510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172471

B. Purpose for Submission:

New Device

C. Measurand:

Human CD30 antigen

D. Type of Test:

Immunohistochemistry

E. Applicant:

Ventana Medical Systems, Inc.

F. Proprietary and Established Names:

VENTANA CD30 (Ber-H2) Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5550 Immunoglobulin (light chain specific) immunological test system.

2. Classification:

   Class II

3. Product code:

   DEH

4. Panel:

   Hematology

H. Intended Use:

1. Intended use(s):

   The VENTANA CD30 (Ber-H2) Assay is intended for laboratory use in the qualitative detection of the CD30 protein in formalin-fixed, paraffin-embedded tissue stained with a VENTANA BenchMark ULTRA instrument and OptiView DAB IHC Detection Kit. CD30 positive staining results may aid in the identification of classical Hodgkin lymphoma (cHL), anaplastic large cell lymphoma (ALCL) and cutaneous T-cell lymphoma (CTCL). This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls. This antibody is intended for in vitro diagnostic (IVD) use.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   BenchMark ULTRA automated staining instrument in combination with the VENTANA OptiView DAB IHC Detection Kit.

I. Device Description:

The VENTANA CD30 (Ber-H2) Assay is a recombinant mouse monoclonal antibody (IgG1, kappa) produced as purified cell culture supernatant. It is diluted in 0.05M Tris buffered saline, 0.01M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide preservative. Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 1.23 ug/mL. The assay includes sufficient reagent for 50 tests.

The assay is for use with the OptiView DAB IHC Detection Kit and accessories not provided with the assay. A negative reagent control antibody is specifically matched for this assay and is used in place of the primary antibody to evaluate nonspecific staining (required not provided with assay).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   DAKO Corporation's Monoclonal Mouse Anti-Human Ki-1 antigen, CD30, Clone Ber-H2
2. Predicate 510(k) number(s):
   K965022
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Product Name	VENTANA CD30 (Ber-H2) Assay	Monoclonal Mouse Anti-Human Ki-1 antigen, CD30, Clone Ber-H2
510(k) Number	K172471	K965022
Intended Use	The VENTANA CD30 (Ber-H2) Assay is intended for laboratory use in the qualitative detection of the CD30 protein in formalin-fixed, paraffin-embedded tissue stained with a VENTANA BenchMark ULTRA instrument and OptiView DAB IHC Detection Kit. CD30 positive staining results may aid in the identification of classical Hodgkin lymphoma (cHL), anaplastic large cell lymphoma (ALCL) and cutaneous T-cell lymphoma (CTCL). This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls. This antibody is intended for in vitro diagnostic (IVD) use.	Monoclonal mouse anti-human Ki-1 antigen, CD30, Clone Ber-H2 (Ber-H2), may be used as one member of a panel of antibodies to aid in the differential diagnosis of large anaplastic cells of undetermined origin. This antibody stains cell membranes of most cases of anaplastic large cell lymphomas (ALCL), often called Ki-1 lymphomas. It also stains cell membranes and/or cytoplasm of most Hodgkin's lymphomas. Ber-H2 is a valuable aid in the assessment of cutaneous lymphoid infiltrates, particularly when large atypical cells are present. Most nonhematolymphoid neoplasms are negative with Ber-H2, although there are several significant exceptions. Membrane positivity is seen with embryonal carcinomas, and weak, diffuse cytoplasmic positivity is seen in pancreatic and salivary gland carcinomas. The staining pattern is most often described as strong membranous and weaker cytoplasmic, specifically paranuclear

Similarities		
Item	Device	Predicate
		dot-positivity of the Golgi region. The weak, diffuse cytoplasmic staining is considered to be unexpected, non-specific labeling. It may be reduced or removed by changing the protease pretreatment of the paraffin sections and/or further dilution of the Ber-H2 antibody.
Target	Human CD30	Same
Specimen Type	Formalin fixed, paraffin embedded human tissue	Acetone fixed, frozen and formalin or B5 fixed, paraffin-embedded tissues
Target Population	Hodgkin's Lymphoma and Anaplastic Large Cell Lymphoma, Cutaneous T Cell lymphoma (CTCL)	Same
Assay Method	Immunohistochemistry with secondary detection	Same
Primary Antibody	CD30 (BerH2) mouse anti-human monoclonal	Same

