VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

REF 740-5065

07727917001

IVD Σ 50

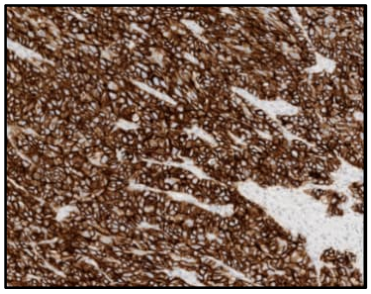

Figure 1. Ovarian carcinoma tissue stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

INTENDED USE

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is a qualitative immunohistochemical assay using mouse monoclonal anti-FOLR1, clone FOLR1-2.1, intended for laboratory 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.

SUMMARY AND EXPLANATION

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay utilizes a mouse monoclonal hybridoma antibody produced against a recombinant protein as a cell culture supernatant, and purified using protein-G. The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay antibody demonstrates cytoplasmic and membranous staining. However, only membrane staining is evaluated for the determination of FOLR1 status.

PRINCIPLE OF THE PROCEDURE

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay utilizes a mouse monoclonal primary antibody that binds to the FOLR1 protein in formalin-fixed, paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit. Refer to the OptiView DAB IHC Detection Kit package insert for further information. Results are interpreted using a light microscope.

Clinical cases must be evaluated with appropriate tissue controls. In addition to staining with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay antibody, a second slide should be stained with VENTANA Negative Control (Monoclonal). This slide must be negative for specific staining to be considered acceptable.

MATERIAL PROVIDED

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay contains approximately 28 µg of a mouse monoclonal (FOLR1-2.1) antibody.

The antibody is diluted in 0.05 M Tris-HCL with carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 5.6 µg/mL.

There is no known non-specific antibody reactivity observed in this product.

VENTANA FOLR1 (FOLR1-2.1) RxDx Assay utilizes a mouse monoclonal antibody produced as a cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet (package insert) for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials are required but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. Drying oven capable of maintaining a temperature of 60°C ± 5°C
5. Bar code labels
6. Xylene (Histological grade)
7. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
8. Deionized or distilled water
9. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
10. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
11. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
12. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
15. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
16. General purpose laboratory equipment
17. BenchMark ULTRA instrument
18. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
19. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
20. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
21. Light microscope
22. Absorbent wipes

STORAGE AND STABILITY

Upon receipt of the assay and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded (FFPE) tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for 6 to 72 hours.[1]

Please also refer to the 'Specific Limitations' section below.

Alcohol-formalin-acetic acid (AFA), 95% alcohol and Prefer fixatives demonstrated a loss of specific FOLR1 protein expression at all fixation times tested (1 to 72 hours), and are not recommended for use with this assay. Use of Zinc Formalin or Z-5 are not recommended due to variability in percent tumor cell staining.

Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of “Recommended Slide Storage and Handling” for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
7. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[2,3]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. For further information on the use of this device, refer to the BenchMark ULTRA instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, a reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark ULTRA instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must verify results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet (package insert) for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 740-5065.

Table 2. Recommended staining protocol for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay with OptiView DAB IHC Detection Kit on BenchMark ULTRA instruments.

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

* Selectable by customer

** Baking is optional. May be performed on-board the instrument or performed offline.

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, a second slide should be stained with the appropriate negative control reagent.

POSITIVE AND NEGATIVE TISSUE CONTROL

A tissue control must be included with each instrument run. This helps identify any failures applying reagents to slides on the staining run. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. See Figure 2. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known 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. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of a positive and negative control tissue for this antibody is normal Fallopian tube. FOLR1 expression is largely restricted to the luminal surface of the epithelial cells of the normal Fallopian tube. FOLR1 staining in normal Fallopian tube tissue exhibits circumferential membrane staining and absence of staining in the stroma. The VENTANA FOLR1 (FOLR1-2.1) RxDx Assay requires the use of a moderate circumferential staining case to use as a positive run control.

Table 3. Positive and negative control tissue evaluation for 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 normal fallopian tube FOLR1 staining.

