EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
CINtec Histology

DECISION SUMMARY

A. DEN Number:

DEN160019

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation of the CINtec Histology device

C. Measurand:

$p16^{INK4a}$ protein

D. Type of Test:

Immunohistochemistry, qualitative

E. Applicant:

Ventana Medical Systems, Inc.

F. Proprietary and Established Names:

CINtec Histology

G. Regulatory Information:

1. Regulation section:

   21 CFR § 864.1865

2. Classification:

   Class II (special controls)

3. Product code:

   PRB

4. Panel:

   88 - Pathology

H. Indications for use:

1. Indications for 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.

2. Special conditions for use statement(s):

   For *in vitro* diagnostic (IVD) use only

   For prescription use only

3. Special instrument requirements:

   VENTANA BenchMark ULTRA instrument

I. Device Description:

The CINtec Histology test is a single dispenser 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 primary antibodies and is comprised of 6 dispensers packaged together in one box. The components of the OptiView DAB IHC Detection Kit are as follows:

Table 1: OptiView DAB 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) (<50µg/mL) in a buffer containing protein with ProClin 300, a preservative.

OptiView HRP Multimer	Mouse monoclonal anti-HQ HRP labeled tertiary antibody (<40µg/mL) in a buffer containing protein with ProClin 300.
OptiView DAB	0.2% 3, 3'-diaminobenzidine tetrahydrochloride (DAB) in 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 table below lists the ancillary reagents required to perform the CINtec Histology assay.

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.
Hematoxylin II Counterstain	Dispenser / Prediluted / 25 mL	Hematoxylin (≤60%); contains glycol and acetic acid stabilizing solution	Stains cellular nuclei. This is a modified Mayer's hematoxylin.
Bluing Reagent	Dispenser / Prediluted / 25 mL	Solution of 0.1 M lithium carbonate in 0.5 M sodium carbonate	Changes the hue of the hematoxylin to a blue color. Applied after hematoxylin.

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 with negative staining 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 for 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 is part of the assay kit to evaluate nonspecific staining. The 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 incubation period for the negative reagent control antibody should be the same as that for the primary antibody.

J. Standard/Guidance Document Referenced:

CLSI I/LA28-A2: Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline – Second Edition

Guidance for Submission of Immunohistochemistry Applications to the FDA. 1998

K. Test Principle:

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.

L. 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.

A positive result is defined as “diffuse,” when there 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 Histology may be nuclear and/or cytoplasmic.

A negative result is defined as either focal staining of the cervical epithelium or absence of 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.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

      i. Within-Day (Repeatability) and Day-to-Day Precision

      Within-Day (repeatability) and Day-to-Day precision were evaluated in a study of 24 cervical punch biopsy FFPE tissue specimens [3 cervical squamous cell carcinoma (SCC), 6 CIN3, 6 CIN2, 6 CIN1, and 3 normal cervix cases]. Two replicate slides from each specimen were stained with the 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 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 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.

      For Within-Day precision (Repeatability), CINtec Histology status for each specimen was compared between duplicates on a single run, with data pooled over the 5 days. The estimate of Within-Day precision was 100%. For Day-to-Day precision, CINtec Histology status of slides from each specimen was compared across all days, using pooled data of all possible pairings. The estimate of Day-to-Day precision was 100%. Results of the study are presented in Table 3.

Table 3: Within-Day (Repeatability) and Day-to-Day Precision: 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[1] (100.0%)	29/29[1] (100.0%)
	CIN Category		29/29[1] (100.0%)	29/29[1] (100.0%)
LSIL[2]- 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[3]- 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[1] (100.0%)	239/239[1] (100.0%)
	CIN Category	170/170 (100.0%)	69/69[1] (100.0%)	239/239[1] (100.0%)

[1]CINtec Histology staining not evaluable for one study sample due to background staining
[2]LSIL - Low Grade Squamous Intraepithelial Lesion
[3]HSIL - High Grade Squamous Intraepithelial Lesion

ii. Instrument-to-Instrument Precision

Precision of the CINtec Histology assay across 3 BenchMark ULTRA instruments was determined by staining 3 replicate slides of 28 cervical punch biopsy cases (8 normal cervix, 6 CIN1, 6 CIN2, 4 CIN3, and 4 cervical carcinoma cases) using the OptiView DAB IHC Detection kit. Appropriate control tissue slides were also stained in each run. All slides were randomized, and then evaluated by a single pathologist, who was blinded to the case diagnosis, for positive or negative

CINtec Histology status. Each CINtec Histology slide was then paired with an H&E slide from the same case. After randomization of the paired slides, a single pathologist evaluated the CIN categories (No CIN, LSIL-histology, HSIL-histology, Cancer) based on the H&E and CINtec Histology slides.

