510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K212176

B Applicant

Ventana Medical Systems, Inc.

C Proprietary and Established Names

CINtec Histology

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PRB	Class II	21 CFR 864.1865 - Cervical Intraepithelial Neoplasia (CIN) Test System	PA - Pathology

II Submission/Device Overview:

A Purpose for Submission:

Change in the CINtec® Histology device from the hybridoma anti-p16 (E6H4) antibody to recombinant anti-p16 (E6H4) antibody.

B Measurand:

$p16^{INK4a}$ protein

C Type of Test:

Immunohistochemistry, qualitative

III Intended Use/Indications for Use:

A Intended Use(s):

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.

B Indication(s) for Use:

Same as above

C Special Conditions for Use Statement(s):

For *in vitro* diagnostic (IVD) use only
Rx - For Prescription Use Only

D Special Instrument Requirements:

VENTANA BenchMark ULTRA instrument

IV Device/System Characteristics:

A Device Description:

CINtec® Histology is an immunohistochemical (IHC) assay system comprised of an anti-p16 primary antibody optimized for use with the BenchMark ULTRA automated slide staining instrument and the OptiView DAB IHC Detection Kit. The antibody is diluted in a Tris-HCl buffer containing carrier protein and 0.1% ProClin 300 as a preservative, and provided as a ready-to-use liquid in a FloLock dispenser. CINtec Histology is available in a 50 test size and a 250 test size.

The OptiView DAB IHC Detection Kit (OptiView) is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit IgG primary antibodies and is comprised of 6 dispensers packaged together in one box. The components of the OptiView DAB IHC Detection Kit are provided in Table 1.

Table 1: OptiView DAB IHC Detection Kit Components

Component	Content
OptiView Peroxidase Inhibitor	3.0% hydrogen peroxide solution
OptiView HQ Universal Linker	Cocktail of HQ-labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit IgG) (<50 µg/mL) in a buffer containing protein with ProClin 300, a preservative
OptiView HRP Multimer	Mouse monoclonal anti-HQ HRP labeled tertiary antibody (<40 µg/mL) in a buffer containing protein with ProClin 300
OptiView DAB	0.2% 3, 3'-diaminobenzidine tetrahydrochloride (DAB) in a stabilizer solution in preservative
OptiView H2O2	0.04% hydrogen peroxide in a phosphate buffer solution
OptiView Copper	Copper sulfate (5.0 g/L) in an acetate buffer in preservative

The ancillary reagents required to perform the CINtec Histology assay are provided in Table 2.

Table 2: Ancillary Reagents

Reagent	Format Provided	Contents	Purpose
EZ Prep	Bulk / 10X Concentrate / 2 liter	Detergent	Removes paraffin from the tissue specimen
Reaction Buffer	Bulk / 10X Concentrate / 2 liter	Tris based buffer solution with detergent and preservative	Provides stable environment for antibody-antigen interactions and enzyme reactions. Also used as a rinse solution to remove reagents between assay steps
ULTRA High Temperature Liquid Coverslip (LCS)	Bulk / 2 liter	Low density paraffinic hydrocarbon and other oils	Functions as a barrier between aqueous solutions and air (i.e., prevents evaporation of reagents during incubation periods on the slide)
ULTRA Cell Conditioning 1 Solution (CC1)	Bulk / Prediluted / 1 liter	Tris based buffer solution with detergent and preservative	Disrupts covalent bonds at high temperatures formed by formalin in tissue Increases antibody accessibility.

Reagent	Format Provided	Contents	Purpose
Hematoxylin II Counterstain	Dispenser / Prediluted / 25 mL	Hematoxylin (≤60%); contains glycol and acetic acid stabilizing solution	A modified Mayer's hematoxylin used for staining cellular nuclei.
Bluing Reagent	Dispenser / Prediluted / 25 mL	Solution of 0.1 M lithium carbonate in 0.5 M sodium carbonate	Applied after hematoxylin and changes the hue of the hematoxylin to a blue color.

Controls:

Positive and negative tissue controls that are fixed and processed in the same manner as the test specimens should be used when performing this test. Positive and negative control tissue is used to confirm that the assay performed as expected. For optimal quality control, cervical carcinoma or CIN2/3 cervical tissue positive for CINtec Histology staining is suitable for use as a positive tissue control, and normal cervical tissue is suitable for use as a negative tissue control. Normal human tonsil tissue is also suitable for use as a tissue control, as tonsil contains both positive and negative staining elements when stained 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.