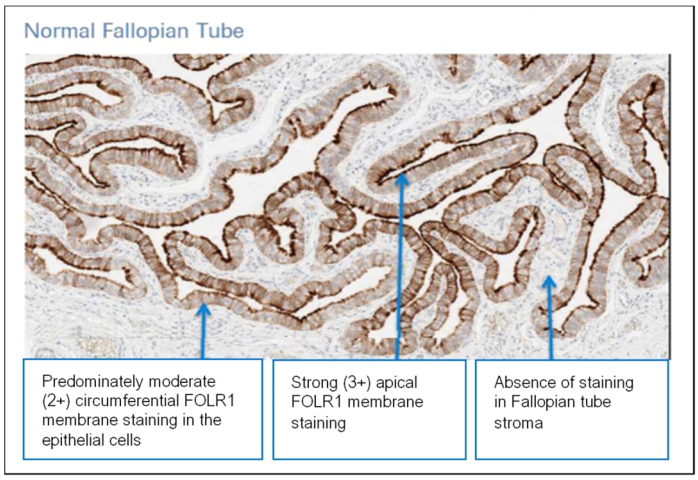

Figure 2. Acceptable Staining of Control Normal Fallopian Tube Tissue.

STAINING INTERPRETATION/EXPECTED RESULTS

The cellular staining pattern for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay is membranous and cytoplasmic in EOC 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). The percentage of tumor cells staining at each intensity [negative, weak (1+), moderate (2+), strong (3+)] will be assessed from specimens containing a minimum of approximately 100 viable tumor cells. Only moderate and strong stain intensities will contribute to the FOLR1 status determination using the scoring method. EOC tissue cases are considered positive for FOLR1 status if ≥ 75% of viable tumor cells (TC) demonstrate moderate and/or strong membrane staining. FOLR1 staining percentage at each intensity is determined by a trained pathologist. EOC tissue must be evaluated according to the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay scoring algorithm, provided in Table 4. Refer to the Interpretation Guide for (P/N 1015089US) for additional instructions and representative images.

Table 4. VENTANA FOLR1 (FOLR1-2.1) RxDx Assay scoring algorithm.

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

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. The patient's final result with regard to FOLR1 staining (positive and negative) should be obtained by either a majority rule or by consensus among the pathologists.

SPECIFIC LIMITATIONS

This antibody demonstrates the following specific limitations:

1. VENTANA FOLR1 (FOLR1-2.1) RxDx Assay has been optimized on the BenchMark ULTRA instrument in combination with the OptiView DAB IHC Detection Kit at a 32 minute primary antibody incubation time. Incubation times and temperatures other than those specified may give erroneous results.
2. Any deviation from recommended test procedures may invalidate test results. Users who deviate from recommended test procedures, as specified in Table 2, are responsible for validation of any modifications.
3. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
4. Tissue staining is dependent on the handling and processing of the tissue before staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or incorrect results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
5. Fixation of 6 - 72 hours in 10% Neutral Buffered Formalin (NBF) was evaluated in representative models. For small specimens, such as biopsies, fixation time of 6 hours or more is recommended. For thicker specimens, such as resections, fixation of 12 hours or more is recommended.
6. Patient tissue should be stained within 45 days of sectioning from the tissue block. Loss of staining performance may be observed on sections that have been stored at room temperature (15-25°C) or 5°C ± 3°C for longer than 45 days.

Slides should be desiccated and stored at room temperature. Because environmental factors are known to affect antigen stability on cut slides, laboratories should validate cut slide stability within their own environment when storing beyond 45 days.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

FOLR1 positive status was observed in 31.2% (68/218) of cases in the cohort of unique EOC resection tissues for prevalence. FOLR1 negative status was observed in 68.8% (150/218) of cases in the cohort of unique EOC resection tissues for prevalence. In addition, an array of neoplastic tissues was evaluated for FOLR1 staining with VENTANA FOLR1 (FOLR1-2.1) Assay.

Table 5. Specificity of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay determined by testing FFPE non-neoplastic tissues.

Tissue	# positive / total cases	Tissue	# 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

Table 6. Sensitivity of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay determined by testing FFPE neoplastic tissues.

