VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

REF 740-5065

07727917001

IVD Σ 50

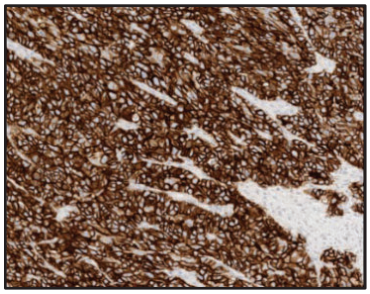

Figure 1. Ovarian carcinoma tissue stained with the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

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 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 (Cat. No. 760-700 / 06396500001). Refer to the OptiView DAB IHC Detection Kit package insert for further information. Results are interpreted using a light microscope and a VENTANA FOLR1 Stain Intensity Reference Slide (FOLR1 SIR).

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) (Cat. No. 760-2014 / 05266670001). This slide must be negative for specific staining to be considered acceptable.

The FOLR1 SIR slide is a ready to use stained slide containing a section of normal human fallopian tube intended to be used in the interpretation of stain intensity by VENTANA FOLR1 (FOLR1-2.1) RxDx Assay.

FOLR1 SIR slides (Cat No. 09382780001) will be distributed independent of the assay by a Roche Affiliate. Additional unlabeled slides without tissue present are included for packaging purposes; these slides are not intended for use. For more information, refer to the Interpretation Guide for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining of Epithelial Ovarian Cancer, Primary Peritoneal, and Fallopian Tube Cancer (P/N 1015089US).

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. VENTANA FOLR1 Stain Intensity Reference Slide (Cat No. 09382780001)
4. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
5. Drying oven capable of maintaining a temperature of 60°C ± 5°C
6. Bar code labels
7. Xylene (Histological grade)
8. 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
9. Deionized or distilled water
10. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
11. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
12. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
13. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
14. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
15. Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
16. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
17. General purpose laboratory equipment
18. BenchMark ULTRA instrument
19. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
20. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
21. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
22. Light microscope
23. 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.

Upon receipt of the FOLR1 SIR slide, store at 15-30°C. Do not freeze. When not in use place slides in a light occluding container and store in the dark.

FOLR1 SIR slides are expiration dated. When properly stored, the slides are stable to the date indicated on the label. Do not use slides 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 12 to 72 hours.[1]

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 dialog.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	Contaminated work clothing should not be allowed out of the workplace.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	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 mixture of: 5-chloro-2-methyl-4-isothiazolin-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

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. 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.

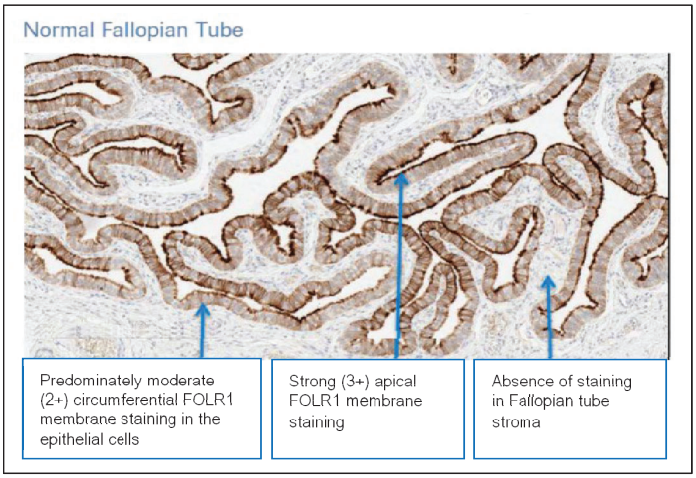

Figure 2. FOLR1 stain intensity criteria for normal Fallopian tube (SIR Slide).

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, moderate, strong) 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 using the FOLR1 SIR slide as the baseline for moderate stain intensity. Each FOLR1 SIR contains at least one region of 10 or more contiguous cells expressing moderate (2+) circumferential membrane staining. Prior to utilizing the FOLR1 SIR as a stain intensity reference tool for interpreting EOC cases stained with the FOLR1 Assay, pathologists should first review the FOLR1 SIR for a moderate staining region according to the criteria described in Figure 2. After locating the moderate staining region in the FOLR1 SIR, the pathologist should use this region to aid in the identification of moderate and stronger staining in EOC slides. 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 Positive 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. 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 28.75% (274/953) of cases in the commercial cohort of unique EOC resection tissues for prevalence. FOLR1 negative status was

observed in 71.25% (679/953) of cases in the commercial 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

Pathology	# positive / total cases
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	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)

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	10.0	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
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 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	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)

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
	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)

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	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 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	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)

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 12 readers (4 readers per site). Each case had 20 results per site (60 results in total). 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 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 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% (0/55)
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% (0/55)
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% (0/55)
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% (0/55)
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% (0/55)
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% (0/15)	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% (0/15)	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)
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 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	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 and it 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. Results of the analysis for 28 cases of 11 readers versus 12 readers 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.