A negative reagent control mouse monoclonal antibody shall be used to evaluate nonspecific staining. This negative reagent control should be used to stain an adjacent section of the patient specimen tissue on a separate slide from the CINtec Histology slide. The staining protocol for the negative reagent control antibody should be the same as that for the primary antibody.

B Principle of Operation:

CINtec Histology is an immunohistochemistry device used to stain FFPE cervical punch biopsy tissue slides for the visualization of the $p16^{INK4a}$ protein. The test process involves the sequential application of antibodies and a chromogen, with interposed washing steps. The assay steps are as follows: 1) the anti-p16 antibody specifically binds to an epitope in the $p16^{INK4a}$ protein; 2) a HQ-labeled secondary antibody binds to the primary antibody (HQ is a proprietary hapten covalently linked to the secondary antibody); 3) a tertiary horseradish peroxidase (HRP)-labeled antibody directed against HQ binds to the HQ-labeled secondary antibody; and 4) the resulting complex is visualized with hydrogen peroxide and DAB, due to the formation of a visible brown precipitate at the antigen site. The specimen slide is then counterstained with hematoxylin and cover slipped. Results are interpreted using a light microscope by a pathologist.

Table 3: CINtec Histology, Staining Protocol

Staining Procedure: CINtec Histology	
Procedure Type	**Parameter Input**
Deparaffinization	Selected Not modifiable
Cell Conditioning (Antigen Unmasking)	ULTRA CC1, 48 minutes
Pre-Primary Peroxidase Inhibitor	Selected Not modifiable
Antibody (Primary)	CINtec Histology OR negative reagent control
Antibody (Primary) Incubation	12 minutes, 36°C
OptiView HQ Linker	8 minutes Not modifiable
OptiView HRP Multimer	8 minutes Not modifiable
Counterstain	Hematoxylin II, 4 minutes Not modifiable
Post Counterstain	Bluing, 4 minutes Not modifiable

Interpretation of Results

CINtec Histology is a qualitative test. The results are interpreted as either positive or negative based on the p16 staining pattern in the FFPE cervical tissue section.

Positive Result: A positive result is defined as "diffuse" staining which is continuous staining of cells in the basal and parabasal cell layers of the cervical squamous epithelium, with or without staining of the intermediate or intermediate to superficial cell layers. Diffuse staining of any intensity is considered to be positive for CINtec Histology status. In some specimens, nuclear expression may be faint or undetectable, but nuclear p16 staining is not required to interpret the CINtec Histology status. Cellular p16 staining for CINtec Hisology may be nuclear and/or cytoplasmic.

Negative Result: A negative result is defined as either focal staining of the cervical epithelium or absence of the p16 staining in the cervical epithelium. Focal staining is defined as staining of isolated cells or small cell clusters, i.e., a non-continuous staining pattern, and particularly not in the basal and parabasal cells.

C Instrument Description Information:

1. Instrument Name:

   VENTANA BenchMark ULTRA instrument which fully automates the processes of baking, deparaffinization, and staining. The BenchMark ULTRA instrument is composed of four main modular components that work together as a system: 1) a stainer subassembly where all slide processing operations are performed containing a reagent carousel, dispenser mechanism, barcode readers, heating elements and other components; 2) an automated fluidics subassembly that provides the compressed air and bulk fluids required by the stainer subassembly; 3) a waste bottle subassembly that collects the waste generated by the system during staining operations; and, 4) a personal computer (PC) running on a Microsoft Windows platform that controls and monitors the system through the Ventana System Software (VSS) host operating software.

2. Specimen Identification:

   Specimen identification is performed through the VSS host operating software. Barcodes reflecting the specific protocols for the CINtec Histology assay are printed by the user and affixed to the appropriate patient specimen slides. Prior to starting the assay run, the barcodes on the slides and dispensers are scanned and qualified by the host software to ensure all elements are in place to complete the staining run. The specific automated staining procedure is performed for each slide, based on the protocol associated to the key code from the slide barcode.

3. Specimen Sampling and Handling:

   Cervical punch biopsy specimens are collected by the clinician and placed in 10% neutral buffered formalin. Tissue sections are prepared from formalin-fixed, paraffin-embedded (FFPE) cervical punch biopsy specimens and mounted on glass slides. Slides are placed on the VENTANA BenchMark ULTRA instrument for staining with the CINtec Histology device.