Pathology	# 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 invasive 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

Precision

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

Intermediate Precision

Twenty-four unique EOC tissue cases were enrolled (12 FOLR1 positive and 12 FOLR1 negative) in the Intermediate Precision study. The study design for evaluation of staining precision on EOC stained with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay included:

- Three lots of FOLR1 antibody
- Three BenchMark ULTRA instruments
- Three OptiView DAB IHC Detection Kits
- Across three non-consecutive days
- One pathologist, 2 replicates

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

Table 7. Median and Range of %TC for Cases 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	5 to 10	0 (0/18)	100 (18/18)
2	Negative	20.0	20 to 20	0 (0/18)	100 (18/18)
3	Negative	25.0	20 to 30	0 (0/18)	100 (18/18)
4	Negative	30.0	25 to 35	0 (0/18)	100 (18/18)
5	Negative	30.0	25 to 30	0 (0/18)	100 (18/18)
6	Negative	35.0	30 to 40	0 (0/18)	100 (18/18)
7	Negative	45.0	45 to 50	0 (0/18)	100 (18/18)
8	Negative	45.0	40 to 45	0 (0/18)	100 (18/18)
9	Negative	50.0	50 to 55	0 (0/18)	100 (18/18)
10	Negative	55.0	55 to 60	0 (0/18)	100 (18/18)
11	Negative	65.0	65 to 65	0 (0/18)	100 (18/18)
12	Negative	70.0	60 to 75	11.1 (2/18)	88.9 (16/18)
13	Positive	75.0	70 to 75	55.6 (10/18)	55.6 (10/18)
14	Positive	80.0	75 to 80	100 (18/18)	100 (18/18)
15	Positive	90.0	85 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	85 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	85 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 95	100 (18/18)	100 (18/18)
22	Positive	90.0	85 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	97 to 98	100 (18/18)	100 (18/18)

Variability of %TC values for 24 cases was evaluated and the following precision components were calculated: repeatability (within-pathologist), between-day, between-antibody kit, between-detection kit, between-instrument and total. Results are summarized in the table below.

Table 8. Precision Components for Cases in Intermediate Precision Study.

					Standard Deviation					
Sample ID	Majority	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	5 to 10	0	2.89	0	0	0	2.89
2	Negative	18	20	20 to 20	0	0	0	0	0	0
3	Negative	18	25	20 to 30	0	2.89	5.00	0	0	5.77
4	Negative	18	30	25 to 35	0	2.89	0	0	0	2.89
5	Negative	18	30	25 to 30	0	2.89	0	0	0	2.89
6	Negative	18	35	30 to 40	1.18	2.36	0	0	4.56	5.27
7	Negative	18	45	45 to 50	0	2.89	0	0	0	2.89
8	Negative	18	45	40 to 45	0	2.89	0	0	0	2.89
9	Negative	18	50	50 to 55	0	0	2.89	2.89	2.89	5.00
10	Negative	18	55	55 to 60	0	0	2.89	0	0	2.89
11	Negative	18	65	65 to 65	0	0	0	0	0	0
12	Negative	18	70	60 to 75	0	2.89	0	4.08	0	5.00
13	Positive	18	75	70 to 75	0	2.89	0	0	0	2.89
14	Positive	18	80	75 to 80	0	2.89	0	0	0	2.89
15	Positive	18	90	85 to 90	0	0	0	0	2.89	2.89
16	Positive	18	90	90 to 90	0	0	0	0	0	0
17	Positive	18	90	85 to 90	0	0	2.89	0	0	2.89
18	Positive	18	90	90 to 90	0	0	0	0	0	0
19	Positive	18	90	85 to 90	0	2.89	0	0	0	2.89
20	Positive	18	90	90 to 90	0	0	0	0	0	0
21	Positive	18	90	90 to 95	0	0	2.89	0	2.89	4.08
22	Positive	18	90	85 to 90	0	0	0	2.89	0	2.89
23	Positive	18	95	95 to 95	0	0	0	0	0	0
24	Positive	18	98	97 to 98	0	0.58	0	0	0	0.58

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

Table 9. Intermediate Precision of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay of EOC specimens.

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between- Antibody Lots	PPA	66/66	100.0	(94.5, 100.0)
	NPA	74/78	94.9	(90.5, 100.0)
	OPA	140/144	97.2	(94.4, 100.0)
Between-Instruments (BenchMark ULTRA)	PPA	66/66	100.0	(94.5, 100.0)
	NPA	76/78	97.4	(95.2, 100.0)
	OPA	142/144	98.6	(97.2, 100.0)
Between-Detection Kits	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-Day	PPA	66/66	100.0	(94.5, 100.0)
	NPA	76/78	97.4	(95.2, 100.0)
	OPA	142/144	98.6	(97.2, 100.0)
Within-Run	PPA	108/108	100.0	(96.6, 100.0)
	NPA	108/108	100.0	(96.6, 100.0)
	OPA	216/216	100.0	(98.3, 100.0)

Note: Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), Overall Percent Agreement (OPA).

