Diagnosis (XP1)

Endpoint	H&E	H&E + CINtec Histology	Difference	p-value
PPA % (95% CI)	87.8% (82.9, 91.8)	92.9% (89.7, 95.6)	5.2% (2.8, 8.0)	<.0001
NPA % (95% CI)	99.0% (98.7, 99.2)	97.5% (97.0, 97.9)	-1.5% (-2.0, -1.1)	<.0001

Table 23. Positive (PPA) and Negative (NPA) Agreement Rates of Community Pathologists Reads for Interpretation of non-LAST cases on H&E versus H&E + CINtec Histology with Expert-derived H&E + CINtec Histology Reference Diagnosis (XP2)

Endpoint	H&E	H&E + CINtec Histology	Difference	p-value
PPA % (95% CI)	73.1% (66.8, 79.1)	84.1% (79.3, 88.1)	11.0% (7.8, 14.1)	<.0001
NPA % (95% CI)	99.2% (99.0, 99.5)	98.5% (98.1, 98.8)	-0.8% (-1.1, -0.5)	<.0001

These findings show that the community pathologists achieved statistically and clinically significant gains in both PPA and NPA for the detection of ≥CIN2 in cases for which they requested an adjunctive p16 stain based on the morphologic interpretation of the H&E-stained tissue per LAST criteria (i.e., cases with a differential diagnosis between high-grade CIN and a morphologic mimic, cases for which a CIN2 diagnosis is considered, and cases categorized as ≤CIN1 with a higher risk of missed disease based on other risk factors, such as a preceding cytologic HSIL diagnosis). Statistically and clinically significant gains in PPA for the detection of ≥CIN2 were also observed in cases that the community pathologists did not identify as cases requiring adjunctive p16 staining. This substantially higher PPA provided by the adjunctive use of CINtec Histology also in non-LAST cases was associated with a small, but statistically significant decrease in NPA in these non-LAST cases (-1.5% for H&E Reference Diagnosis; -0.8% for H&E + CINtec Histology Reference Diagnosis).

CINtec Histology Staining Performance

The secondary objective of this study was to assess the staining performance of the CINtec Histology assay as determined by the community pathologists during review of the study slides. A total of 19,250 CINtec 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 24).

Table 24. CINtec Histology Staining Performance

Endpoint	Number of Interpretations n/N	Rate
Staining Acceptability	19,074 / 19,250	99.09%
Morphology Acceptability	19,249 / 19,250	99.99%
Background Acceptability	19,249 / 19,250	99.99%

Conclusions

The use of CINtec 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, a clinically and statistically significant increase in PPA for the detection of ≥CIN2 is observed in both LAST cases (i.e., cases for which the pathologists requested adjunctive p16 staining per LAST recommendations) and non-LAST cases. There is also a significant increase of NPA in LAST cases, and a small, but statistically significant decrease of NPA in non-LAST cases. Furthermore, the consistency of diagnoses between community pathologists with each other and with an expert panel improves.

REFERENCES

1. Darragh TM, Colgan TJ, Cox JT, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012;16:205-242. Erratum in J Low Genit Tract Dis. 2013;17:368.