4. Calibration:

   Not applicable. See section titled Controls above.

5. Quality Control:

   See Controls in section IV.A above.

V Substantial Equivalence Information:

A Predicate Device Name(s):

CINtec® Histology

B Predicate 510(k) Number(s):

DEN160019

Device Name	CINtec® Histology
Regulation Number	21 CFR 864.1865
Regulation Name	A cervical intraepithelial neoplasia (CIN) test system
Regulatory Classification	Class II
Product Code	PRB
DEN#	DEN160019

C Table 4: Comparison with Predicate(s):

Device & Predicate Device(s):	**K212176**	**DEN160019**
Device Trade Name	CINtec® Histology	CINtec® Histology
General Device Characteristic Similarities		
Intended Use/ Indications For Use	Same	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.
Antibody	Same	anti-p16
Clone	Same	E6H4
Differences		
Method of antibody production	Recombinant technology	Hybridoma technology

Cell line	Chinese hamster ovary cells (CHO)	Murine hybridoma cell line (clone E6H4)

VI Standards/Guidance Documents Referenced:

The subject and predicate device (CINtec Histology) have special controls in the device-specific classification regulation 21 CFR 864.1865. The special controls as outlined in the predicate device De Novo Decision Summary (DEN160019) are unchanged except the results of the performance testing that continues to meet current acceptance criteria. For a detailed description of the performance testing, refer to Sections 13: Labeling and Section 18: Performance Testing of this Traditional 510(k) Notification.

VII Performance Characteristics (if/when applicable):

A summary of performance testing is provided below. The subject recombinant CINtec Histology performs equivalently to the predicate hybridoma CINtec Histology and both meet current acceptance criteria.

A Analytical Performance:

1. Precision/Reproducibility:
   i. Between-Lot precision

Three production lots of the recombinant CINtec® Histology formulated with three independent recombinant anti-p16 antibody raw material lots were used in this study to stain each of the 26 cervical tissue cases (6 No CIN, 6 CIN1, 6 CIN2, 6 CIN3, and 2 cervical squamous cell carcinomas (SCC). These cases were qualified based on H&E staining. Eighteen sections were cut from each tissue block. For each lot, duplicate slides from each case were stained with H&E, CINtec® Histology and a Negative Control (Monoclonal). The H&E stained slides were adjacent to the CINtec® Histology slides. All CINtec® Histology and Negative Control (Monoclonal) stained slides were run on the same BenchMark ULTRA instrument. Appropriate control tissue slides were also stained in each run.

Each CINtec® Histology stained slide was than paired with its adjacent H&E and Negative Control (Monoclonal) slides. The order of the 26 slide sets was randomized. The sections stained with recombinant CINtec® Histology were evaluated by a study pathologist for p16 staining intensities (0-4), p16 staining pattern (diffuse, focal, negative) and background (acceptable, unacceptable) and assigned a CINtec® Histology status (positive, negative) according to scoring criteria described in the section titled “Interpretation of Results” above. Slide sets were also assessed for histological diagnosis (No CIN, CIN1, CIN2, CIN3 or invasive carcinoma) based on conjunctive evaluation of the H&E and recombinant CINtec® Histology stained slides and assigned to CIN category (CIN2+ vs. CIN1-). Agreement across lots based on CINtec® Histology status (positive/negative) and by CIN status categories (CIN1-/CIN2+) were evaluated.

Acceptance Criteria: Stain intensity shall not vary more than 0.5 point from the median score on a 0-4 scale of each sample on greater than or equal to 85% between lots; all samples shall show equal to or higher than 90% positive/negative agreement for CINtec® Histology status; the

antibody shall demonstrate background/cross-reactivity less than or equal to 0.5 points on a 0-4 scale in 90% or greater of the tissue samples stained.

Results of the study are presented in Tables 5 and 6 below.