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%. Results of the study are presented in Table 4.

Table 4: Instrument-to-Instrument Precision: 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%)
	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%)

iii. Lot-to-Lot Precision

Lot-to-Lot precision of the CINtec Histology was evaluated by testing 3 lots of the CINtec Histology primary antibody on duplicate slides of 18 cervical punch biopsy tissue specimens (4 normal cervix, 4 CIN1, 4 CIN2, 4 CIN3, and 2 cervical carcinoma cases) on one BenchMark ULTRA instrument using the OptiView DAB IHC Detection kit. Appropriate control tissue slides were also stained in each run.

Each CINtec Histology slide was paired with an adjacent H&E 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, LSL-histology, HSIL-histology, Cancer] were determined based on adjunctive interpretation of H&E and CINtec Histology slide.

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%. Results of the study are presented in Table 3.

Table 5: Lot-to-Lot Precision: 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 = 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%)

iv. Reader Precision

Within- and Between-Reader precision were evaluated on 50 cervical cases (16 normal cervix, 12 CIN1, 12 CIN2, 6 CIN3, and 4 cervical carcinoma cases) stained with the CINtec Histology and the OptiView DAB IHC Detection kit. All slides were randomized, and subsequently evaluated by 3 pathologists for CINtec Histology status. Pathologists were blinded to the case diagnosis. The CINtec Histology 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, LSL-histology, HSIL-histology, Cancer) was evaluated by 3 pathologists based on adjunctive interpretation of the H&E and 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.

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 3 pathologists, using pooled data of all possible pairings. The estimate of Reader-to-Reader agreement was 98.7%. The study results are provided in Table 6.

Table 6: Reader Precision: Number of Observations 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 = 19	N = 19
	CINtec Histology Status		112/113[1] (99.1%)	112/113[1] (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[1] (99.3%)	297/299[1] (99.3%)
	CIN Category	138/156 (88.5%)	125/143[1] (87.4%)	263/299[1] (88.0%)

[1] A single observation with unevaluable CINtec Histology status by Reader 2 was excluded

v. Reproducibility:

An inter-laboratory reproducibility study (Laboratory-to-Laboratory precision study) for the 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, LSL-histology, HSIL-histology, Cancer) based on adjunctive interpretation of the H&E and CINtec Histology slides. Pathologists were blinded to the case diagnoses. The study results are provided in Table 5.

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.

Table 7: Reproducibility: Number of Observations 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 = 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-	# of cases	N = 2	N = 2	N = 4

		Modal CINtec Histology Status		
Modal CIN Category		**Positive**	**Negative**	**Total**
Histology	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%)

Forty three 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.

b. *Linearity/assay reportable range:*

Not Applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

i. <u>Assay Reagent stability:</u>

Product expiration dating and shipping conditions were established based on testing with three lots of the CINtec Histology reagent. The intended storage condition (2-8 ºC) was tested on 3 replicates of two cervical biopsy specimens (CIN2/CIN3) and one tonsil specimen by staining with CINtec Histology device. Additionally one slide from each of the tissues was stained with the negative reagent to assess background staining. The testing time points were as follows: point zero, month 3, 6, 8, 9, 11, 12, 14, 18, 20, 24, and 26 for all three lots. The staining pattern and staining intensity were assessed at each testing time point. The resulting stability data supported expiration dating of 24 months when the product is stored at 2-8 ºC.