Differences		
Item	Device	Predicate
Instrument	BenchMark Ultra	DAKO Autostainer

K. Standard/Guidance Document Referenced (if applicable):

June 1998 "Guidance for Submission of Immunohistochemistry Applications to the FDA"

L. Test Principle:

CD30 antigen is expressed in mononuclear Hodgkin's cells and multinucleated Reed Sternberg cells of Hodgkin Lymphoma as well as on anaplastic large cell lymphomas. The CD30 (Ber-H2) monoclonal antibody produces membranous, cytoplasmic, and Golgi staining of both lymphoma cells and of scattered large activated B and T cells in lymph nodes, spleen, tonsil, and thymus. Approximately 4um thick sections are cut for staining. After CD30 binding to treated tissue sections, the specific antibody is localized using a secondary antibody followed by multimer anti-hapten conjugate.

The OptiView DAB IHC Detection Kit is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit primary antibodies. The OptiView DAB IHC Detection Kit produces a visible dark brown precipitate (3, 3'-Diaminobenzidine) via a horseradish peroxidase (HRP) enzymatic reaction at the antigen site. The reaction product precipitates at the antigen sites localized by VENTANA CD30 (Ber-H2) Assay. The cellular staining pattern for VENTANA CD30 (Ber-H2) Assay is membranous, cytoplasmic and/or Golgi.The pathologist evaluates the brown precipitate using bright-field microscopy. A negative control reagent and tissue controls with positive and negative elements are run with each assay as process controls.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Inter-Antibody Lot, Kit, Inter-Detection Lot, and Intra-Instrument Intermediate Precision Study

Between-lot and intra-instrument intermediate precision of VENTANA CD30 (Ber-H2) staining was evaluated by verifying that replicate slides from a panel of lymphoma model tissue samples result in concordant CD30 (Ber-H2) status when stained with full factorial combinations of 3 lots of CD30 (Ber-H2), 3 lots of OptiView DAB IHC Detection Kit, and on 3 BenchMark ULTRA instruments using twenty-four lymphoma tissue specimens, including 10 positive specimens, 10 negative specimens, and 4 borderline cases, were evaluated by one reader.

The studies were required to demonstrate the same clinical CD30 positive/negative status with 85% concordance of the 95% lower bound confidence interval (CI) using the recommended staining protocol. Results in Table 1 show that percent positive agreement (PPA) and negative percent agreement (NPA) were >93% when borderline cases were included for antibody lot, detection kit lot, and instrument precision studies.

Table 1. Inter-antibody lot, Inter-detection lot and Intra-instument Precision Summary of CD30 Status

Study Parameter	***Excluding*** Borderline Cases		***Including*** Borderline Cases		
	PPA n/N (%) (95% CI)	**NPA n/N (%) (95% CI)**	**OPA n/N (%) (95% CI)**	**PPA n/N (%) (95% CI)**	**NPA n/N (%) (95% CI)**
Inter-antibody Lot Intermediate Precision	257/269 (95.5%) (92.4-97.4)	270/270 (100%) (98.6-100.0)	634/647 (98.0%) (96.6-98.8)	337/350 (96.3%) (93.7-97.8)	297/297 (100%) (98.7-100.0)

Inter-detection Kit Lot Intermediate Precision	257/269 (95.5%) (92.4-97.4)	270/270 (100%) (98.6-100.0)	634/647 (98.0%) (96.6-98.8)	337/350 (96.3%) (93.7-97.8)	297/297 (100%) (98.7-100.0)
Intra-platform Intermediate Precision	257/269 (95.5%) (92.4-97.4)	270/270 (100%) (98.6-100.0)	634/647 (98.0%) (96.6-98.8)	337/350 (96.3%) (93.7-97.8)	297/297 (100%) (98.7-100.0)

Intra-day Repeatability and Inter-day Intermediate Precision Study

The objective of this study was to evaluate the VENTANA CD30 (Ber-H2) Assay staining repeatability and intermediate precision on lymphoma tissue samples representing the CD30 (Ber-H2) staining range by verifying concordance of CD30 (Ber-H2) status and/or acceptability. Twenty four lymphoma tissue cases were enrolled in this study for both intra-day and inter-day analyses, including 4 borderline cases. Slides were randomized and blinded by assigning a unique identifier to each slide and scored by one reader.