Table 5: Between-Lot Precision for CINtec® Histology

		Modal CINtec Histology Status		
Modal CIN Category		**Positive**	**Negative**	**Total**
No CIN	# of cases	N = 0	N = 8	N = 8
	CINtec Histology Status		48/48 (100.0%)	48/48 (100.0%)
	CIN Category		48/48 (100.0%)	48/48 (100.0%)
LSIL-Histology	# of cases	N = 1	N = 3	N = 4
	CINtec Histology Status	6/6 (100.0%)	18/18 (100.0%)	24/24 (100.0%)
	CIN Category	6/6 (100.0%)	18/18 (100.0%)	24/24 (100.0%)
HSIL-Histology	# of cases	N = 12	N = 0	N = 12
	CINtec Histology Status	71/71 (100.0%)		71/71 (100.0%)
	CIN Category	69/71 (97.2%)		69/71 (97.2%)
Cancer	# of cases	N = 2	N = 0	N = 2
	CINtec Histology Status	12/12 (100.0%)		12/12 (100.0%)
	CIN Category	12/12 (100.0%)		12/12 (100.0%)
Total	# of cases	N = 15	N = 11	N = 26
	CINtec Histology Status	89/89 (100.0%)	66/66 (100.0%)	155/155 (100.0%)
	CIN Category	87/89 (97.8%)	66/66 (100.0%)	153/155 (98.7%)

Note: A single observation with unevaluable CINtec Histology status for Lot 3 was excluded from analysis.

Table 6. Comparisons of CIN categories across Three Lots

			CIN category-Comparison 2		
Lot Pair	**Replicate Pair**	**CIN category-Comparison 1**	**CIN2+**	**CIN1-**	**Total**
All lot pairs	All replicate pairs	CIN 2+	157	5	162
		CIN 1-	1	145	146
		Total	158	150	308
		Overall Percent Agreement: 98.1%			
		Average Positive Agreement: 98.1%			
		Average Negative Agreement: 98.0%			

ii. Immunoreactivity

This study evaluated the immunoreactivity of recombinant CINtec Histology in various normal [Tour of Body (TOB)] and malignant tissues [Tour of Tumor (TOT)]. Additionally, the study evaluated if the positive and negative staining of these same tissue types with recombinant CINtec Histology was consistent with the staining of hybridoma CINtec Histology. Background/cross-reactivity and morphology was also evaluated. The sections from TOB and TOT tissue microarrays and 20 additional individual cases were evaluated in this study. One section from each array or case was stained with recombinant CINtec Histology and hybridoma CINtec Histology and Negative Control (Monoclonal) on BenchMark ULTRA using OptiView DAB IHC detection kit. Furthermore, sections from one prequalified tonsil case were stained with hybridoma CINtec Histology and Negative Control (Monoclonal) were used as a run control. The stained slides were evaluated by pathologist for staining intensities (0-4), background and morphology (acceptable/ unacceptable).

Acceptance Criteria: Background/cross-reactivity less than or equal to 0.5 points on a 0-4 scale in 90% or greater of the tissue samples stained. The recommended staining protocol shall preserve tissue morphology as noted by the qualified reader in a minimum of 90% of interpretable samples stained.

Table 7: CINtec Histology staining in FFPE normal tissues (TOB)

Tissue types	Recombinant CINtec Histology (# of positive cases/total cases)	Hybridoma CINtec Histology (# of positive cases/total cases)	CINtec Histology (type of positive cells in at least 1 of 3 cases)
Adrenal gland	3/3	3/3	Adrenocortical epithelial cells
Appendix	0/3	0/3	NA
Bladder	3/3	3/3	Urothelial cells
Bone marrow	2/3	2/3	NA
Breast	3/3	3/3	Myoepithelial cells, luminal epithelial cells, stromal cells
Cerebellum	3/3	3/3	Purkinje cells
Cerebrum	4/4	4/4	Glial cells
Cervix	3/3	3/3	Squamous epithelial cells, endocervical cells, stromal cells

Colon	3/3	3/3	Epithelial cells (few)
Esophagus	3/3	3/3	Squamous epithelial cells (few)
Heart	0/3	0/3	NA
Kidney	3/3	3/3	Tubular epithelial cells, glomeruli mesangial cells (few)
Liver	0/3	1/5	Hepatocytes
Lung	3/3	3/3	Pneumocytes, bronchial epithelial cells
Lymph node	3/3	3/3	Lymphocytes, follicular dendritic cells
Mesothelium	0/3	0/3	NA
Ovary	3/3	3/3	Stromal cells (few)
Pancreas	3/3	3/3	Islets of Langerhans, acinar cells
Parathyroid	2/3	2/3	Chief cells
Peripheral nerve	4/4	3/3	Schwann cells
Pharynx/Oral cavity	2/3	2/3	Respiratory epithelial cells, striated duct epithelial cells, mucous acinar cells
Pituitary	3/3	3/3	Anterior pituitary epithelial cells
Prostate	3/3	3/3	Acinar cells, basal cells
Salivary gland	3/3	3/3	Striated duct epithelial cells, serous acinar cells
Skeletal muscle	0/3	0/3	NA
Small intestine	3/3	3/3	Epithelial cells (few)
Soft tissue	3/3	3/3	Endothelial cells, fibroblasts, ductal cells
Spleen	3/3	3/3	Lymphocytes, follicular dendritic cells
Stomach	3/3	3/3	Epithelial cells, fundic glands
Testis	3/3	3/3	Spermatogenic cells, Leydig cells
Thymus	3/3	3/3	Epithelial reticular cells, lymphocytes, Hassall's corpuscles
Thyroid	3/3	3/3	Follicular cells, parafollicular cells (few)
Tonsil	5/5	5/5	Squamous epithelial cells, lymphocytes, follicular dendritic cells
Uterus	3/3	3/3	Endometrial glandular cells, stromal cells
Skin	0/3	0/3	NA