Simulated shipping conditions (heated ship stress at 30ºC and 15ºC and freeze/thaw cold ship stress at -20ºC) were also tested using 3 lots of the device on 3 replicates of two cervical biopsy specimens (CIN2/CIN3) and one tonsil specimen. Additionally, 1 slide from each of the tissues was stained with the negative reagent to assess background staining. Three lots of the device were held at heated ship stress condition of 30ºC for 192 hours, at 15ºC for 192 hours and at the freeze/thaw cold ship stress condition of -20 ºC for 192 hours. The device was then placed at intended storage (2-8°C) for the duration of testing. The testing time points were the same as the assay reagent stability testing time points. Results for all tested simulated shipping conditions were acceptable.

ii. Cut-Slide stability and storage:

The impact of storage time and temperature on tissue section mounted slides prior to staining was assessed. Slides cut from two multi-tissue blocks, each containing an invasive carcinoma (diffuse staining pattern), a CIN1 (focal staining pattern), and a normal cervical epithelium were stained in duplicate on Day 0. Additional slides from each block were then stored at 30°C and 2-8°C and then stained in duplicate. Slides were tested at point zero and at weeks 2, 4, 5, 6, 7, 8, 9, 10, 14, 18, 22, 26. Results for all slides were compared to the Day 0 results. Results showed no change in staining intensity or background staining up to the 26-week testing time point. Cut-slide stability is set at 24 weeks when stored at 2-8°C or 30°C.

d. Detection limit:

Not Applicable

e. Analytical Reactivity:

i. Western Blot:

Western Blot analysis was conducted to demonstrate that the CINtec Histology anti-p16 primary antibody specifically detects the p16 ^{INK4a} protein. Two different cell lysates were used. The HeLa cell line is known to express p16 ^{INK4a} and was used as a positive control, while the P693 cell line does not express p16 ^{INK4a} and was used as a negative control. It was expected that one single band would be

detected between 15 kD and 20 kD in the HeLa cells, while the P693 cell lysate should have no signal. The lanes containing the HeLa lysate showed a single band ~18 kDa, and, as expected, the lanes containing the negative control lysate showed no detectable signal.

ii. Peptide Inhibition Study:

A peptide inhibition study was conducted to evaluate antibody specificity of the CINtec Histology. The anti-p16^{INK4a} (E6H4) antibody was pre-incubated at 1:1 dilutions 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) and then applied to stain tissue cases that were known to express p16. The peptide concentrations were selected to span a range of molar ratios: approximately a 1000-fold, 100-fold, and 10-fold molar excess of peptide. 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 (CWQHQPEDRPNFAIILERIEY, MW=2658 g/mol) and 1:1 with diluent only (no peptide control).

All stained slides were evaluated for stain intensity and non-specific background on a 0-4+ scale. Non-specific background was scored as acceptable (≤ 0.5) or unacceptable (> 0.5). The study confirmed specificity of the anti-p16^{INK4a} (E6H4) primary antibody to only its immunizing peptide.

iii. Immunoreactivity:

Analytical specificity and sensitivity were determined by staining a variety of normal and neoplastic human tissues with the CINtec Histology. For the purposes of this study, any nuclear and/or cytoplasmic staining was considered as positive staining, unless otherwise specified. 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 ^{INK4a} protein in cell cycle regulation. No unexpected staining was observed with the CINtec Histology on the normal and neoplastic tissues.

Table 8: 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

Tissue	# Positive / Total cases	Cell Type
Parathyroid gland	1/1	Chief cells
Hypophysis	3/3	Anterior pituitary epithelial cells
Testis	2/3[c]	Spermatogenic and Leydig cells
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

Tissue	# Positive / Total cases	Cell Type
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 9: CINtec Histology staining in a variety of FFPE neoplastic tissues

Neoplasm	# Positive / Total cases	Neoplasm	# Positive / Total cases
Glioblastoma	1/1	Hepatocellular carcinoma	0/1
Atypical meningioma	0/1	Hepatoblastoma	0/1
Malignant ependymoma	1/1	Renal clear cell carcinoma	0/1
Malignant oligodendroglioma	0/1[a]	Prostatic adenocarcinoma	1/2
Ovarian Serous papillary adenocarcinoma	1/1	Leiomyoma	1/1
Ovarian adenocarcinoma	0/1	Uterine endometrial adenocarcinoma	1/1
Islet cell carcinoma	0/1	Uterine endometrial clear cell carcinoma	1/1[a]
Pancreatic adenocarcinoma	0/1	Cervical squamous cell carcinoma	2/2
Seminoma	0/1	Embryonal rhabdomyosarcoma	1/1
Embryonal carcinoma	0/1	Malignant melanoma	1/1
Thyroid Medullary carcinoma	1/1[a,b]	Basal cell carcinoma*	1/1
Thyroid Papillary carcinoma	0/1	Squamous cell carcinoma	0/1
Breast intraductal carcinoma	1/1	Neurofibroma	0/1
Breast invasive ductal carcinoma	2/2	Neuroblastoma	0/1