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 for EOC tissues. The study included three readers (pathologists). Readers scored all specimens twice, with a minimum of two weeks between reads. Each case 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 below.

Table 10. Precision Components for Cases in Reader Precision Study.

					Standard Deviation			
Case Category	# Case	# Read	Range of Median %TC	Range of %TC (Min to Max)	Within-Reader	Between-Reader	Total	Percent Positive Results
Negative	32	192	0 to 20	0 to 20	3.56	3.88	5.27	0.0 (0/192)
	5	30	25 to 40	21 to 40	7.90	10.1	12.8	0.0 (0/30)
	10	60	42.5 to 62.5	41 to 64	9.19	9.81	13.4	6.7 (4/60)
Borderline Negative	5	30	65 to 72.5	65 to 74	4.72	9.91	11.0	26.7 (8/30)
Borderline Positive	20	120	75 to 85	75 to 85	3.99	6.56	7.68	90.8 (109/120)
Positive	25	150	87.5 to 95	86 to 95	4.40	5.45	7.00	99.3 (149/150)
	3	18	99 to 100	96 to 100	2.52	0.37	2.55	100.0 (18/18)

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

Table 11. Within-Reader and Between- Reader and Precision of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay of EOC specimens.

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	276/288	95.8	(93.4, 98.2)
	ANA	300/312	96.2	(93.9, 98.4)
	OPA	288/300	96.0	(93.7, 98.3)
Between-Reader	APA	266/288	92.4	(88.2, 96.0)
	ANA	290/312	92.9	(89.3, 96.3)
	OPA	278/300	92.7	(88.7, 96.0)

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

Inter-Laboratory Reproducibility 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 specimens (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 6 readers (2 readers per site). Each case had 10 results per site (30 results in total). Performance was evaluated and the following precision components were calculated: between-reader, between-day, between-site and total. Results are presented in the tables below.

Table 12. Precision Components for Cases in the Inter-Laboratory Reproducibility Study

				Standard Deviation (SD)			Percent Positive Results				
Sample ID	Majority FOLR1	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 1	0.1	0.0	0.0	0.1	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
2	Negative	10.0	1 to 25	5.2	0.0	7.6	9.3	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
3	Negative	20.0	5 to 30	0.9	3.2	4.9	5.9	0.0% (0/10)	0.0% (0/10)	0.0% (0/10))	0.0% (0/30)
4	Negative	27.5	2 to 55	0.0	2.9	16.2	16.5	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
5	Negative	40.0	6 to 80	23.0	2.8	40.0	46.2	0.0% (0/10)	10% (1/10)	0.0% (0/10)	3% (1/30)
6	Negative	40.0	10 to 60	5.1	0.0	8.3	9.7	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
7	Negative	42.5	15 to 70	16.8	5.9	39.3	43.2	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
8	Negative	50.0	10 to 70	24.0	2.9	55.0	60.0	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
9	Negative	50.0	25 to 75	6.8	0.0	13.2	14.9	10% (1/10)	0.0% (0/10)	0.0% (0/10)	3% (1/30)
10	Negative	50.0	10 to 80	15.8	0.0	40.6	43.6	10% (1/10)	0.0% (0/10)	0.0% (0/10)	3% (1/30)
11	Negative	50.0	0 to 70	17.6	13.2	35.6	41.9	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
12	Negative	60.0	30 to 80	5.3	10.1	0.0	11.4	20% (2/10)	0.0% (0/10)	30% (3/10)	17% (5/30)
13	Negative	60.0	40 to 70	0.0	0.0	0.0	0.0	0.0% (0/10)	0.0% (0/10)	0.0% (0/10)	0.0% (0/30)
14	Negative	60.0	40 to 80	2.2	6.7	0.0	7.0	10% (1/10)	0.0% (0/10)	0.0% (0/10)	3% (1/30)
15	Positive	75.0	40 to 90	24.8	2.9	46.8	53.1	50% (5/10)	90% (9/10)	40% (4/10)	60% (18/30)
16	Positive	77.5	55 to 95	15.2	0.0	29.4	33.1	60% (6/10)	100% (10/10)	10% (1/10)	57% (17/30)
17	Positive	80.0	70 to 95	6.9	3.4	16.2	17.9	90% (9/10)	100% (10/10)	90% (9/10)	93% (28/30)
18	Positive	80.0	0 to 90	4.1	19.4	12.5	23.4	70% (7/10)	100% (10/10)	63% (5/8)	79% (22/28)
19	Positive	80.0	70 to 95	6.5	2.0	13.3	14.9	90% (9/10)	100% (10/10)	100% (10/10)	97% (29/30)
20	Positive	80.0	40 to 95	17.0	3.8	29.5	34.3	60% (6/10)	90% (9/10)	60% (6/10)	70% (21/30)
21	Positive	81.5	72 to 100	4.1	3.3	12.0	13.1	100% (10/10)	100% (10/10)	90% (9/10)	97% (29/30)
22	Positive	90.0	80 to 100	4.0	1.5	10.8	11.6	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
23	Positive	92.5	75 to 100	7.9	0.0	10.1	12.8	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
24	Positive	92.5	80 to 100	1.6	2.3	7.5	8.0	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
25	Positive	95.0	73 to 100	0.0	0.0	7.9	7.9	100% (10/10)	100% (10/10)	90% (9/10)	97% (29/30)
26	Positive	95.0	80 to 99	5.9	2.5	0.0	6.4	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30)
27	Positive	98.0	80 to 100	0.0	0.0	4.4	4.4	100% (10/10)	100% (10/10)	100% (10/10)	100% (30/30))
28	Positive	98.5	0 to 100	3.4	23.7	0.0	23.9	100% (10/10)	100% (10/10)	80% (8/10)	93% (28/30)