For intra-day intermediate repeatability, 5 slides per case were stained with CD30 (Ber-H2) and 1 slide per case with Mouse Monoclonal Negative Control Ig to serve as negative reagent control.

For inter-day intermediate precision, duplicate slides per case were stained with CD30 (Ber-H2) and 1 slide per case with Mouse Monoclonal Negative Control Ig on 5 non-consecutive days spanning at least a 20-day period. Two CD30 (Ber-H2)-stained slides per case from intra-day repeatability were used as Day 1 slides for inter-day intermediate precision.

The studies were required to demonstrate the same clinical CD30 positive/negative status with 85% concordance of the 95% lower bound CI using the recommended staining protocol. Results summarized in Table 2 show PPA and NPA were at least 88% for the 95% lower bound confidence interval. Data were analyzed for morphology acceptability, background acceptability, and intra-day repeatability and inter-day precision by case-level CD30 (Ber-H2) status concordance.

Table 2.Intra-day and Inter-day Precision Results (Including Borderline Cases)

Study Parameter	**OPA**		**PPA**		**NPA**	
	OPA % (n/N)	95% CI	PPA % (n/N)	95% CI	NPA % (n/N)	95% CI
Intra-day Repeatability	98.33 (118/120)	94.13, 99.54	96.67 (58/60)	88.64, 99.08	100.00 (60/60)	93.98, 100.00

Inter-day Intermediate Precision	100.00 (240/240)	98.42, 100.0	100.00 (120/120)	96.90, 100.00	100.00 (120/120)	96.90, 100.00

Inter-Reader/Intra-Reader Precision Study

A study was conducted to assess precision between readers and within readers using 50 lymphoma cases including 6 borderline cases. Slides were blinded and randomized and read by three pathologists. After a four week washout period, the slides were read by the same pathologists again. Results in Table 3 for intra-reader and Table 4 for inter-reader agreement below show both APA and ANA were 98.7%, thus surpassing the acceptance criteria of ≥85%.

Table 3. Intra-Reader Agreement of CD30 Status

		Clinical Assessment		
Comparison	Clinical Assessment	Positive	Negative	Total
Average Intra-Reader Agreement	Positive	74	0	74
	Negative	2	74	76
	Total	76	74	150
Overall Percent Agreement: n/N | % | 95% CI		148/150	98.7	96.7,100.0
Average Positive Agreement: n/N | % | 95% CI		148/150	98.7	96.4,100.0
Average Negative Agreement: n/N | % | 95% CI		148/150	98.7	96.3,100.0

Table 4. Inter-Reader Agreement of CD30 Status

		Clinical Assessment		
Comparison	Clinical Assessment	Positive	Negative	Total
Average Inter-Reader Agreement	Positive	296	4	300
	Negative	4	296	300
	Total	300	300	600
Overall Percent Agreement: n/N | % | 95% CI		592/600	98.7	96.7,100.0
Average Positive Agreement: n/N | % | 95% CI		592/600	98.7	96.4,100.0
Average Negative Agreement: n/N | % | 95% CI		592/600	98.7	96.3,100.0

Inter-laboratory Reproducibility Study

A multi-site reproducibility study was conducted to assess reproducibility of the assay for determining CD30 status at 3 external sites with 2 readers each. The study was carried out over 5 non-consecutive days using 24 deidentified formalin-fixed, paraffin-embedded (FFPE) specimens. Specimens included both MTCL and CTCL and spanned a range of staining with 4 borderline cases. Prior to staining, slides were blinded and randomized. The performance of the VENTANA CD30 (Ber-H2) Assay was to be considered acceptable if both the PPA and NPA rates across all observations met or exceeded 85% for the lower bound of the 95% confidence

intervals. The PPA and NPA rates for the agreement of reader-assigned CD30 status with the consensus CD30 status in the combined analysis were 93.6% and 99.7%, respectively as shown in Table 5.