Neoplasm	# Positive / Total cases	Neoplasm	# Positive / Total cases
Diffuse B-cell lymphoma	1/3	Epithelial malignant mesothelioma	1/1
Lung small cell undifferentiated carcinoma	1/1	Hodgkin lymphoma	1/1
Lung squamous cell carcinoma	0/1	Anaplastic large cell lymphoma	1/1
Lung adenocarcinoma	1/1	Bladder transitional cell carcinoma	0/1
Esophageal squamous cell carcinoma	0/1	Low grade leiomyosarcoma	1/1
Esophageal adenocarcinoma	0/1	Osteosarcoma	1/1
Gastric mucinous adenocarcinoma	1/1	Spindle cell rhabdomyosarcoma	1/1
Gastrointestinal adenocarcinoma	3/3	Intermediate grade leiomyosarcoma	1/1
GIST	3/3		

[a] few cells staining; [b] nuclear staining only

f. Robustness:

i. Tissue Thickness:

Robustness of staining performance due to 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. Results for all samples were compared to the recommended 4 micron thickness. All tissue thicknesses demonstrated appropriate specific staining and background levels with CINtec Histology.

ii. Fixation:

The impact of pre-analytical factors (fixation type and fixation time) on the $p16^{INK4a}$ antigen as detected by the CINtec Histology was assessed using fresh xenograft tumors derived from cancer cell line. Tumors were fixed for 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours and 72 hours with each of the following fixatives: 10% neutral buffered formalin (10% NBF), zinc formalin, alcohol formalin, alcohol formalin acetic acid (AFA), Prefer solution and Z-fix. The resulting fixed tissues were paraffin embedded, then sectioned and stained with the CINtec Histology. Slides were read by a single reader and scored for p16 staining intensity and background. Results for all fixation types and times were

compared to those for tissues fixed for 12 hours with 10% NBF. Tissues fixed in 10% NBF, zinc formalin, and Z-Fix across all seven fixation times performed equivalently to tissue fixed in 10% NBF for 12 hours. It is recommended that tissues be fixed with 10% NBF for a minimum of 6 hours before staining with the CINtec Histology device. Alcohol formalin and Prefer fixatives are not recommended for use with CINtec Histology due to demonstrated weaker or variable staining.

iii. Staining Options:

All user selectable options in the CINtec Histology staining procedure on the BenchMark ULTRA stainer were validated using 6 cervical samples (1 CIN1, 3 CIN2, 2 normal cervix) stained with all possible combinations allowed within the CINtec Histology staining procedure, for a total of 6 staining conditions. Results from each sample were compared against the recommended protocol. All samples tested for all staining conditions yielded equivalent results to the recommended condition with regard to both the CINtec Histology staining pattern and CINtec Histology positive/negative status.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

FFPE uterine cervical punch biopsy specimen is the only recommended matrix.

3. Clinical studies:

To demonstrate that the CINtec Histology results in an improvement in consistency of the diagnosis of cervical intraepithelial neoplasia (CIN), levels of agreement between Community pathologists' (CP) and Expert pathologists' (XP) readings of cervical punch biopsy tissue 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. Study slides were assessed by CPs reviewing H&E stained slides only in the first round (Round 1, CP1) and by reviewing H&E and CINtec Histology stained slides in the second round (Round 2, CP2) for each study case by same pathologists after a 4-week washout period. The results were compared to XP diagnosis for each study case to evaluate positive, negative and overall agreements. 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. After a minimum of 4 week washout period, the same XPs evaluated both the H&E and CINtec Histology slides to establish their diagnosis (No CIN, LSIL-histology, HSIL-histology, (ACIS, or invasive carcinoma). 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, ACIS, or invasive carcinoma). 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, HSIL-histology, ACIS, or invasive carcinoma). The CPs noted the CINtec Histology status (positive = diffuse CINtec Histology staining; negative = focal or no CINtec Histology staining) along with their histological diagnosis using both the H&E-stained slide along with the CINtec Histology stained slide.

A) *Cases for which p16 staining was required according to LAST 2012 recommendations by majority of CP (LAST Cases)*
   There were 436 cases for which p16 staining was required by the majority of CPs per Round 1 questionnaire. For these cases, the levels of agreement between EPs using H&E alone or both H&E and CINtec Histology is shown in Table 10 below.