Performance for 28 cases by 6 readers is also summarized by the table below.

Table 13. Percent of Positive and Negative FOLR1 Results for Different Ranges of %TC

%TC Range (Median Values)	N of cases	Percent Positive results	Percent Negative results
< 50	7	0.5%	99.5%
(50-75)	7	3.8%	96.2%
75	1	60.0%	40.0%
(75-85)	6	82.0%	18.0%
> 85	7	98.6%	1.4%

In addition, a qualitative analysis of different precision components was performed. Results of the analysis for 28 cases are summarized in the table below.

Table 14. Inter-Laboratory Reproducibility for overall agreement rates for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay of EOC specimens.

External Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	371/418	88.8	(80.8, 95.9)
	NPA	411/420	97.9	(96.4, 99.3)
	OPA	782/838	93.3	(89.2, 97.0)
Within-Site	PPA	366/398	92.0	(81.6, 96.6)
	NPA	426/440	96.8	(94.8, 98.8)
	OPA	792/838	94.5	(91.4, 97.3)
Within-Reader	PPA	368/383	96.1	(93.9, 97.9)
	NPA	443/455	97.4	(96.0, 98.7)
	OPA	811/838	96.8	(95.3, 98.1)

Note: Positive Percent Agreement (PPA), Negative Positive Agreement (NPA), Overall Percent Agreement (OPA).

Overall agreements were calculated by referring case-level mode.

Within-Site agreements were calculated by referring within-site case-level mode.

Within-Reader agreements were calculated by referring within-reader case-level mode.

In addition, pairwise comparisons were made Between-site, Between-Reader and Between-Day for FOLR1 status. The data in the table below indicates assay reproducibility across 5 days, 3 sites, and 6 readers.

Table 15. Additional pairwise external reproducibility results of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay of EOC specimens.

External Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-sites	APA	6686/7566	88.4	(80.5, 94.6)
	ANA	8274/9154	90.4	(86.1, 94.9)
	OPA	7480/8360	89.5	(83.8, 94.7)
Within-Site Between-Readers	APA	340/380	89.5	(81.2, 95.7)
	ANA	418/458	91.3	(86.6, 96.0)
	OPA	379/419	90.5	(84.4, 95.9)
Within-Reader Between-days	APA	1416/1515	93.5	(90.4, 96.0)
	ANA	1730/1829	94.6	(92.7, 96.4)
	OPA	1573/1672	94.1	(91.8, 96.3)

CLINICAL PERFORMANCE

The efficacy of ELAHERE (mirvetuximab soravtansine) was investigated in a single-arm study (Study IMGN853-0417, NCT04296890) of patients with FRα (FOLR1) positive, platinum-resistant EOC (n=104). Patients received one to three prior lines of therapy, including at least 1 line of therapy containing bevacizumab. All patients received ELAHERE (mirvetuximab soravtansine) 6 mg/kg AIBW as an IV infusion until disease progression or unacceptable toxicity. The major efficacy outcome measures were investigator-assessed overall response rate (ORR) (primary endpoint) and duration of response (DOR; secondary endpoint) evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (v1.1). The primary endpoint of ORR was calculated based on the Investigator EE (n=104) population.

