SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: VENTANA FOLR1 (FOLR-2.1) RxDx Assay

Device Trade Name: VENTANA FOLR1 (FOLR-2.1) RxDx Assay

Device Procode: QUL

Applicant's Name and Address: Ventana Medical Systems Inc.
1910 E. Innovation Park Drive,
Tucson, AZ 85755

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P220006

Date of FDA Notice of Approval: November 14, 2022

II. INDICATIONS FOR USE

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is a qualitative immunohistochemical assay using mouse monoclonal anti-FOLR1, clone FOLR1-2.1, intended for use in the assessment of folate receptor alpha (FOLR1) protein in formalin-fixed, paraffin-embedded epithelial ovarian, fallopian tube or primary peritoneal cancer tissue specimens by light microscopy. This assay is for use with OptiView DAB IHC Detection Kit for staining on a BenchMark ULTRA instrument.
FOLR1 expression clinical cut-off is ≥ 75% viable tumor cells (TC) with membrane staining at moderate and/or strong intensity levels.
This assay is indicated as an aid in identifying patients with epithelial ovarian, fallopian tube, or primary peritoneal cancer who may be eligible for treatment with ELAHERE (mirvetuximab soravtansine).
Test results of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.
This product is intended for *in vitro diagnostic* (IVD) use.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay labeling.

V. DEVICE DESCRIPTION

A. Device Kit Components

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay contains optimized reagents required to complete an immunohistochemical staining procedure for formalin-fixed paraffin embedded (FFPE) specimens on the BenchMark ULTRA automated staining instrument visualized using the OptiView DAB IHC Detection Kit. VENTANA FOLR1 (FOLR1-2.1) RxDx Assay utilizes a mouse monoclonal hybridoma antibody produced against a recombinant protein as a cell culture supernatant, purified using protein G. The antibody and detection reagents are provided as ready-to-use dispensers and each dispenser contains sufficient reagent for 50 tests. A Stain Intensity Reference (SIR) slide is required for assay interpretation. The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Components and description is provided in the table below.

Table 1: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Components

Components	Packaged Form	Description
VENTANA FOLR1 (FOLR1-2.1) Mouse Monoclonal Primary Antibody	1 Flo-Lok Dispenser: 50 tests	One 5 mL dispenser FOLR1 reagent contains approximately 28µg of a mouse monoclonal FOLR1-2.1 antibody (approximately 5.6 µg/mL). The antibody is diluted in 0.05 M Tris- HCL with carrier protein and 0.10% ProClin 300, a preservative.
OptiView DAB IHC Detection Kit	Set of 6 Flo-Lok dispensers, packaged in a kit: 250 tests	OptiView Peroxidase Inhibitor contains 3.0% hydrogen peroxide solution.
		OptiView HQ Universal Linker contains a cocktail of HQ-labeled (HQ is a proprietary hapten covalently attached to the goat antibodies) antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (<50 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		OptiView HRP Multimer contains a mouse monoclonal anti-HQ- labeled HRP tertiary antibody (<40 µg/mL) in a buffer containing protein with ProClin 300, a preservative.
		OptiView H_2O_2 contains 0.04% hydrogen peroxide in a phosphate buffer solution.
		OptiView DAB contains 0.2% 3, 3′- diaminobenzidine tetrahydrochloride (DAB) in a proprietary stabilizer solution with a proprietary preservative.
		OptiView Copper contains copper sulfate (5.0 g/L) in an acetate buffer with a proprietary preservative.

BenchMark ULTRA automated staining instrument and Ventana System Software (VSS) software	Instrument installed with the VSS host system software, Version 12.3 and 12.3.1	A personal computer (PC) that runs on Microsoft Windows and controls the BenchMark ULTRA instrument via the host operating software.
Negative Control (Monoclonal)	1 Flo-Lok dispenser: 250 tests	A mouse monoclonal antibody intended for laboratory use as a control for nonspecific binding of the primary antibody in sections of FFPE tissue. One 25 mL dispenser contains approximately 25 µg (1 µg/mL) of mouse monoclonal antibody. The antibody is diluted in phosphate buffered saline containing carrier protein and ProClin 300, a preservative.
VENTANA Stain Intensity Reference (SIR) Slide	2 SIR slides packed in a slide mailer	Intended to be used as an aid when assessing the stain intensity of DAB in FOLR1 tumor cell staining in epithelial ovarian, fallopian tube or primary peritoneal cancer tissue. A section of normal fallopian tube tissue embedded on a glass slide and stained with FOLR1 Assay

Table 2: Ancillary Reagents Required for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Reagents
EZ Prep (10x)
Reaction Buffer (10x)
ULTRA Liquid CoverSlip (LCS), pre-dilute
ULTRA Cell Conditioning Solution (CC1)
Hematoxylin II counterstain
Bluing Reagent

B. Device Instrumentation and Software

The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is performed on the BenchMark ULTRA automated staining instrument using VSS versions 12.3 or 12.3.1. The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay staining protocol is assay specific. To ensure that all system reagents are used together, the software has been designed to recognize and group reagents required for staining per the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay staining protocol.

C. Specimen Preparation

Routinely processed FFPE tissues are suitable for use with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Tissue is fixed in 10% neutral buffered formalin (NBF) for 12 to 72 hours. Use of alcohol-formalin-acetic acid (AFA), 95% alcohol, PREFER fixatives and Zinc Formalin or Z-5 are not recommended due to loss of specific FOLR1 protein expression.

Tissue sections should be cut at approximately 4 µm thickness and mounted on positively-charged glass slides. Slides should be stained immediately, as antigenicity of

cut tissue sections may diminish over time. See device labeling (package insert) for additional details.

D. Quality Control Procedures

Run controls are included in each staining run to establish the validity of the test results. The following controls must be run with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

1. **Positive/Negative Tissue Control:** Normal Fallopian tube is used as a positive control tissue for this antibody. Positive and negative staining elements for the FOLR1 protein present in fallopian tube tissue are used to confirm that the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay functioned properly. Positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. The interpretation of the FOLR1 staining in normal fallopian tube when used as a positive/negative tissue control is given in the table below.

Table 3: Positive Control Tissue Evaluation, Normal Fallopian Tube

Status	Staining Pattern
Acceptable	Predominately moderate circumferential* FOLR1 membrane staining in the epithelium of normal Fallopian tube and absence of specific staining in normal Fallopian tube stroma.
Not Acceptable	Absence of staining, or predominantly weak or strong circumferential* FOLR1 membrane staining in the epithelium of normal Fallopian tube and/or Non-Specific FOLR1 background staining that interferes with interpretation.

* Note: Apical staining of the first layer of the luminal cells must not be considered in evaluating the acceptability of fallopian tube FOLR1 staining.

2. **Negative Reagent Control:** A matched negative reagent control slide should be run for every specimen to aid in the interpretation of results. Negative Reagent (Monoclonal), a negative reagent control antibody, is specifically matched for this VENTANA FOLR1 (FOLR1-2.1) RxDx Assay and is used in place of the primary antibody to evaluate nonspecific staining in the patient tissue that may result from a reaction with the detection chemistry and not the anti-FOLR1 primary antibody.