[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. Arch Pathol Lab Med - Vol 136, October 2012

Table 10: XP Agreement, H&E only vs. H&E and CINtec Histology (LAST Cases)

		Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel H&E Only					Total
		No CIN	CIN1	CIN2	CIN3	ACIS or Cancer	
Reference Diagnosis = Majority/ Consensus by Expert Panel H&E and CINtec Histology	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

The levels of agreement between CPs using H&E alone vs. the Reference Diagnosis by XPs using H&E alone and agreements between CPs with H&E and CINtec Histology vs. the Reference Diagnosis by XPs with H&E and CINtec Histology are presented in Table 11 below.

Table 11: CP Agreement with Reference Diagnosis, H&E only vs. H&E+CINtec histology (LAST Cases)

	H&E Only				H&E and CINtec Histology			
	Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel				Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel			
	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

	H&E Only Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel		H&E and CINtec Histology Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel	
	≤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

The estimate of positive percent agreement (PPA) was based on the comparison of the agreement between CPs using H&E and CINtec Histology vs. Reference Diagnosis by XPs using H&E and CINtec Histology for cases with reference diagnoses of ≥HSIL-histology and the agreement between CPs using H&E vs Reference Diagnosis by XPs using H&E for cases with reference diagnoses of ≥CIN2. The estimate of negative percent agreement (NPA) was based on the comparison of the agreement between CPs using H&E and CINtec Histology vs. Reference Diagnosis by XPs using H&E and CINtec Histology for cases with reference diagnoses of ≤LSIL-histology and the agreement between CPs using H&E vs. Reference Diagnosis by XPs using H&E for cases with reference diagnoses of ≤CIN1. The clinical study data demonstrated a statistically significant improvement in consistency of the diagnoses by CPs when using CINtec Histology staining as summarized in Table 12.

Table 12: Positive and Negative Percent Agreements (LAST Cases)

Agreement	H&E and 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%

B) *All cases regardless of LAST 2012 recommendations* (ALL Cases)
There were 1,100 cases in the study. The levels of agreement between EPs using H&E alone or both H&E and CINtec Histology are shown in Table 13 below.

Table 13: EP Agreement, H&E only vs. H&E and CINtec Histology (ALL Cases)

		Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel **H&E Only**					Total
		No CIN	CIN1	CIN2	CIN3	ACIS or Cancer	
Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel **H&E +p16 (CINtec histology)**	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

The levels of agreement between CPs using H&E alone vs. the Reference Diagnosis by XPs using H&E alone and agreements between CPs with H&E and CINtec Histology vs. the Reference Diagnosis by XPs with H&E and CINtec Histology are presented in Table 14 below.

Table 14: CP Agreement with Reference Diagnosis, H&E only vs. H&E+CINtec histology (ALL Cases)

	H&E Only					**H&E +p16 (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	12.6	13.2	10.6	12.9		12.8	13.6	15.2	16.0

averaged over all cases					
	≤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

The estimate of PPA was based on the comparison of the agreement between CPs using H&E and CINtec Histology vs. Reference Diagnosis by XPs using H&E and CINtec Histology for cases with reference diagnoses of ≥HSIL-histology and the agreement between CPs using H&E vs Reference Diagnosis XPs using H&E for cases with reference diagnoses of ≥CIN2. The estimate of NPA was based on the comparison of the agreement between CPs using H&E and CINtec Histology vs. Reference Diagnosis by XPs using H&E and CINtec Histology for cases with reference diagnoses of ≤LSIL-histology and the agreement between CPs using H&E vs. Reference Diagnosis by XPs using H&E for cases with reference diagnoses of ≤CIN1. The clinical study data demonstrated a statistically significant improvement in consistency of the diagnoses by CPs when using CINtec Histology staining as summarized in Table 15.

Table 15: Positive and Negative Percent Agreements, 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%

C) 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 alone is shown in Table 16. CINtec Histology positive results showed an increasing trend with increasing severity of CIN diagnosis.

Table 16: Percent of CINtec Histology positive results by CIN Diagnosis

	Reference Diagnosis = Majority/Consensus Diagnosis by Expert Panel with H&E				
	No CIN	CIN1	CIN 2	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: Fifteen Observations with unevaluable CINtec Histology status were excluded, 14 were No CIN and 1 was CIN1 by expert panel using H&E only

D) CINtec Histology Staining Performance

A total of 19,250 CINtec Histology interpretations were rendered during the study by the 70 CPs. The staining performance criteria assessed included overall staining acceptability, background staining acceptability (background does not interfere with the clinical interpretation of the stain) and morphology acceptability (cellular elements of interest are visualized allowing clinical interpretation of the stain). The results are presented in Table 17 below.