2. Sano T, Oyama T, Kashiwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol. 1998;153:1741-1748.
3. Negri G, Egarter-Vigl E, Kasal A, et al. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immuncytochemical correlations. Am J Surg Pathol. 2003;27:187-193.
4. von Knebel Doeberitz M, Vinokurova S. Host factors in HPV-related carcinogenesis: cellular mechanisms controlling HPV infections. Arch Med Res. 2009;40(6):435-442.
5. Voorhoeve PM, Agami R. The tumor-suppressive functions of the human INK4A locus. Cancer Cell. 2003;4:311-319.
6. Klaes R, Friedrich T, Spitkovsky D, et al. Overexpression of p16(INK4a) as a specific marker for dysplasia and neoplastic epithelial cells of the cervix uteri. Int J Cancer. 2001;92:276-284.
7. Klaes R, Benner A, Friedrich T, et al. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002;26:1389-1399.
8. Negri G, Vittadello F, Romano F, et al. p16INK4a expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. Virchows Arch. 2004;445:616-620.
9. Wentzensen N, von Knebel Doeberitz M. Biomarkers in cervical cancer screening. Dis Markers. 2007;23(4):315-330.
10. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol Biomarkers Prev. 2008;17:2536-2545.
11. Tsoumpou I, Arbyn M, Kyrgiou M, et al. p16INK4a immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009;35:210-220.
12. Dijkstra MG, Heideman DA, de Roy SC, et al. p16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. J Clin Pathol. 2010;63(11):972-977.
13. Bergeron C, Ordi J, Schmidt D, et al. European CINtec Histology Study Group. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. Am J Clin Pathol. 2010;133:395-406.
14. Galgano MT, Castle PE, Atkins KA, et al. Using biomarkers as objective standards in the diagnosis of cervical biopsies. Am J Surg Pathol. 2010;34:1077-1087.
15. Massad LS, Einstein M, Huh W, et al. 2012 Updated Consensus Guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1-S27.
16. Arbyn M, Kyrgiou M, Simoens C, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: metaanalysis. BMJ. 2008;337:a1284.
17. Albrechtsen S, Rasmussen S, Thoresen S, et al. Pregnancy outcome in women before and after cervical conisation: population based cohort study. BMJ. 2008;337:a1343.
18. Sadler L, Saftlas A, Wang W, et al. Treatment for cervical intraepithelial neoplasia and risk of preterm delivery. JAMA. 2004;291:2100-2106.
19. Park KJ, Soslow RA. Current concepts in cervical pathology. Arch Pathol Lab Med. 2009;133(5):729-738.
20. Stoler MH, Schiffman M. Atypical squamous cells of undetermined significance-low-grade squamous intraepithelial lesion triage study (ALTS) group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. JAMA. 2001;285:1500-1505.
21. Stoler MH, Ronnett BM, Joste NE, et al. New Mexico HPV Pap registry steering committee. The interpretive variability of cervical biopsies and its relationship to HPV status. Am J Surg Pathol. 2015;39(6):729-736.
22. Reuschenbach M, Wentzensen N, Dijkstra MG, et al. p16INK4a immunohistochemistry in cervical biopsy specimens: A systematic review and meta-analysis of the interobserver agreement. Am J Clin Pathol. 2014;142(6):767-772.
23. De Vet HCW, Knipschild PG, Schouten HJA, et al. Interobserver variation in histopathological grading of cervical dysplasia. J Clin Epidemiol. 1990;43:1395-1398.
24. Creagh T, Bridger JE, Kupek E, et al., Pathologist variation in reporting cervical borderline epithelial abnormalities and cervical intraepithelial neoplasia. J Clin Pathol, 1995. 48(1): p. 59-60.
25. Ceballos KM, Chapman W, Daya D, et al., Reproducibility of the histological diagnosis of cervical dysplasia among pathologists from 4 continents. Int J Gynecol Pathol, 2008. 27(1): p. 101-107.
26. Gurrola-Díaz CM, Suárez-Rincón AE, Vázquez-Camacho G, et al. p16INK4a immunohistochemistry improves the reproducibility of the histological diagnosis of cervical intraepithelial neoplasia in cone biopsies. Gynecol Oncol. 2008;111:120-124.
27. Horn LC, Reichert A, Oster A, Arndal SF, et al. Immunostaining for p16INK4a used as a conjunctive tool improves interobserver agreement of the histologic diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2008;32:502-512.
28. Sayed K, Korourian S, Ellison DA, et al. Diagnosing cervical biopsies in adolescents: the use of p16 immunohistochemistry to improve reliability and reproducibility. J Low Genit Tract Dis. 2007;11:141-146.
29. Stoler, M, Bergeron, C, Colgan, TJ, et al. Tumours of the uterine cervix. In Kurman, RJ, Carcangiu, ML, Herrington, CS, et al. (Eds.), WHO classification of tumours of female reproductive organs. Lyon, France: IARC and WHO, 2014;169-206.
30. Clark JL, Lu D, Kalir T, et al.. Overdiagnosis of HSIL on cervical biopsy: errors in p16 immunohistochemistry implementation. Human Pathol. 2016;55:51–56.
31. Mills A, Paquette C, Castle P, et al. Risk stratification by p16 immunostaining of CIN1 biopsies. A retrospective study of patients from the quadrivalent HPV vaccine trials. Am J Surg Pathol. 2015;39:611-617.
32. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology, 2nd edition. St. Louis: C.V. Mosby Company; 1980.
33. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
34. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
35. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2011
36. CLSI. Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays: Approved Guideline-Second Edition. CLSI document I/LA28-A2 (ISBN 1-56238-745-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2011.
37. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
38. Omata M, et al. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol. 1980;73(5):626-632.
39. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med. 1983;14:767.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

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

www.ventana.com