Table 5. Inter-laboratory Reproducibility of VENTANA CD30 (Ber-H2) Assay staining in lymphoma (CTCL and MTCL) tissue specimens.

Inter-laboratory Reproducibility	Overall Percent Agreement (95% CI)	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
Agreement of reader-assigned CD30 status	96.7 (95.1- 97.7%)	93.6 (90.6- 95.7%)	99.7 (98.4- 100.0%)

Tissue Thickness

Tissue thickness was evaluated using human lymphoma tissue. Tissues were sectioned and tested in duplicate at 3, 4, 5, 6, and 7 microns. Tissue thicknesses of 4 microns demonstrated appropriate specific staining for CD30 and appropriate background levels with VENTANA CD30 (Ber-H2) Assay. Tissue thicknesses cut at 3 microns did have a difference in CD30 status when compared to the 4 to 7 µm sections for one case. Additional cases cut from 3 to 7 microns did not have any change in clinical interpretation; however, it was noted that at thicknesses of ≥ 5 microns the background increased causing neoplastic cells to become more difficult to identify. Ventana recommends that specimens be cut at 4 microns for the assay

Functional Characterization of the Lymphoma Model Tissue

b. Linearity/assay reportable range:

Not applicable.

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

Kit Stability

The objective of this study was to evaluate the Real Time Stability of three production lots of VENTANA CD30 (Ber-H2) Assay under intended storage and simulated Ship Stress conditions.

Three lots of VENTANA CD30 (Ber-H2) Assay were tested in triplicate on lymphoma tissues for a minimum of eleven time points while kept at intended storage (2-8°C) and nine time points post Ship Stressing at Category A (30±5°C), Category B (15±5°C), and Cold Ship Stress (Freeze/Thaw -20±5°C) conditions. When not being tested, all lots were kept at intended storage prior to and post ship stressing for the duration of the study.

All three production lots passed the acceptance criteria. Open Vial and On-Board Stability have been completed for all three lots, and these data satisfy regional requirements for open vial and on-board stability. The real time and ship stress stability data demonstrate that VENTANA CD30 (Ber-H2) Assay Mouse Monoclonal Primary Antibody is stable for 12 months at the intended use storage of 2-8ºC.

Cut Slide Stability

This study was carried out to evaluate the changes in CD30 antigenicity in lymphoma tissue sectioned on positively charged microscope slides after storage at two temperatures, 2-8° and 30°C. Two qualified multi-tissue blocks (MTBs) were sectioned at Day 0 (up to approximately 100 sections) and the slides were stored at 2-8°C and 30°C. Slides were read by three readers using two instruments. Testing established day 0 baseline staining and interpretation of the lymphoma cases. Subsequent testing was compared to the baseline staining until loss of specific staining was reached. All three readers noted a decrease in staining intensity for slides stored at 2-8°C at the month 2 time point for tissue 2 while the 30°C storage condition passed up to month 6 for MTB D. Therefore the recommended maximum storage time for cut unstained lymphoma slides using the VENTANA CD30 (Ber-H2) Assay is 1 month for slides stored at 2-8ºC and 5 months for slides stored at 30 ºC.

Controls

A negative antibody reagent control is used with used with the assay. Positive and negative control slides should be stained with each staning run.

d. Detection limit:

Not applicable

e. Analytical specificity:

A peptide inhibition study was conducted by incubating a specific peptide for the CD30 epitope or a nonspecific peptide with a Hodgkin lymphoma specimen. Results show the specific peptide reduced CD30 staining consistent with a reduction in the CD30 antibody titer, whereas the nonspecific peptide did not reduce CD30 staining caused by the Ber-H2 antibody. It was noted that the CD30 statining was not completely eliminated by the specific peptide. To address this issue specificity of the assay was evaluated by demonstrating binding of the Ber-H2 antibody to CD30 in Western Blots. The antibody recognized endogenous CD30 in whole cell lysates from HH and AT cell lines but did not bind proteins in SKMel5 cell line which does not express CD30. The Ber-H2 antibody also specifically recognized recombinant CD30 protein but not recombinant FGF19. The staining in the two cell lines expressing CD30 showed a reactivity pattern to the expected molecular weight as well as weaker signal for breakdown products of CD30 protein.

f. Assay cut-off:

For HL and ALCL any percent positivity (≥1%) was counted toward positivity.