Table 17: CINtec Histology Staining Performance

	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%

4. Clinical cut-off:

   Refer to Section M – Interpretation of Results.

5. Expected values/Reference range:

   Not applicable

N. System Descriptions:

1. Modes of Operation:

The CINtec Histology assay is performed on the BenchMark ULTRA instrument which includes the staining system with embedded software, host PC with installed VSS software system (version 12.2), and system peripherals (printer, slide labeler, mouse & keyboard). Reagents are loaded onto the instrument to perform the assay. The CINtec Histology assay protocol is assay specific. The system performs all operations required to automatically process slides for IHC staining with the CINtec Histology device.

2. Software:

FDA has reviewed the applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X__ or No _____

3. **Calibration and Quality Controls:**
   See discussion of controls in section I above

O. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

P. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

Q. Identified Risks to Health and Identified Mitigations:

Identified Risks to Health	Identified Mitigations
Inaccurate test results, such as false positive or false negative results	General controls and special controls (1) and (2)
Failure to correctly interpret test results can lead to false positive or false negative results	General controls and special controls (1) and (2)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	This is an immunohistochemistry test intended to improve the consistency of CIN diagnosis by community pathologists in punch biopsies of the cervix uteri, providing a clinically important medical need. In unselected specimens, this device provides an improvement in the consistent diagnosis of cervical pre-cancers when used in combination with H&E, as compared to H&E alone. In cases that are considered by pathologists to be challenging, difficult, or of critical clinical importance, the device is expected to provide a larger benefit in diagnosis.

Summary	
Summary of the Risk(s)	There is minimal potential risk associated with use of this device given the combination of required general controls and special controls. The primary risks to patients are related to the consequences of clinical decisions based on false negative and false positive results due to inaccurate test results or failure to correctly interpret test results. A false positive could lead to an additional colposcopy and possibly to a cervical loop electrosurgical excision procedure (LEEP). These are relatively low risk procedures. A false negative or no result could lead to a delay in diagnosis of a cervical high grade squamous intraepithelial lesion (HSIL). Such a delay, unless it is on the order of years, is unlikely to result in progression of HSIL to invasive carcinoma, and is mitigated by the concomitant use of H&E with the device, and continued follow-up of patients with other abnormal tests indicating potential uterine cervical disease. There are additional risks associated with collection of tissue specimens for testing with the device. The test requires that a colposcopic biopsy of the cervix uteri be obtained. This is a standard procedure in clinical care, and the risk to patients is minimal. Further, the risk to laboratory workers is no greater than that for the routine collection and handling of tissue specimens, given that the test is for use by laboratory professionals in a clinical laboratory setting. These risks are mitigated by the appropriate CLIA (Clinical Laboratory Improvement Amendments) categorization of the device and labeling. The risks of the test are mitigated since the results from this test are intended to be used with results from other clinical, cervical cytology and HPV testing results. The assay is not to be used as a standalone diagnostic and is to be used in conjunction with other clinical and laboratory findings such as results of patient Pap tests and HPV tests. The risks are further mitigated by the special controls established for this device.
Summary of Other Factors	None
Conclusions Do the probable benefits outweigh the probable risks?	Yes, the probable benefits of this device, which allows evaluation of p16 staining by community pathologists for the diagnosis of CIN on a more consistent basis, outweigh the probable risks, given the combination of required general controls and special controls established for this device.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 864.1865.

FDA believes that the stated special controls and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PRB
Device Type: A cervical intraepithelial neoplasia (CIN) test system
Class: II (special controls)
Regulation: 21 CFR 864.1865

(a) *Identification.*
A cervical intraepithelial neoplasia (CIN) test system is a device used to detect a biomarker associated with CIN in human tissues. The device is indicated as an adjunct test and not to be used as a stand-alone device. The test results must be interpreted in the context of the patient's clinical history including, but not limited to, prior and current cervical biopsy results, Papanicolaou (Pap) test results, human papillomavirus (HPV) test results, and morphology on hematoxylin and eosin (H&E) stained sections. This device is not intended to detect the presence of HPV.