Table 8: CINtec Histology staining in a variety of FFPE neoplastic tissues (TOT)

Organ/Anatomic Site	Tumor Type	Recombinant CINtec Histology (# of positive cases/total cases)	Hybridoma CINtec Histology (# of positive cases/total cases)
	Meningioma	1/1	1/1
Cervix	Adenocarcinoma (Endocervical)	1/1	1/1
	Squamous Cell Carcinoma	1/1	1/1
Colon	Adenocarcinoma	1/1	1/1
	Adenosquamous carcinoma	1/1	1/1
Esophagus	Adenocarcinoma	1/1	1/1
	Squamous Cell Carcinoma	0/1	0/1
Head and Neck	Adenocarcinoma	1/1	1/1
	Squamous Cell Carcinoma	0/1	0/1
Kidney	Papillary Renal Adenoma	1/1	1/1
	Renal Cell Carcinoma	1/2	1/1
Liver	Cholangiocarcinoma	0/1	0/1
	Hepatocellular Carcinoma	1/1	1/1
Lymph Node	Anaplastic Large Cell Lymphoma	1/1	1/1
	Follicular Lymphoma	1/1	1/1
	Hodgkin's Lymphoma	1/1	1/1
Lung	NSCLC-Adenocarcinoma	1/1	1/1
	NSCLC-Squamous Cell Carcinoma	0/1	0/1
	Small Cell Lung Carcinoma	1/1	1/1
Mesothelium	Mesothelioma	1/1	1/1
	Pleural Solitary Fibrous Tumor	1/1	1/1
Muscle	Alveolar Rhabdomyosarcoma	0/1	0/1
	Myxoma	1/1	1/1
Ovary	Granulosa Cell Tumor	1/1	1/1
	Serous Carcinoma	1/1	1/1
	Teratoma	1/1	1/1
Pancreas	Islet Cell Tumor	1/1	1/1
	Pancreatic Ductal Adenocarcinoma	1/1	1/1
Peripheral Nerve	Neurofibrosarcoma	1/1	1/1
	Schwannoma	1/1	1/1
Prostate	Adenocarcinoma	2/2	2/2
	Pleomorphic Adenoma	1/1	1/1

Salivary Gland	Warthin's Tumor	1/1	1/1
Small Intestine	Adenocarcinoma	0/1	0/1
	Gastrointestinal Stromal Tumor (GIST)	1/1	1/1
Soft Tissue	Angiosarcoma	1/1	1/1
	Liposarcoma	1/1	1/1
Spleen	Diffuse Large B-Cell Lymphoma	0/1	0/1
Stomach	Adenocarcinoma	1/1	1/1
	Gastrointestinal Stromal Tumor (GIST)	1/1	1/1
Testis	Embryonal Carcinoma	1/1	1/1
	Seminoma	1/1	1/1
Thyroid	Follicular Thyroid Cancer	1/1	1/1
	Papillary Thyroid Cancer	0/1	0/1
Uterus	Clear Cell Carcinoma	1/1	1/1
	Endometrioid or Mucinous carcinoma	1/1	1/1
	Leiomyoma	0/1	0/1
	Leiomyosarcoma	1/1	1/1
Skin	Basal Cell Carcinoma	1/1	1/1
	Invasive Melanoma	1/1	1/1
	Squamous Cell Carcinoma	0/1	0/1

The recombinant CINtec Histology and hybridoma CINtec Histology performed equivalently when used to stain a variety of normal and neoplastic tissue types. The tissues stained with hybridoma CINtec Histology exhibited same staining pattern as tissues stained with recombinant CINtec Histology.