2. Comparison studies:

   a. Method comparison with predicate device:

   A method comparison study was performed between the VENTANA CD30 (Ber-H2) Assay and the predicate device using 115 lymphoma cases with four pathologists. A second read of the slides was conducted following a four week wash out period. Results in Table 6 show >85% agreement based on the 95% lower bound confidence interval and demonstrate the VENTANA CD30 (Ber-H2) Assay is equivalent to the predicate.

Table 6. Agreement of CD30 status between the VENTANA CD30 (Ber-H2) Assay and DAKO assay

Readers 1 - 4, first and second reads	Overall Percent Agreement	739/796 (92.8)	90.8, 94.7
	Average Positive Agreement	373/387 (96.4)	94.4, 98.1
	Average Negative Agreement	366/409 (89.5)	85.9, 92.7

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

   The test is intended for qualitative use.

5. Expected values/Reference range:

   Tour of Body/Tour of Tumor
   The purpose of this study was to establish how the VENTANA CD30 (Ber-H2)

Assay performs when staining a variety of different normal and neoplastic tissues. The Tour of Body and Tour of Tumor Arrays containing 90 normal and 54 neoplastic tissues were evaluated for CD30 expression. An additional 13 normal tonsil cases were also evaluated for CD30 expression, all of which demonstrated specific staining for CD30 as shown in Table 7. Of the neoplastic tissues, 3 exhibited CD30 expression, including: seminoma, embryonal carcinoma, and Hodgkin lymphoma, as shown in Table 8.

Analytical sensitivity of the VENTANA CD30 (Ber-H2) Assay was tested on 1378 lymphoma model tissues. The 1378 lymphoma model tissues consisted of 97 CTCL cases, 35 Mature T-Cell Lymphoma (MTCL, also referred to as Peripheral T Cell Lymphoma (PTCL)) cases, 1120 DLBCL cases, 40 ALCL cases, and 86 HL cases. When any staining was observed (≥1% for CD30 staining), the prevalence for ALCL was found to be 97.5% (39/40) and for HL was found to be 100% (86/86).

Table 7. Specificity of VENTANA CD30 (Ber-H2) Assay was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Lung	0/4
Cerebellum	0/3	Heart	0/5
Adrenal gland	0/3	Esophagus	0/3
Ovary	0/3	Stomach	0/3
Uterus	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	0/3	Liver	0/3
Hypophysis	0/3	Salivary gland	0/3
Testis	0/3	Kidney	0/3
Thyroid	0/4	Prostate	0/3
Breast	0/3	Cervix	0/2
Spleen	0/3	Skin	0/3
Tonsil	15/16	Nerve	0/3
Thymus	0/3	Skeletal muscle	0/2
Myeloid (bone marrow	0/3		

Table 8. Sensitivity of VENTANA CD30 (Ber-H2) Assay was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Meningioma	0/1
Anaplastic ependymoma	0/1
Oligodendroglioma	0/1
Ovarian adenocarcinoma	0/2
Pancreatic neuroendocrine neoplasm	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	1/1
Embryonal carcinoma	1/1
Medullary thyroid carcinoma	0/1
Papillary thyroid carcinoma	0/1
Breast intraductal carcinoma	0/1
Breast invasive ductal carcinoma	0/2
B-Cell Lymphoma; NOS	0/1
Lung small cell carcinoma	0/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Esophageal squamous cell carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma	0/1
Small Intestine, Gastrointestinal Stromal Tumor	0/1
Colon adenocarcinoma	0/1
Colon, Gastrointestinal Stromal Tumor	0/1
Rectal adenocarcinoma	0/1
Rectum, Gastrointestinal Stromal Tumor	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1

Pathology	# positive / total cases
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/2
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Cervical squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	0/1
Cutaneous squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Lymph Node B-Cell Lymphoma; NOS	0/2
Hodgkin Lymphoma	1/1
Anaplastic large cell lymphoma	0/1
Bladder Urothelial carcinoma	0/1
Leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Malignant melanoma	0/1

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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