(b) *Classification*. Class II (special controls). A cervical intraepithelial neoplasia (CIN) test system must comply with the following special controls:

1. Premarket notification submissions must include the following information:

i. The indications for use must specify the biomarker that is intended to be identified and its adjunct use (e.g., adjunct to examination of H&E stained slides) to improve consistency in the diagnosis of CIN.

ii. Summary of professional society recommendations, as applicable.

iii. A detailed device description including:

A. A detailed description of all test components, including all provided reagents and required, but not provided, ancillary reagents.
B. A detailed description of instrumentation and equipment, including illustrations or photographs of non-standard equipment or manuals.
C. If applicable, detailed documentation of the device software, including, but not limited to, standalone software applications and hardware-based devices that incorporate software.
D. A detailed description of appropriate positive and negative controls that are recommended or provided.
E. Detailed specifications for sample collection, processing, and storage.
F. A detailed description of methodology and assay procedure.
G. A description of the assay cut-off (the medical decision point between positive and negative) or other relevant criteria that distinguishes

positive and negative results, including the rationale for the chosen cut-off or other relevant criteria and results supporting validation of the cut-off.

H. Detailed specification of the criteria for test results interpretation and reporting.

iv. Detailed information demonstrating the performance characteristics of the device, including:

A. Analytical specificity studies such as, but not limited to, antibody characterization (e.g., Western Blot, peptide inhibition analysis), studies conducted on panels of normal tissues and neoplastic tissues, interference by endogenous and exogenous substances as well as cross-reactivity, as applicable.

B. Device analytical sensitivity data generated by testing an adequate number of samples from individuals with the target condition including limit of blank, limit of detection, and limit of quantification, as applicable.

C. Device precision/reproducibility data to evaluate within-run, between-run, between-day, between-lot, between-site, between-reader, within-reader and total precision, as applicable, using a panel of samples covering the device measuring range and /or the relevant disease categories (e.g. No CIN, CIN1, CIN2, CIN3, cervical cancer) and testing in replicates across multiple, nonconsecutive days.

D. Device robustness/guardbanding studies to assess the tolerance ranges for various critical test and specimen parameters.

E. Device stability data, including real-time stability and shipping stability under various storage times, temperatures, and freeze-thaw conditions.

F. Data from a clinical study demonstrating clinical validity using well-characterized prospectively or retrospectively obtained clinical specimens, as appropriate, representative of the intended use population. The study must evaluate the consistency of the diagnosis of CIN, for example, by comparing the levels of agreements of diagnoses rendered by community pathologists to those rendered by a panel of expert pathologists. Agreement for each CIN diagnostic category (e.g., No CIN, CIN1, CIN2, CIN3, cancer) and for alternate diagnostic categories (e.g., No CIN, low grade squamous intraepithelial lesion (LSIL)-histology, high grade squamous intraepithelial lesion (HSIL)-histology, cancer) between reference diagnosis by expert pathologist and community pathologist must be evaluated, as applicable. In addition, agreements for CIN binary categories as ≥ CIN2 (i.e., CIN2 or CIN3 or cancer) and ≤ CIN1 (i.e., No CIN or CIN1) between reference diagnosis by expert pathologist with H&E staining and community pathologist with H&E staining and agreements for alternate CIN binary categories as ≥HSIL-histology (i.e., HSIL-histology or cancer) and ≤LSIL-histology (i.e., No CIN or LSIL-histology) between reference diagnosis by expert pathologist

with H&E+[biomarker specified in paragraph (1)(i) of this section] and community pathologist with H&E+[biomarker specified in paragraph (1)(i) of this section] must be evaluated and compared, as applicable.

G. The staining performance of the device as determined by the community pathologists during review of the study slides must be evaluated. The staining performance criteria assessed must include overall staining acceptability, background staining acceptability, and morphology acceptability, as applicable.

H. Appropriate training requirements for users, including interpretation manual, as applicable.

I. Identification of risk mitigation elements used by the device, including a description of all additional procedures, methods, and practices incorporated into the instructions for use that mitigate risks associated with testing.

2. The device's 21 CFR 809.10(b) compliant labeling must include a detailed description of the protocol, including the information described in paragraph (1)(ii) of this section, as applicable, and a detailed description of the performance studies performed and the summary of the results, including those that relate to paragraph (1)(ii) of this section, as applicable.