E. Principles of Operation

The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is fully automated for use on the BenchMark ULTRA automated slide stainer from deparaffinization through counterstaining. Patient FFPE tissue specimens are cut to approximately 4 µm thickness and mounted on positively charged glass slides. These slides are loaded into the Benchmark ULTRA instrument. This system first removes the paraffin wax from the tissue (deparaffinization), and then subjects the tissue to heated antigen retrieval (cell conditioning). Endogenous peroxidases that could potentially react with the horseradish peroxidase conjugates (HRP) are blocked with OptiView Inhibitor (3% H_2O_2). After the endogenous peroxidase block, the VENTANA FOLR1 (FOLR1-2.1) Mouse Monoclonal Primary Antibody is dispensed during the antibody incubation

step and allowed to bind to its antigen. The slides are then incubated with the reagents in the OptiView DAB IHC Detection Kit, which is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit primary antibodies and which produces a visible dark brown precipitate (3,3'-Diaminobenzidine) via a horseradish peroxidase (HRP) enzymatic reaction at the antigen site. Slides are then counterstained using Hematoxylin II and Bluing Reagent to create brown/blue contrast to aid the pathologist when reviewing the slides using bright field microscopy. The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay staining protocol is shown in the table below.

Table 4: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining Protocol on BenchMark ULTRA Instrument

Procedure Type	Protocol Parameter
Baking	Optional, maybe performed off-line
Deparaffinization	4 minutes (default), 72°C
Cell Conditioning (Antigen Unmasking) Pre-Primary Peroxidase Inhibitor	ULTRA CC1, 64 minutes, 100°C 4 minutes, 36°C
Antibody (Primary)	Ventana FOLR1-2.1 RxDx Assay Ab (32 minutes, 36°C) Or Negative Control Ab (32 minutes, 36°C)
OptiView HQ Linker	8 minutes, 36°C
OptiView HRP Multimer	8 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes, 36°C
Post Counterstain	Bluing, 4 minutes, 36°C

F. Slide Review and Interpretation of FOLR1 Staining

The cellular staining pattern for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is membranous and cytoplasmic in epithelial ovarian, fallopian tube or primary peritoneal cancer tissue, with varying ranges of stain intensity; only membranous staining is evaluated for the determination of FOLR1 status. Membrane staining pattern may be apical or circumferential (partial or complete).

i. **Hematoxylin & Eosin (H&E) Slide:**
The pathologist will determine whether the H&E slide contains sufficient tumor tissue (it is recommended that approximately 100 viable tumor cells are present) consistent with epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer to allow interpretation of the case-matched IHC slides. If the H&E is not acceptable, the case-matched NRC and FOLR1 slides will not be evaluated.

ii. **System-level Control Slide(s)-Positive and Negative Tissue Control Slide(s):**
Normal fallopian tube tissue contains both positive-staining and negative-staining elements with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Normal fallopian tube tissue stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay contains both specific FOLR1 staining in the luminal surface of the epithelial cells and absence of FOLR1 staining in the stroma, both of which

should be evaluated to confirm that the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is performing correctly.

iii. **Negative Reagent Control (NRC) Slide:**
The NRC slide is evaluated based on the level of non-specific staining (background). If the NRC slide is not acceptable, slide will not be evaluated, and the assay should be repeated.

iv. **SIR Slide:**
FOLR1 staining percentage at each intensity is determined by a trained pathologist using the FOLR1 SIR slide as the baseline for moderate stain intensity. Each FOLR1 SIR slide contains at least one region of 10 or more contiguous cells expressing moderate (2+) circumferential membrane staining. Prior to utilizing the FOLR1 SIR slide as a stain intensity reference tool for interpreting epithelial ovarian, fallopian tube or primary peritoneal cancer cases stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, pathologists should first review the FOLR1 SIR slide for a moderate staining region. After locating the moderate staining region in the FOLR1 SIR slide, the pathologist should use this region to aid in the identification of moderate and stronger staining in epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer slides. These tissues must be evaluated according to the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay scoring algorithm, provided in Table 5. Refer to the Interpretation Guide for additional instructions.

v. **Patient Tissue Slide:**
FOLR1 status will only be assigned if the H&E slide, the system-level control slide, the NRC slide, and the FOLR1 slide (including background, morphology, and overall staining) are all acceptable. Patient specimens should have approximately 100 viable tumor cells identified on the H&E in order to determine FOLR1 status. The percentage of tumor cells staining at each intensity (negative, weak, moderate, strong) will be assessed but only moderate and strong stain intensities will contribute to the FOLR1 status determination using the scoring method. Epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer tissue cases are considered positive for FOLR1 status if ≥ 75% of viable tumor cells (TC) demonstrate moderate and/or strong membrane staining.

Due to the histological characteristics of epithelial ovarian carcinoma, primary peritoneal adenocarcinoma and primary fallopian tube carcinoma they are grouped together as epithelial ovarian cancer (EOC) in this document. The scoring algorithm for the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is provided in the table below.

Table 5: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Scoring Algorithm for EOC

FOLR1 Status	Staining Description
Positive*	≥ 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Negative*	< 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Not Evaluable	Artifacts making interpretation not possible.

* Re-reading by Additional Pathologists for FOLR1 Scoring

Re-reading by Additional Pathologists for FOLR1 Scoring: To decrease variability of FOLR1 results for cases with %TC near the threshold of 75% [65% to 85%], re-reading of the slide by a second pathologist is recommended. The case result with %TC between 65-85% by a pathologist should be adjudicated by one or two independent pathologists. In these cases, the patient's result with regard to FOLR1 status (positive/negative) should be obtained by either a majority rule or by consensus among the pathologists.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved assay available for detection of FOLR1 in FFPE epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer tissues to estimate the likelihood of response for patients treated with ELAHERE (mirvetuximab soravtansine).

VII. MARKETING HISTORY

The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is intended for in vitro diagnostic (IVD) use only. As with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. Failure of the device to perform as expected or failure to correctly interpret test results may lead to improper patient management decisions.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

Non-clinical studies were performed using the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay to establish analytical performance of the device in epithelial ovarian cancer, fallopian tube cancer and primary peritoneal cancer. These studies were performed using the BenchMark ULTRA instrument using the VSS software version 12.3 and 12.3.1. These studies were conducted to characterize the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, demonstrate the impact of pre-analytical variables on assay performance, evaluate assay precision and robustness and establish assay stability. The study results detailed below establish assay sensitivity, specificity, precision, robustness, stability, external reproducibility, and other performance characteristics of the device.

1. Analytical Sensitivity

Analytical sensitivity of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay was assessed based on the prevalence of FOLR1 staining on a sample set which included 953 unique EOC cancer resection tissue cases. The slides were read by one pathologist and scored using the scoring method specified in Table 5 above.

FOLR1 positive status was observed in 28.75% (274/953) of cases in the commercial cohort of EOC resection tissues for prevalence reporting. FOLR1 negative status was observed in 71.25% (679/953) of cases and FOLR1 borderline status was observed in 224 of 953 cases. Borderline status is defined as 75% ± 10% tumor cell (TC) staining. Positive borderline accounted for 15.95% (152/953) of cases and 7.56% (72/953) cases as negative borderline. Results are also provided in the table below.

Table 6: Prevalence of FOLR1 in EOC stained with VENTANA FOLR1 (FOLR1-2.1) Assay

FOLR1 Status	Prevalence at 75% Cutoff (% n/N)	Borderline Prevalence (% n/N)
Positive	28.75% (274/953)	15.95% (152/953)
Negative	71.25% (679/953)	7.56% (72/953)

Note: In different populations, prevalence of FOLR1 IHC scores may be different from the prevalence presented in the above table.