The median age of the patients was 62 years (range: 35 to 85), the majority were white (96%) and all patients had an ECOG PS of 0 (57%) or 1 (43%). Fifty-one percent of patients had 3 prior systemic therapies. All patients received prior bevacizumab and 47% had received a prior PARP inhibitor. Positive FRα expression of the tumor was defined by the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay. Efficacy results for Study IMGN853-0417 are summarized below.

Table 16. Efficacy results in study IMGN853-0417.

Endpoint	ELAHERE (mirvetuximab soravtansine) (N=104)
Confirmed Overall Response Rate[a] (95% CI)	31.7% (22.9, 41.6)
Complete response rate	4.8%
Partial response rate	26.9%
Duration of Response	
Number of responders	33
Median Duration of Response, months (95% CI)	6.9 (5.6, 9.7)

[a] Investigator assessment.

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

Concordance Study of VENTANA FOLR1 (FOLR1-2.1) RxDx Assay without SIR for IMGN853-0417

The efficacy of ELAHERE (mirvetuximab soravtansine) was investigated in IMGN853-0417 in patients with platinum-resistant EOC, whose tumors were FRα (FOLR1) positive, as determined by VENTANA FOLR1 (FOLR1-2.1) RxDx Assay with a Stain Intensity Reference (SIR) slide. A bridging study was conducted to determine the simulated efficacy results for investigator-assessed ORR and DOR evaluated according to RECIST v1.1 amongst patients with platinum-resistant EOC who could have been enrolled into IMGN853-0417 had VENTANA FOLR1 (FOLR1-2.1) RxDx Assay without a SIR slide been used for enrollment rather than VENTANA FOLR1 (FOLR1-2.1) RxDx Assay with a SIR slide.

A concordance analysis was conducted to assess the similarity of FOLR1 status in the IMGN853-0417 patient population defined by the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay without a SIR slide versus with a SIR slide, as the comparator, was calculated. Among all the clinical samples from the original IMGN853-0417 study with evaluable results for both VENTANA FOLR1 (FOLR1-2.1) RxDx Assay with a SIR slide and without a SIR slide, the concordance results are shown in Table 17.

Table 17. FOLR1 Status Agreement Between Reads without SIR Slide Compared to Reads with SIR Slide as the Comparator.

Agreement			
Measure	n/N	%	95% CI
PPA	87/104	83.7	(75.4, 89.5)
NPA	224/234	95.7	(92.3, 97.7)
OPA	311/338	92.0	(88.6, 94.5)

CI=confidence interval; NPA=negative percent agreement; OPA=overall percent agreement; PPA=positive percent agreement

Additional analyses were conducted to simulate efficacy results for clinical outcome data in 10 patients with platinum-resistant EOC whose tumors would have been FOLR1 positive upon testing with VENTANA FOLR1 (FOLR1-2.1) RxDx Assay without the SIR using a multiple imputation (MI) approach. The point estimates for ORR (median of MI is 33.0%) and DOR (median of MI is 5.9) in the data set that included the imputed efficacy results were comparable to the original IMGN853-0417 study results, suggesting that nearly identical clinical outcomes may be expected whether or not the SIR slide was used to assist in the interpretation of the sides stained using the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

TROUBLESHOOTING

If a problem cannot be attributed to any of these causes, or if the suggested corrective action fails to resolve the problem, consult your local support representative.

Table 18. Troubleshooting guidance for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that U FOLR1 (FOLR1-2.1) RxDx Assay procedure was used.
		Verify that FOLR1-2.1 RxDx Assay Ab was selected for Primary Antibody
	Degradation of tissue	Verify tissue was stained within the recommended time frame following sectioning.
	Dispenser malfunction	Verify nozzle cap is removed.
		Ensure dispenser is primed
		Check the priming chamber for foreign materials or particulates, such as fibers or precipitate
		Refer to inline dispenser package insert associated with P/N 740-5065 located at www.ventana.com
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that U FOLR1 (FOLR1-2.1) RxDx Assay procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

REFERENCES

1. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
C	Updates to Specimen Preparation section. Updates to Specific Limitations section.

INTELLECTUAL PROPERTY

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