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

a. Western Blot

The primary objective of this study was to confirm the specificity of the recombinant anti-$p16^{INK4a}$ antibody to bind $p16^{INK4a}$ protein. In addition, the results from the Western blot assay using recombinant antibody were compared with the results using hybridoma Millipore anti-$p16^{INK4a}$ (E6H4) antibody. The study also addressed the sensitivity of the recombinant anti-$p16^{INK4a}$ antibody by evaluating the lysates from cell lines expressing various amounts of $p16^{INK4a}$ protein.

One lot of recombinant anti-p16 antibody and one lot of hybridoma anti-p16 antibody were evaluated. Lysates from four cell lines with various expression levels of $p16^{INK4a}$ protein and purified recombinant $p16^{INK4a}$ protein (positive control) and recombinant GADPH protein (negative control) were used in this study.
Acceptance Criteria: Single band between 15-20 kDa (~16 kDa) must be detected on the Western blot membrane for those lanes loaded with recombinant $p16^{INK4a}$ protein or with lysates from $p16^{INK4a}$ -expressing cell lines and consequently probed with recombinant anti- $p16^{INK4a}$ antibody or hybridoma anti- $p16^{INK4a}$ antibody.

Both hybridoma and recombinant anti- $p16^{INK4a}$ (E6H4) antibody lots performed equivalently in the Western Blot assay demonstrating same sensitivity and specificity for $p16^{INK4a}$ recombinant and endogenous protein, with no significant unexpected off target reactivity observed.

b. Peptide Inhibition

The objective of this study is to confirm the specificity of CINtec® Histology formulated with the recombinant anti-p16 antibody for p16 protein in the context of the IHC assay.

One lot of the recombinant CINtec® Histology was used on a BenchMark ULTRA instrument for this study. The anti-$p16^{INK4a}$ (E6H4) antibody was pre-incubated at 1:1 dilution with three different concentrations ($3x10^{-6}$ M, $3x10^{-7}$ M, and $3x10^{-8}$ M) of p16 INK4a epitope-specific peptides (AGGTRGSNHARIDAAEGPSDIDP, MW=2265 g/mol). The non-specific peptide used in this study is These three concentrations were selected to span a range of molar ratios: approximately a 1000-fold, 100-fold, and 10-fold molar excess of peptide compared to the final concentration of anti-p16 antibody in the solution ($3.3x10^{-9}$ M). The primary antibody was also diluted 1:1 with three different concentrations ($3x10^{-6}$ M, $3x10^{-7}$ M, and $3x10^{-8}$ M) of a non-specific peptide control [Alk-a (amino acid sequence: CWQHQPEDRPNFAIILERIEY), MW=2658 g/mol].

CINtec® Histology/no peptide control solution CINtec® Histology reagent was diluted at a 1:1 ratio with the diluent only. All these solutions contained only 50% of the CINtec® Histology reagent. A multi-tissue block (MTB) containing one case of each CIN3, CIN1, and normal cervical epithelium was used in this study.

Duplicate MTB slides were stained with these prepared solutions and also with undiluted CINtec® Histology reagent. In addition, a single slide was stained with the negative reagent control [Negative Control (Monoclonal)]. All stained slides were scored for p16 staining intensity on a 0-4 scale for each tissue. **Note:** The p16 staining intensities determined for the cervical tissues stained with CINtec® Histology/no peptide control solution were used as a reference, since this solution contains the same amount of anti-p16 antibody as the solutions containing p16- specific or non-specific peptides (50% only).

Acceptance Criteria: Decreased staining in the tissues stained with recombinant CINtec Histology reagent containing p16 epitope-specific peptide when compared to the tissues stained with recombinant CINtec Histology reagent containing diluent or non-specific peptide.

This study confirmed the specificity of recombinant anti-p16 (E6H4) antibody in the context of the IHC assay. A nearly complete inhibition of the anti-p16 antibody binding to its target protein

in tissues was detected when the p16 specific peptide was present in the recombinant CINtec® Histology reagent and partial restoration of specific staining was observed when the p16 specific peptide concentration in recombinant CINtec® Histology reagent was reduced. In contrast, no reduction in the signal intensities was observed when unrelated non-specific peptide was present in recombinant CINtec® Histology reagent. No staining was detected when the tissues were stained with Negative Control (Monoclonal) or diluent only.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Not applicable.