2. Analytical Specificity

The antibody used in the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is Mouse Anti-Human FOLR1 Monoclonal Antibody (Clone FOLR1-2.1). The FOLR1 antibody detects the glycosylated form of the FOLR1 protein, which has a molecular weight of 40 kDa. The following studies were conducted with FOLR1 (FOLR1-2.1) antibody to establish antibody specificity.

a. Western Blot Studies

Western blot (WB) analysis was performed on whole cell lysates from 4 cell lines with varying expression levels of FOLR1. The 4 cell lines were KB (Epithelial Carcinoma), Igrov-1 (Ovarian Adenocarcinoma), Ishikawa (Endometrial Adenocarcinoma) and Calu-3 (Lung Adenocarcinoma). VENTANA FOLR1 (FOLR1-2.1) reacted with a ~40kD band in cell lysates prepared from the KB cells (Epithelial Carcinoma) which expresses high levels of FOLR1 protein by IHC staining (3+); (Ovarian Adenocarcinoma), Igrov-1 cells (2+); and (Endometrial Adenocarcinoma) Ishikawa cells (1+). No band of this size was observed in the IHC negative Calu-3 (Lung Adenocarcinoma) cell line (0+). Independent confirmation of the relative expression levels was based on assessment of mRNA expression levels for FOLR1. No unexpected staining or background was observed in any of the whole cell lysates. An additional WB analysis was performed to ensure that VENTANA FOLR1 (FOLR1-2.1) antibody is specific for FOLR1 and does not cross-react with the other FOLR proteins. VENTANA FOLR1 (FOLR1 2.1) antibody showed no reactivity for human FOLR2 or FOLR3 proteins in the WB assay.

b. Blast Results for FOLR1 Epitope

An NCBI Blast search comparison of FOLR1 with FOLR2 and FOLR3 protein sequences showed that these proteins share 77-85% sequence identity. However, sequence analysis surrounding the Asn 69 N-linked glycosylation site in FOLR1 show this potential glycosylation site does not exist in FOLR2 or FOLR3, as the motif Asn-X-Ser/Thr is

absent in this region of FOLR2 and FOLR3. Both of these proteins do contain additional potential N-linked glycosylation sites downstream.

c. Immunoreactivity in Human Tissues [Tour of Body (TOB) and Tour of Tumor (TOT)]

The purpose of this study was to assess the analytical specificity (Tour of Body and Tour of Tumor) including non-specific staining, background and cross-reactivity of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay on non-neoplastic (TOB) and neoplastic tissue (TOT) samples. One lot each of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay and Negative Control reagent were used to stain slides containing multi-tissue arrays (TMA) of non-neoplastic and neoplastic tissue. Slides were evaluated by a FOLR1 trained pathologist for FOLR1 reactivity, acceptable background staining and stain intensity, and potential cross reactivity of the assay.

TOB: In this study, 128 non-neoplastic tissues along with 99 cores of non-neoplastic TMA and 2 Non-neoplastic Bladder Single-Tissue Cases were analyzed.

Result: FOLR1 reactivity was observed in 6 out of 128 non-neoplastic tissues, occurring in adrenal gland, kidney, and larynx tissues. FOLR1 reactivity was observed in 57 out of 148 neoplastic tissues, 0 of 2 individual bladder tissues, 4 of 99 TOB cores occurring in kidney and ovary tissues. FOLR1 reactivity was not observed in non-neoplastic bladder or parathyroid tissue cases. Results for non-neoplastic tissues are shown in the table below.

Table 7: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining of FFPE Non-neoplastic Tissues

Tissue	Number of positive/total cases	Tissue	Number of positive/total cases
Cerebrum	0/4	Stomach	0/4
Cerebellum	0/4	Small Intestine	0/4
Adrenal gland	1/4	Colon	0/4
Ovary	0/9	Liver	0/4
Pancreas	0/4	Salivary gland	0/4
Parathyroid gland	0/3	Kidney	4/4
Hypophysis	0/3	Prostate	0/4
Testis	0/4	Endometrium	0/4
Thyroid	0/4	Cervical	0/4
Breast	0/4	Skeletal Muscle	0/3
Spleen	0/3	Skin	0/4
Tonsil	0/3	Peripheral (Nerve)	0/3
Thymus gland	0/3	Mesothelium	0/3
Myeloid (Bone)	0/3	Retina	0/3
Lung	0/4	Larynx	1/3
Heart	0/3	Bladder	0/3
Esophagus	0/4	Rectal	0/1

TOT: In this study, 148 neoplastic tissues along with 95 cores of neoplastic TMA were analyzed.

Result: FOLR1 reactivity was observed in 57 out of 148 neoplastic cores/cases and 4 of 95 cores from the TOT arrays. Results for neoplastic tissues are shown in **Table 8.**

Table 8: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining of FFPE Neoplastic Tissues

Pathology	Number of positive/ total cases
Meningioma, fibroblastic (Cerebrum)	0/1
Astrocytoma (Cerebrum)	0/1
Meningioma, fibroblastic (Cerebellum)	0/1
Malignant meningioma (Cerebellum)	0/1
Adenoma, cortical (Adrenal Gland)	0/1
Adrenocortical carcinoma (Adrenal Gland)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/2
Follicular carcinoma (Thyroid)	0/1
Follicular papillary adenocarcinoma (Thyroid)	0/1
Fibroadenoma (Breast)	0/2
Invasive ductal carcinoma (Breast)	0/3
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	0/1
Small cell carcinoma (Lung)	0/1
Metastatic cancer from gastrointestinal site (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/3
Adenocarcinoma (Stomach)	0/3
Adenoma (Small Intestine)	0/1
Adenocarcinoma (Small Intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Hepatocellular carcinoma (Liver)	0/4
Metastatic colon adenocarcinoma (Liver)	0/1
Pleomorphic adenoma (Salivary Gland)	0/1
Adenoid cystic carcinoma (Salivary Gland)	0/1
Adenocarcinoma (Oral Cavity)	0/1
Squamous cell carcinoma (Oral Cavity)	0/1
Nasopharyngeal carcinoma, NPC (Nasopharynx)	0/1
Melanoma (Nasal cavity)	0/1
Clear cell carcinoma (Kidney)	1/2
Adenocarcinoma (Prostate)	0/2
Adenocarcinoma (Endometrium)	0/2
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Transitional cell carcinoma (Bladder)	0/2
Adenocarcinoma (Rectum)	0/3
Reactive (Lymph node)	0/1

Hodgkin lymphoma (Lymph node)	0/1
Non-Hodgkin B-cell lymphoma (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/2
Metastatic breast ductal carcinoma (Lymph node)	0/1
Metastatic esophagus squamous cell carcinoma (Lymph node)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Endometrioid adenocarcinoma (Ovary)	9/16
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Serous adenocarcinoma (Ovary)	39/42
Clear cell carcinoma (Ovary)	5/8
Mucinous adenocarcinoma (Ovary)	3/10

3. Precision:

Precision of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay on BenchMark ULTRA was evaluated in three studies: Intermediate Precision study, Reader (pathologist) Precision study and Inter-Laboratory and Inter-Reader Precision (Reproducibility) study.

a. Intermediate Precision

Twenty-four unique EOC tissue specimens were enrolled (12 FOLR1 positive and 12 FOLR1 negative) in the intermediate precision study. The study design for evaluation of precision of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay included:

- Three lots of FOLR1 antibody
- Three BenchMark ULTRA instruments
- Three OptiView DAB IHC Detection Kits
- Study performed over three non-consecutive days
- One pathologist reader
- 2 replicates per condition

All slides were blinded and randomized and then evaluated using the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay scoring algorithm specified in table 5 above. Each case had 18 results and a majority FOLR1 result was assigned based on 18 results. For each case, the median %TC and the range of %TC of 18 results were calculated. In addition, percent positive (%TC≥75%, "Eligible" with regard to FOLR1 therapy) results was calculated. Results are summarized in the tables below.

Table 9. Median and Range of %TC for Samples in the Intermediate Precision Study

Sample ID	Majority FOLR1 Result	Median %TC	Range %TC (Min to Max)	Percent Positive Results	Percent Agreement with Majority FOLR1 Result
1	Negative	10.0	10 to 10	0 (0/18)	100 (18/18)
2	Negative	20.0	20 to 25	0 (0/18)	100 (18/18)
3	Negative	25.0	25 to 25	0 (0/18)	100 (18/18)
4	Negative	25.0	25 to 25	0 (0/18)	100 (18/18)
5	Negative	30.0	25 to 30	0 (0/18)	100 (18/18)
6	Negative	35.0	35 to 35	0 (0/18)	100 (18/18)

7	Negative	45.0	45 to 50	0 (0/18)	100 (18/18)
8	Negative	45.0	45 to 45	0 (0/18)	100 (18/18)
9	Negative	50.0	45 to 50	0 (0/18)	100 (18/18)
10	Negative	55.0	55 to 55	0 (0/18)	100 (18/18)
11	Negative	65.0	60 to 75	5.6 (1/18)	94.4 (17/18)
12	Negative	70.0	60 to 70	0 (0/18)	100 (18/18)
13	Positive	75.0	70 to 75	94.4 (17/18)	94.4 (17/18)
14	Positive	80.0	80 to 85	100 (18/18)	100 (18/18)
15	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
16	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
17	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
18	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
19	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
20	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
21	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
22	Positive	90.0	90 to 90	100 (18/18)	100 (18/18)
23	Positive	95.0	95 to 95	100 (18/18)	100 (18/18)
24	Positive	98.0	98 to 98	100 (18/18)	100 (18/18)

Table 10: Precision Components for Samples in Intermediate Precision Study

					Standard Deviation					
Sample ID	**Majority Call FOLR1 Result**	**Number of Results**	**Median %TC**	**Range %TC (Min to Max)**	**Repeatability (Within Run)**	**Between Day**	**Between Antibody Lot**	**Between Detection Kit**	**Between Instrument**	**Total**
1	Negative	18	10.0	10 to 10	0	0	0	0	0	0
2	Negative	18	20.0	20 to 25	1.16	0	0	0	0	1.16
3	Negative	18	25.0	25 to 25	0	0	0	0	0	0
4	Negative	18	25.0	25 to 25	0	0	0	0	0	0
5	Negative	18	30.0	25 to 30	0	0	0	0	1.01	1.01
6	Negative	18	35.0	35 to 35	0	0	0	0	0	0
7	Negative	18	45.0	45 to 50	0	1.00	1.00	0	0	1.42
8	Negative	18	45.0	45 to 45	0	0	0	0	0	0
9	Negative	18	50.0	45 to 50	1.16	0	0	0	0	1.16
10	Negative	18	55.0	55 to 55	0	0	0	0	0	0
11	Negative	18	65.0	60 to 75	0	2.21	0	0.81	0	2.36
12	Negative	18	70.0	60 to 70	0	0	0	0	2.01	2.01
13	Positive	18	75.0	70 to 75	0	1.29	0	0	0	1.29
14	Positive	18	80.0	80 to 85	0	0	0	0.85	0	0.85
15	Positive	18	90.0	90 to 90	0	0	0	0	0	0
16	Positive	18	90.0	90 to 90	0	0	0	0	0	0
17	Positive	18	90.0	90 to 90	0	0	0	0	0	0
18	Positive	18	90.0	90 to 90	0	0	0	0	0	0
19	Positive	18	90.0	90 to 90	0	0	0	0	0	0
20	Positive	18	90.0	90 to 90	0	0	0	0	0	0

					Standard Deviation					
Sample ID	Majority Call FOLR1 Result	Number of Results	Median %TC	Range %TC (Min to Max)	Repeatability (Within Run)	Between Day	Between Antibody Lot	Between Detection Kit	Between Instrument	Total
21	Positive	18	90.0	90 to 90	0	0	0	0	0	0
22	Positive	18	90.0	90 to 90	0	0	0	0	0	0
23	Positive	18	95.0	95 to 95	0	0	0	0	0	0
24	Positive	18	98.0	98 to 98	0	0	0	0	0	0

In addition, a qualitative analysis of different components was performed. Results are summarized in the table below.

Table 11: Intermediate Precision of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between- Antibody Lots	PPA	72/72	100.0	(94.9, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	144/144	100.0	(97.4, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	72/72	100.0	(94.9, 100.0)
	NPA	71/72	98.6	(97.2, 100.0)
	OPA	143/144	99.3	(98.6, 100.0)
Between-Detection Kits	PPA	71/72	98.6	(97.2, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	143/144	99.3	(98.6, 100.0)
Between-Day	PPA	71/72	98.6	(97.2, 100.0)
	NPA	71/72	98.6	(97.2, 100.0)
	OPA	142/144	98.6	(97.2, 100.0)
Within-Run	PPA	107/108	99.1	(98.1, 100.0)
	NPA	107/108	99.1	(98.1, 100.0)
	OPA	214/216	99.1	(98.1, 100.0)

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA)

b. Reader Precision Study

In the Reader Precision study for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, Within-Reader and Between-Reader components of precision for EOC tissue reads were evaluated. The study included 100 unique EOC specimens (50 FOLR1 positive and 50 FOLR1 negative) that were stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Specimens were blinded and randomized prior to evaluation for FOLR1 status using the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay scoring algorithm specified in table 5 above. The study included three readers (pathologists). Readers scored all sample twice, with a minimum of two-week wash-out period between reads. Each sample had 6 reads (2 reads by each of three readers). Variability of %TC values for 100 cases was evaluated and following precision components were calculated: within-reader, between-reader and total. Results are summarized in the tables 12 and 13 below:

Table 12: Precision Components for Samples in Reader Precision Study

				Standard Deviation			
Sample Category	**#Samples**	**# Read**	**Range of Median %TC**	**Within Reader**	**Between Reader**	**Total**	**Percent Positive Results**
Negative	30	180	0 to 20	3.57	2.23	4.21	0.0 (0/180)
	7	42	21 to 40	12.1	8.68	14.9	0.0 (0/42)
	6	36	41 to 64	8.36	9.44	12.6	0.0 (0/36)
Borderline Negative	7	42	65 to 74	7.82	10.6	13.2	14.3 (6/42)
Borderline Positive	17	102	75 to 85	5.75	6.77	8.88	90.2 (92/102)
Positive	22	132	86 to 95	6.52	5.21	8.35	99.2 (131/132)
	11	66	96 to 100	2.58	4.55	5.24	100.0 (66/66)

In addition, a qualitative analysis of different precision components was performed. The agreement rates for these studies are summarized in the table 13 below.