6. Detection Limit:

   Not applicable.

7. Assay Cut-Off:

   See Interpretation of Results in section IV.B above.

8. Accuracy (Instrument):

   Please see comparison study below.

9. Carry-Over:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed to compare the performance of the CINtec Histology formulated with the recombinant anti-p16 antibody formulation and the CINtec Histology formulated with the hybridoma anti-p16 antibody (predicate device). The study was conducted using 249 cervical cases [114 No CIN, 17 CIN1, 53 CIN2, 51 CIN3, and 14 cervical SCC cases] run on one BenchMark ULTRA instrument. The cases (H&E stained slides) were reviewed and enrolled into the study by an experienced pathologist. Individual slides were stained with the recombinant CINtec Histology and hybridoma CINtec Histology device, randomized and evaluated for CINtec Histology status (positive/negative) by another pathologist (study pathologist) who was not involved in the selection of study cases.

Acceptance Criteria: Overall percent agreement (OPA) shall demonstrate a lower bound for the two-sided 95% confidence interval (LBCI) of greater than or equal to 85%. Background should be (less than or equal to 0.5 points (on a 0-4 scale) in 90% or greater of the tissue samples stained. Tissue morphology should be preserved as noted by the qualified reader in a minimum of 90% of interpretable samples stained.

A summary of the study results is provided in Table 9 below.

Table 9. Agreement on CINtec Histology Status for Recombinant and Hybridoma p16 Antibodies by Enrollment Diagnosis

		Hybridoma CINtec Histology Result [a]			Agreement		
Enrollment Diagnosis	Recombinant CINtec Histology Result [b]	Positive	Negative	Total	Measure[c]	% (n/N)	95% CI [d]
Normal	Positive	4	0	4	PPA	100.0 (4/4)	(51.0, 100.0)
	Negative	0	107	107	NPA	100.0 (107/107)	(96.5, 100.0)
	Total	4	107	111	OPA	100.0 (111/111)	(96.7, 100.0)
CIN 1	Positive	10	0	10	PPA	100.0 (10/10)	(72.2, 100.0)
	Negative	0	6	6	NPA	100.0 (6/6)	(61.0, 100.0)
	Total	10	6	16	OPA	100.0 (16/16)	(80.6, 100.0)
CIN 2	Positive	48	0	48	PPA	98.0 (48/49)	(89.3, 99.6)
	Negative	1	1	2	NPA	100.0 (1/1)	(20.7, 100.0)
	Total	49	1	50	OPA	98.0 (49/50)	(89.5, 99.6)
CIN 3	Positive	45	1	46	PPA	97.8 (45/46)	(88.7, 99.6)
	Negative	1	0	1	NPA	0 (0/1)	(0.0, 79.3)
	Total	46	1	47	OPA	95.7 (45/47)	(85.8, 98.8)
SCC	Positive	11	0	11	PPA	100.0 (11/11)	(74.1, 100.0)
	Negative	0	3	3	NPA	100.0 (3/3)	(43.9, 100.0)
	Total	11	3	14	OPA	100.0 (14/14)	(78.5, 100.0)

[a] Hybridoma CINtec Histology result is the final status determined for each slide stained using hybridoma p16 antibody.
[b] Recombinant CINtec Histology result is the final status determined for each slide stained using recombinant p16 antibody.
[c] PPA = Positive Percent Agreement; NPA = Negative Percent Agreement; OPA = Overall Percent Agreement.
[d] Two-sided 95% confidence interval calculated using the Wilson score method.
Note: Cases that do not have evaluable status for slides stained with either Hybridoma or Recombinant p16 antibodies were excluded.

Table 10. Comparison of histology status between Recombinant CINtec® Histology and hybridoma CINtec® Histology

	Hybridoma CINtec® Histology Status		
Recombinant CINtec® Histology Status –	**Positive**	**Negative**	**Total**
Positive	118	1	119
Negative	2	117	119

Total	120	118	238
Overall Percent Agreement (OPA) 98.7% (235/238) 95% CI (96.4, 99.6)			
Positive Percent Agreement (PPA) 98.3% (118/120) 95% CI (94.1, 99.5)			
Negative Percent Agreement (NPA) 99.2% (117/118) 95% CI (95.4, 99.9)			

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

See Interpretation of Results in section IV.B above.

E Expected Values/Reference Range:

Not applicable

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

The labelling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.