Table 13: Within-Reader and Between-Reader Precision of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Precision	**Agreement**			
	Type	**n/N**	**%**	**95% CI**
Within-Reader	APA	286/295	96.9	(95.1, 98.6)
	ANA	296/305	97.0	(95.1, 98.7)
	OPA	291/300	97.0	(95.0, 98.7)
Between-Reader	APA	276/296	93.2	(89.4, 96.8)
	ANA	284/304	93.4	(89.9, 96.8)
	OPA	280/300	93.3	(90.0, 96.7)

Average Positive Agreement (APA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA)

4. External Reproducibility (Inter-Laboratory) Study

The Inter-laboratory Reproducibility (ILR) study for the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay was conducted to evaluate reproducibility of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay on the BenchMark ULTRA. The study included 28 EOC samples (14 FOLR1 positive and 14 FOLR1 negative) run across three BenchMark ULTRA instruments on each of 5 non-consecutive days at three external laboratories. Each set of 5 stained slides per sample per staining day was randomized and evaluated by a total of 12 readers (4 readers per site). Each case had 20 results per site (60 results in total). Data showed that the performance of one of 12 readers (8.3%) was significantly different from other eleven readers. Performance of 11 readers (4 readers at site A, 3 readers at site B, and 4 readers at site C) was evaluated and following precision components were calculated: between-reader, between-day, between-site and total. Results are presented in the table below.

Table 14. Precision Components for Samples in the Inter-Laboratory Reproducibility Study

				Standard Deviation (SD)				Percent Positive Results			
Sample ID	Majority Call, FOLR1 Status	Median %TC	Range %TC (Min to Max)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Overall
1	Negative	0.0	0 to 85	1.3	0.0	0.0	1.3	5% (1/20)	0% (0/15)	0% (0/20)	2% (1/55
2	Negative	10.0	3 to 25	3.4	1.5	0.0	3.8	0% (0/20)	0% (0/15)	0% (0/20)	0.0% (0/55)
3	Negative	25.0	5 to 60	0.0	0.0	10.4	10.4	0% (0/20)	0% (0/15)	0% (0/20)	0.0% (0/55)
4	Negative	25.0	5 to 50	5.5	0.0	9.0	10.6	0% (0/20)	0% (0/15)	0% (0/20)	0.0% (0/55)
5	Negative	40.0	15 to 70	0.0	0.0	7.2	7.2	0% (0/20)	0% (0/15)	0% (0/20)	0.0% (0/55)
6	Negative	45.0	20 to 70	0.0	0.0	2.0	2.0	0% (0/20)	0% (0/15)	0% (0/20)	0.0% (0/55)
7	Negative	50.0	30 to 75	2.1	0.0	5.4	5.8	15% 3/20	0% (0/15)	0% (0/20)	5% (3/55
8	Negative	50.0	20 to 75	1.7	3.2	5.2	6.3	10% (2/20)	0% (0/15)	0% (0/20)	4% (2/55
9	Negative	50.0	15 to 75	3.3	0.0	4.7	5.8	10% (2/20)	0.0% (0/15)	0.0% (0/20)	4% (2/55
10	Negative	50.0	0 to 75	8.9	12.1	18.3	23.7	10% (2/20)	0% (0/15)	0% (0/20)	4% (2/55
11	Negative	60.0	25 to 85	3.9	1.8	7.2	8.4	25% (5/20)	0% (0/15)	0% (0/20)	9% (5/55
12	Negative	60.0	40 to 75	1.9	0.0	0.0	1.9	5% (1/20)	0% (0/15)	0% (0/20)	2% (1/55
13	Negative	60.0	30 to 75	0.4	0.0	0.0	0.4	35% (7/20)	0% (0/15)	0% (0/20)	13% (7/55
14	Negative	65.0	22 to 80	1.3	8.0	0.0	8.1	20% (4/20)	0.0% (0/15)	35% (7/20)	20% (11/55)
15	Positive	75.0	55 to 100	8.8	0.0	14.4	16.8	90% (18/20)	80% (12/15)	35% (7/20)	67% (37/55
16	Positive	75.0	40 to 95	12.0	0.0	12.6	17.4	80% (16/20)	73% (11/15)	40% (8/20)	64% (35/55
17	Positive	75.0	40 to 95	11.9	4.2	20.1	23.7	75% (15/20)	80% (12/15)	50% (10/20)	67% (37/55
18	Positive	80.0	0 to 90	3.6	23.9	20.3	31.6	95% (19/20)	93% (14/15)	58% (11/19)	81% (44/54)

				Standard Deviation (SD)				Percent Positive Results			
Sample ID	Majority Call, FOLR1 Status	Median %TC	Range %TC (Min to Max)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Overall
19	Positive	80.0	75 to 100	5.0	0.0	10.7	11.8	100% (20/20)	100% (15/15)	100% (20/20)	100% (55/55)
20	Positive	80.0	65 to 95	3.4	0.0	7.4	8.1	100% (20/20)	93% (14/15)	80% (16/20)	91% (50/55)
21	Positive	85.0	70 to 100	8.1	0.0	13.3	15.5	100% (20/20)	100% (15/15)	85% (17/20)	95% (52/55)
22	Positive	90.0	70 to 100	3.9	1.8	8.4	9.4	100% (20/20)	100% (15/15)	95% (19/20)	98% (54/55)
23	Positive	90.0	75 to 100	5.3	0.0	2.4	5.9	100% (20/20)	100% (15/15)	100% (20/20)	100% (55/55)
24	Positive	90.0	80 to 98	6.4	1.2	7.4	9.9	100% (20/20)	100% (15/15)	100% (20/20)	100% (55/55)
25	Positive	90.0	0 to 100	0.0	2.5	0.0	2.5	95% (19/20)	100% (15/15)	100% (20/20)	98% (54/55)
26	Positive	95.0	75 to 100	2.2	0.0	2.3	3.2	100% (20/20)	100% (15/15)	100% (20/20)	100% (55/55)
27	Positive	95.0	60 to 100	1.7	0.0	0.0	1.7	100% (20/20)	93% (14/15)	100% (20/20)	98% (54/55)
28	Positive	95.0	0 to 100	2.6	23.7	3.2	24.1	100% (20/20)	100% (15/15)	80% (16/20)	93% (51/55)

Performance for 28 cases by 11 readers is also summarized in the table below:

Table 15: Percent of Positive and Negative FOLR1 Results for Different Ranges of %TC

%TC Range (Median Values)	Number of Samples	Percent Positive Results	Percent Negative Results
<50	6	0.3%	99.7%
(50-75)	8	7.5%	92.5%
75	3	66.1%	33.9%
(75-85)	4	89.9%	10.1%
>85	7	99.2%	0.8%

Performance of one of the twelve readers was significantly different from the other 11 readers which showed a high percent of positive results for slides with median %TC values larger than 40%. In addition, a qualitative analysis of different precision components was performed for 28 cases and 12 readers. Results of the analysis are summarized in the table below.

Table 16. Inter-Laboratory Reproducibility for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

		Agreement for 11 Readers			Agreement for 12 Readers		
External Reproducibility	**Type**	**n/N**	**%**	**95% CI**	**n/N**	**%**	**95% CI**
Overall*	PPA	688/769	89.5	(82.6, 95.6)	758/839	90.3	(84.0, 95.9)
	NPA	736/770	95.6	(94.0, 97.0)	763/840	90.8	(88.3, 93.3)
	OPA	1424/1539	92.5	(89.0, 95.6)	1521/1679	90.6	(87.1, 93.7)
Within-Site	PPA	678/739	91.7	(86.3, 96.2)	748/809	92.5	(87.5, 96.5)
	NPA	756/800	94.5	(92.2, 96.5)	783/870	90.0	(87.3, 92.7)
	OPA	1434/1539	93.2	(90.1, 95.8)	1531/1679	91.2	(88.2, 93.9)
Within-Reader	PPA	696/734	94.8	(91.9, 97.3)	805/849	94.8	(92.3, 97.0)
	NPA	779/805	96.8	(95.6, 97.9)	800/830	96.4	(95.3, 97.4)
	OPA	1475/1539	95.8	(94.2, 97.3)	1605/1679	95.6	(94.1, 97.0)

Positive Percent Agreement (PPA), Negative Positive Agreement (NPA), Overall Percent Agreement (OPA).
*0.06% (1 out of 1680) results was not evaluable.

In addition, pairwise comparison calculations were performed for Between-Site, Between-Reader and Between-Day for FOLR1 status. The data in the table below indicates VENTANA FOLR1 (FOLR1-2.1) RxDx Assay reproducibility across 3 days, 3 sites, and 12 readers.

Table 17: External reproducibility, Pairwise Comparison Results

External Reproducibility	**Agreement**			
	Type	**n/N**	**%**	**95% CI**
Between-Sites	APA	(27990/33362	83.9	(77.5, 89.1)
	ANA	(28386/33758	84.1	(79.7, 88.4)
	OPA	(28188/33560	84.0	(78.7, 88.7)
Between-Readers	APA	(2134/2505	85.2	(79.5, 89.9)
	ANA	(2158/2529)	85.3	(81.2, 89.4)
	OPA	(2146/2517)	85.3	(80.5, 89.6)
Between-Days	APA	(3088/3337	92.5	(89.5, 95.1)
	ANA	(3126/3375	92.6	(90.5, 94.8)
	OPA	(3107/3356	92.6	(90.1, 94.9)

5. Robustness:

a. Tissue Thickness

Tissue thickness was evaluated using 5 unique EOC specimens. Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. A 4-micron thick sample was used as a reference for each case. Three, 4-, 5-, 6- and 7-micron thick sections demonstrated concordant FOLR1 status and acceptable background staining when stained with VENTANA FOLR1 (FOLR1-

2.1) RxDx Assay and compared to the reference of 4 microns. Sections that were 2 microns thick exhibited a change in FOLR1 status compared to the reference. Specimens should be cut at 4 microns for staining with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

b. Protocol Limitations and Failure Modes:

The purpose of this study was to identify protocol conditions (protocol parameters and simulated dispense errors) that might lead to a potential false positive, false negative, or unacceptable result and prevent these conditions from affecting the end user.

The test conditions included

- One lot of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay
- One lot of OptiView DAB IHC Detection Kit
- Three BenchMark ULTRA instrument systems
- Replicates: 2 per condition stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay
- One Negative Reagent Control per case per test condition
- One SIR Slide
- One Pathologist (Reader)
- Six EOC samples (Three FOLR1 positive cases (including one borderline positive case) and three FOLR1 negative cases (including one borderline negative case) were enrolled

Parameters tested included Offline baking, On Instrument Baking (Low/mid/high), Counterstain and Post-Counterstain (Bluing, Hematoxylin II, Bluing/Hematoxylin II Stacked) for different time durations, Multimer Over/Under Dispense and Peroxidase Inhibitor Over/Under Dispense and Antibody Over/Under Dispense at different concentrations (1/2, 1/4, 2X or 4X). Other assay parameters were locked and not used as test conditions in this study.

The result of each test sample was compared to its respective sample stained with the final locked staining protocol. Based on the data, there were differences in staining results between the final locked staining protocol parameters and the modified parameters. Therefore, the final staining protocol specified in Table 4should be followed. Refer to the Labeling (Package Insert) for additional instructions.

6. Stability Studies:

a. Cut Slide Stability

This study evaluated the stability of FOLR1 antigen in FFPE tissue that had been sectioned onto positively charged glass microscope slides and stored for an extended duration of time at 5±3°C and 30±5°C. Cut slide stability was evaluated on seven EOC samples with staining that spanned the range of FOLR1 expression. Slides sectioned and stained at the Day 0 time point served as the baseline comparator for the remainder of the time points tested. Tissue was sectioned from each of the seven cases and separated into two different storage conditions for the duration of the study. One set of slides was stored at the refrigerated temperature condition (5±3°C) and one at the incubator temperature condition (30±5°C). Slides were stained at each pre-defined designated time

and staining results for each time point were compared to the Day 0 baseline slides. Based on the study results, the recommended cut slide stability is 45 days at both the 5±3ºC and 30±5ºC storage conditions.

b. Reagent/Ship Stress Stability

The objective of this study was to assess the stability (shelf-life and in-use) and shipping category of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Three final lots of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay were subjected to different stress conditions and then placed at the intended storage condition (2-8°C). The conditions tested were as follows:

i. Intended Storage (2-8°C)
ii. Hot Ship Stress Cat. A 33°C±3ºC 192 hours)
iii. Hot Ship Stress Cat. B 18°C±3ºC 192 hours)
iv. Hot Ship Stress Cat. F 37°C±2ºC 192 hours)
v. Cold Ship Stress Freeze/Thaw (-20°C±5ºC 192 hours)
vi. Cold Ship Stress Freeze/Thaw (-25°C±5ºC 192 hours)

Based on the study results, the stability dating for the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay device is 24 months when stored at 2-8°C and shipping categories A and F.

c. Real-time and Ship Stress Stability of the VENTANA FOLR1 Stain Intensity Reference (Normal Fallopian Tube) Slide:

This study evaluated the stability of IHC staining intensity of the DAB signal across a range of staining on normal fallopian tube tissue when stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. The study design encompassed 14 test conditions [type of storage container (plastic box, card box slide storage box, open slide flat), type of coverslip types (glass, film), type of lighting (fluorescent, dark, intermittent exposure)], 9 total normal fallopian tube tissue samples with two replicates, 1 reader, 1 lot of the VENTANA FOLR1 (FOLR1-2.1) RxDx antibody, 2 lots of OptiView DAB IHC Detection Kit, 6 BenchMark ULTRA Instruments and 9 testing time points; Slides were stained and read at Day 0 and the same slides were read again at months between 1 thru 6, and month 9 and month 12.

Acceptance criteria was as follows: At each time point, slides shall exhibit assay overall stain intensity that is equal to but no more than 1.0 point different compared to the Day 0 stained slide score.

Acceptance criteria for this study were met. Based on the study results, the stability dating for the SIR slide is 9 months when stored in room temperature conditions (15-30˚C).

B. Animal Studies

None

C. Additional Studies

1. Tissue Heterogeneity

This study evaluated the prevalence of tissue heterogeneity in EOC tissue blocks from the same case (multiple blocks from the same case, as well as heterogeneity within a block) when stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay on the BenchMark ULTRA instrument.

a. Within-Block Heterogeneity

The intent of this study was to evaluate FOLR1 tumor cell expression level across multiple sections from the same tissue block when stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Eight FFPE EOC samples encompassing the FOLR1 expression range from negative to positive were enrolled in the study. The case distribution consisted of 3 positive cases (all borderline) and 5 negative cases (inclusive of 1 borderline negative case). For each block, approximately every 10th section was stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Cases were sectioned to exhaustion. Six out of eight cases maintained the FOLR1 expression level throughout the block. Both cases with inconsistent FOLR1 expression level were borderline cases - 1 borderline positive and 1 borderline negative. This demonstrates that some heterogeneity may be observed in the FOLR1 expression level within each EOC tissue block from the same case when stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

b. Within-Case Heterogeneity

The intent of this study was to evaluate EOC case heterogeneity when multiple blocks from the same patient case were stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Twenty-seven cases with two blocks per case and 2 cases with four blocks per case were evaluated in this study. Twenty-six 26) out of 29 patient cases (89.7%) displayed no case level heterogeneity for the 75% cutoff. Case level heterogeneity was observed in 3 out of 29 cases (10.3%) for the 75% cut-off. This demonstrates that variation may be observed in the FOLR1 expression level of different EOC tissue blocks from the same patient case when stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

2. Impact of Tissue Specimen Preparation and Treatment Studies/ Effect of Fixative Type, Time and Delay to Fixation on FOLR1 antigenicity in DU-145 Xenografts:

a. Ischemia Study (Time to Fixation)

The objective of this study was to evaluate the effects of ischemic time on FOLR1 antigenicity as detected by staining with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. This study examined the effects of delay to fixation (Ischemia) for DU-145 xenograft samples at zero hours, 0.5 hours, 1 hour, 2 hours, 6 hours, and 24 hours post excision. All samples were fixed in 10% NBF for 24 hours after being delayed for fixation at their various ischemia time points. The study demonstrated concordant FOLR1 staining results for all samples tested. However, it is recommended that samples are fixed immediately in 10% NBF.

b. Fixation Study

The objective of this study was to evaluate the effects of fixative type and fixation time on FOLR1 antigenicity as detected by staining with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Six DU-145 Xenograft blocks were fixed for 1, 6, 12, 24, 48 and 72 hours in 6 fixatives: 10%

NBF, Zinc formalin, 95% alcohol, AFA, Z-5, and PREFER for a total of 36 samples. The data were then compared to the reference standard of 10% NBF for 24 hours.
Tissues fixed in AFA, PREFER fixative, 95% EtOH and zinc formalin were unacceptable when stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay and therefore are not recommended fixatives. Based on results of this study, specimens for testing with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay should be fixed immediately post-excision in 10% NBF for 12-72 hours.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The objective of this study was to evaluate the performance of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay as a companion diagnostic (CDx) device to identify patients for treatment with ELAHERE (mirvetuximab soravtansine) in patients with platinum-resistant epithelial ovarian cancer, primary peritoneal, or fallopian tube cancer.

A. Study Design

Immunogen Study IMGN853-0417 (NCT04296890) was a Phase 3, single-arm trial to evaluate the efficacy and safety of ELAHERE in patients with Folate Receptor (FRα) positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer. Patients were permitted to receive up to three prior lines of systemic therapy. All patients were required to have received prior bevacizumab. The trial enrolled patients whose tumors were positive for FRα expression (i.e., ≥ 75% of viable TC demonstrated FOLR1 membrane staining at moderate and/or strong staining intensity) as determined by the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay at a central site. The efficacy evaluable population included 104 patients with platinum-resistant disease, who had measurable disease, and received at least one dose of ELAHERE.

1. Clinical Inclusion and Exclusion Criteria

a. Key Trial Inclusion Criteria

Patients had to meet all the following criteria to enter the enrollment phase and receive ELAHERE in this study:

i. Patients must have a confirmed diagnosis of high-grade serous epithelial ovarian, fallopian tube or primary peritoneal cancer
ii. Patients must have had platinum-resistant disease
iii. Patients must have progressed radiographically on or after their most recent line of anticancer therapy
iv. Patients must be willing to provide an archival tumor tissue block or slides, or undergo procedure to obtain a new biopsy using a low-risk, medically routine procedure for immunohistochemistry (IHC) confirmation of FRα positivity
v. Patient's tumor must be positive for FRα expression as defined by the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay
vi. Patients must have received at least 1 but no more than 3 prior systemic lines of anticancer therapy

b. Key Trial Exclusion Criteria

Patients who met any of the following criteria were excluded from study enrollment:

i. Patients with endometrioid, clear cell, mucinous, or sarcomatous histology, mixed tumors containing any of the above histologies, or low-grade/borderline ovarian tumor
ii. Patients with primary platinum-refractory disease, defined as disease that did not respond to (CR or PR) or has progressed within 3 months of the last dose of first-line platinum-containing chemotherapy
iii. Patients with prior wide-field radiotherapy (RT) affecting at least 20% of the bone marrow
iv. Patients with > Grade 1 peripheral neuropathy per Common Terminology Criteria for Adverse Events (CTCAE)
v. Patients with a history of other malignancy within 3 years prior to enrollment

2. Follow-up Schedule

The median follow-up time was approximately 3 months.

3. Primary Clinical Efficacy Endpoints

Primary objective of the IMGN853-0417 study was to evaluate the efficacy of ELAHERE in patients with platinum-resistant epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer and high FRα expression by assessing the ORR, which includes best response of complete response (CR) or partial response (PR) as assessed by the Investigator.

B. Accountability of PMA Cohort

A total of 467 patients were screened, and a total of 106 patients were enrolled into IMGN853-0417 study. Of the 467 patients screened for entry into Study IMGN853-0417, 25 cases failed enrollment criteria associated with study IMGN853-0417 prior to testing and 4 cases were associated with a diagnostic testing (Dx) screen failure (cases did not have an acceptable H&E slide). Tissue specimens from the remaining 438 patients were tested with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Out of the 438 patients, 332 patients were not enrolled because they failed enrollment criteria related to Study IMGN853-0417, 1 patient was enrolled who did not have efficacy results and one patient was excluded from the efficacy evaluable population as this patient did not have platinum-resistant disease. The remaining 104 cases comprised the efficacy population. Patient accountability is summarized in the below table.

Table 18: Accountability of IMGN853-0417 Study PMA Cohort

Patient Disposition for Study IMGN853-0417	N
Total number of patients screened	**467**
-Samples did not meet the study eligibility criteria (prior to testing)	25
-Dx testing screen failure (case did not have an acceptable H&E slide)	4
Number tested with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay	**438**
Failed enrollment criteria related to ImmunoGen Study IMGN853-0417	334
Final number of patients in the trial	**104**

Sixty-six cases were associated with a Dx protocol deviation (specimen slides were scored based on digital slide image reads instead of glass slides). These 66 cases were re-scored using glass slides by same readers at same sites (as in digital reads), with a minimum wash-out period of 2 months. Pairwise comparison was used to calculate the agreement rates between the digital image reads and glass slide reads. The overall concordance between the digital image reads and glass slide reads was 93.9 % (95% CI: 87.7, 98.5). Of the 66 patients who were screened using digital reads, 29 were FOLR1-positive. Of those 29 patients, 27 were enrolled to the trial, with the other 2 being excluded due to other inclusion/exclusion criteria not related to FOLR1 clinical status. None of the remaining 37 patients were enrolled into the clinical trial.

C. Study Population Demographics and Baseline Parameters

Table below summarizes patient demographic and specimen characteristic information.

Table 19: Study Population Demographics and Baseline Parameters-Patient Characteristic

	Enrolled (N=104)	Not Enrolled (N=334)	Overall (N=438)
Age (years)			
Mean (SD)	61.7 (9.73)	62.5 (10.43	62.3 (10.27
Median	62	63	63
Min - Max	35 - 85	30 - 87	30 - 87
Missing	0	4	4
Age Group			
18-64	59 (56.7%	182 (54.5%	241 (55.0%
>=65	45 (43.3%	148 (44.3%	193 (44.1%
Missing	0	4 (1.2%	4 (0.9%
Sex			
Female	104 (100.0%	334 (100.0%	438 (100.0%
Race			
Asian	2 (1.9%	10 (3.0%	12 (2.7%
Black Or African American	0	7 (2.1%	7 (1.6%
White	100 (96.2%	276 (82.6%	376 (85.8%
Not Reported	2 (1.9%	30 (9.0%	32 (7.3%
Missing	0	11 (3.3%	11 (2.5%
Ethnicity			
Hispanic or Latino [b]	2 (1.9%	18 (5.4%	20 (4.6%

Not Hispanic or Latino	97 (93.3%	276 (82.6%	373 (85.2%
Unknown	1 (1.0%	4 (1.2%	5 (1.1%
Not Reported	4 (3.8%	25 (7.5%	29 6.6%)
Missing	0	11 (3.3%	11 (2.5%
Stage at Initial Diagnosis			
IA	0	2 (0.6%	2 (0.5%
IB	0	3 (0.9%	3 (0.7%
IC	2 (1.9%	10 (3.0%	12 (2.7%
IIA	0	2 (0.6%	2 (0.5%
IIB	0	3 (0.9%	3 (0.7%
IIC	0	6 (1.8%	6 (1.4%
IIIA	5 4.8%)	23 (6.9%	28 (6.4%
IIIB	5 (4.8%	20 (6.0%	25 (5.7%
IIIC	51 (49.0%	149 (44.6%	200 (45.7%
IV	40 (38.5%	92 (27.5%	132 (30.1%
Missing	1 (1.0%	24 (7.2%	25 (5.7%
Histology at Diagnosis			
Carcinosarcoma	0	1 (0.3%	1 (0.2%
Clear Cell	0	1 (0.3%	1 (0.2%
Endometrioid	0	5 (1.5%	5 (1.1%
High Grade Serous	104 (100.0%	274 (82.0%	378 (86.3%
Low Grade Serous	0	7 (2.1%	7 (1.6%
Mucinous	0	1 (0.3%	1 (0.2%
Serous Adenocarcinoma	0	18 (5.4%	18 (4.1%
Squamous	0	2 (0.6%	2 (0.5%
Other	0	14 (4.2%	14 (3.2%
Missing	0	11 (3.3%	11 (2.5%
Sample Characteristic			
Sample Collection Method			
Biopsy	19 (18.3%	88 (26.3%	107 (24.4%
Excision/Resection	85 (81.7%	246 (73.7%	331 (75.6%
Tumor Type			
Locoregional Recurrence	22 (21.2%	82 (24.6%	104 (23.7%
Metastasis	8 (7.7%	47 (14.1%	55 (12.6%
Primary	74 (71.2%	205 (61.4%	279 (63.7%
Location (Sample Origin of Primary Tumor)			
Fallopian Tube	5 (4.8%	12 (3.6%	17 3.9%)
Ovary	53 (51.0%	160 (47.9%	213 (48.6%
Peritoneum	15 (14.4%	29 (8.7%	44 (10.0%
Not Applicable*	31 (29.8%	133 (39.8%	164 (37.4%

[a] Patients are classified as Hispanic or Latino if they are Mexican/Hispanic American, Mexican National, Central American, Puerto Rican, Cuban, South American, Caribbean, or Other Hispanic or Latino Origin

*Metastasis or Locoregional Recurrence

C. Safety and Effectiveness Results

1. Safety Results

No adverse events associated with use of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay under study IMGN853-0417 protocol occurred during the clinical study.

2. Effectiveness Results

The major efficacy outcome measures were investigator-assessed ORR (primary endpoint) and DOR (secondary endpoint) evaluated according to RECIST, version 1.1. The primary endpoint of ORR was calculated based on the Efficacy Evaluable (EE; n=104) population. The ORR was 31.7% (22.9, 41.6) with 4.8% of patients showing complete response and 26.9% of patients showing partial response. Considering the 66 patients who were screened using digital slide reading and then re-scored using glass slides, 3 patients had positive FOLR1-2.1 RxDx Assay results based on digital slide reading and negative results based on glass slides reading. Two of the 3 patients were included in the efficacy population. After excluding these 2 patients, the observed ORR for patients had positive FOLR1-2.1 RxDx Assay results based on glass slide reading was 32.4% (23.5, 42.2) with 4.9% of patients showing complete response and 27.5% of patients showing partial response. In addition, one patient with negative result based on digital slide image but positive result based on glass slide re-read may be eligible but was not enrolled into the Study 0417 and therefore not available for CDx efficacy analysis. Efficacy results for IMGN853-Study 0417 are summarized in Table 20.

Table 20: Efficacy Results from Study IMNG853-0417 by Investigator

	ELAHERE (N=104)[b]
Confirmed Overall Response [a] (n) (%; 95% CI)	N=33 (31.7%; 22.9, 41.6)
Complete response (n) (%)	5 (4.8%
Partial response (n) (%)	28 (26.9%
Duration of Response	N=33
Median Duration of Response, months 95% CI)	6.9 (5.6, 9.7)

[a] Investigator assessment

[b] The CDx efficacy population excluded 2 subjects from the drug efficacy population who are digital slide read positive, but glass slide re-read negative. In addition, one subject with digital slide read negative result but glass slide re-read positive result may be eligible but was not enrolled into the clinical trial and therefore not available for CDx efficacy analysis

Response assessment results using independent radiology review were consistent with investigator assessment.

3. Subgroup Analyses

There were no subgroup analyses performed in this trial.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

D. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study investigators did not report any financial conflicts of interest for this study.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA did not raise any new safety and effectiveness questions compared with information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The primary efficacy data in conjunction with the staining performance support the reasonable assurance of safety and effectiveness of use of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay as a companion diagnostic device for ELAHERE treatment in the target EOC patient population. The IMGN853-0417 primary efficacy analysis demonstrated a clinically meaningful ORR for patients with advanced stage EOC whose tumors have high FOLR1 expression as determined by the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. The performance of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay was also supported by the analytical performance validation studies.

B. Safety Conclusions

The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above.

The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is an *in vitro* diagnostic device which is used to test FFPE tumor specimens collected from patients with EOC. No adverse events associated with the diagnostic testing procedure were reported during this study. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as the required biopsies are obtained using a medically routine sampling procedure that does not present a significant risk to the patient.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in the clinical study IMGN853-0417 conducted to support the PMA approval as described above. The clinical performance of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay was demonstrated in the clinical validation studies. As shown in Table 20 above, the ORR among patients selected by the VENTANA FOLR1 (FOLR1- 2.1) RxDx Assay in study IMGN853-0417 was 31.7% with a duration of response of 6.9 months. The studies demonstrated that VENTANA FOLR1 (FOLR1-2.1) RxDx assay appropriately and reproducibly detects FOLR1 antigen in EOC carcinoma tissue and can aid in the assessment of these patients being considered for treatment with ELAHERE.

The primary risk of the VENTANA FOLR1 (FOLR1-2.1) RxDx assay is obtaining a false result. A false positive result could lead to the treatment with reduced probability of benefit. This could unnecessarily expose the patient to toxicity of the drug. A false negative result could deprive a patient of the potential benefit of ELAHERE (mirvetuximab soravtansine) targeted treatment. There is also a risk of delayed results, which may lead to a delay in treatment with ELAHERE (mirvetuximab soravtansine) or other approved therapy depending on the FOLR1 testing result. In conclusion, given the available information above, the data support the use of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay for determination of eligibility for ELAHERE (mirvetuximab soravtansine) treatment in patients with advanced EOC who progressed on previous chemotherapy treatments, who are more likely to benefit from treatment with ELAHERE (mirvetuximab soravtansine) monotherapy, as the probable benefits outweigh the probable risks.

D. Patient Perspective

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

E. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The provided study data support the use of the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay as a companion diagnostic to identify patients with epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer, for treatment with (mirvetuximab soravtansine) ELAHERE.

XIV. CDRH DECISION

CDRH issued an approval order on November 14, 2022.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.