		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)

Note: 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 comparisons were made Between-site, Between-Reader and Between-Day for FOLR1 status. The data in the table below indicates assay reproducibility across 3 days, 3 sites, and 12 readers.

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

	Agreement			
External Reproducibility	**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)

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.

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 17. 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 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

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 dialog.roche.com for definition of symbols used):

GTIN — Global Trade Item Number

UDI — Unique Device Identification

Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

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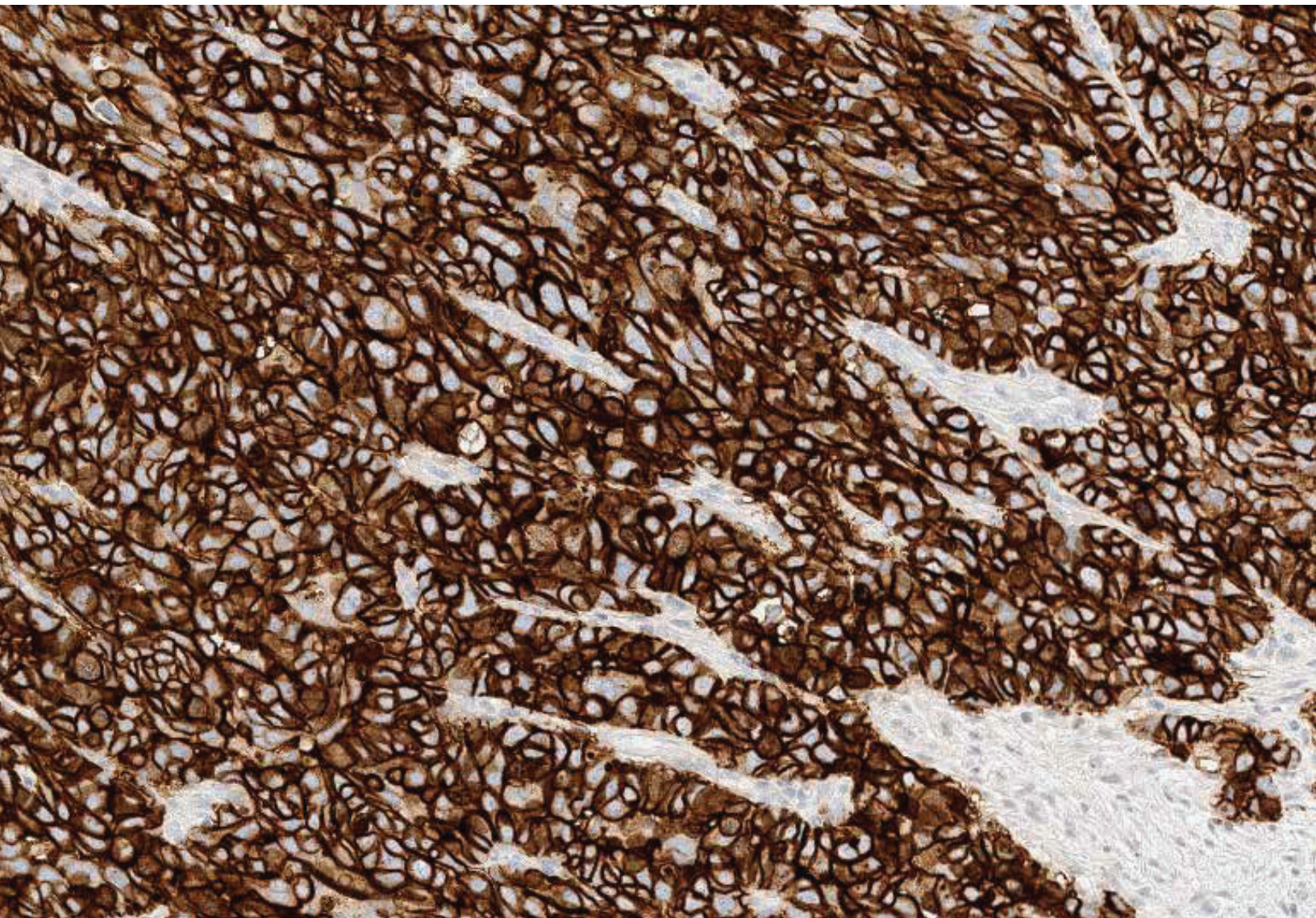
VENTANA FOLR1 (FOLR1–2.1) RxDx Assay Interpretation Guide for Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer



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Introduction

The Folate Receptor 1 protein (FOLR1), also commonly known as Folate Receptor alpha (FRα), is a 38-40 kDA glycosylphosphatidylinositol (GPI)-anchored cell surface protein encoded by the FOLR1 gene, that is largely restricted to malignant tumors compared to normal tissue.[1] The FOLR1 protein mediates the transfer of a carbon unit necessary for the de novo synthesis of purines and thymidylate, and it is required for synthesizing DNA, RNA, and enzyme co-factors. Tumors have increased metabolic demands as a consequence of their enhanced proliferation, and thus, a higher demand for thymidylate and purines compared to normal tissues. Exploiting this biologic necessity for enhanced folate uptake may provide an opportunity for anti-folate cancer therapy.[2] FOLR1 shows limited normal tissue expression and high expression on the surface of solid tumors, particularly epithelial ovarian cancer (EOC), endometrial cancer, non-small cell lung carcinoma, and renal cell cancer. Malignancies that are managed as EOC include primary fallopian tube cancer and primary peritoneal cancer.[3] FOLR1 levels are positively associated with tumor stage and grade, which suggests that FOLR1 might confer a growth advantage to the tumor by modulating folate uptake or by generating regulatory signals.[2,4] The FOLR1 protein is either absent from normal tissues or localized to the luminal surface of certain epithelial cells, where it is inaccessible to the circulation, whereas in tumors, FOLR1 is fully accessible via circulation.[4] Therefore, FOLR1 is frequently exploited as a target for receptor specific delivery of chemotherapy and immunotherapy agents.[1,5] Since folate-linked drugs do not normally accumulate in normal tissues, this approach provides high specificity for FOLR1 expressing tumor cells.

EOC patients often present with advanced disease, and have limited prognosis. Despite considerable improvements in primary therapy, 80% of the patients with advanced EOC are expected to relapse during or after treatment with platinum-containing regimens.[6] Treatment of patients with recurrent EOC is less standardized than treatment of newly diagnosed patients. A recent retrospective study demonstrated that progression-free survival (PFS) and overall survival (OS) decrease by more than 50% as patients move from the first to the fifth relapse.[7] In addition, the study revealed that patients derived little benefit from treatment with current agents beyond the third line of therapy.[8]

Roche Tissue Diagnostics has developed the VENTANA FOLR1 (FOLR1-2.1) RxDx Assay (hereafter VENTANA FOLR1 Assay) for use as a companion diagnostic to aid in identifying folate receptor positive patients who may benefit from FOLR1-targeted therapy. The VENTANA FOLR1 Assay is used with the OptiView DAB IHC Detection Kit as a fully automated immunohistochemistry (IHC) assay on the BenchMark ULTRA IHC/ISH instrument. The sensitivity of the IHC assay enables a reproducible, binary scoring system (Positive or Negative for FOLR1 status) for evaluating the staining results (refer to the package insert for VENTANA FOLR1 (FOLR1-2.1) RxDx Assay (Cat. No. 740-5065/ 07727917001).

Intended Use

Intended Use of Product

Refer to the corresponding VENTANA FOLR1 Assay package inserts for the detailed intended use of this product.

Purpose of Interpretation Guide

This guide is intended to:

- Provide pathologists with a tool to facilitate the clinical evaluation of formalin-fixed, paraffin-embedded (FFPE) epithelial ovarian cancer, primary peritoneal, and fallopian tube cancer sections (all managed as EOC) stained with the VENTANA FOLR1 Assay using the VENTANA FOLR1 Assay Scoring Method in accordance with the proposed product labeling.
- Provide photographic images that illustrate the staining patterns that may result from staining of ovarian carcinoma tissues with The VENTANA FOLR1 Assay. Images of primary peritoneal and primary fallopian tube cancer are not included in visual illustrations due to the rarity of these indications.
- Provide a reference for relating staining patterns and intensities to specific FOLR1 scores.
- Provide example images of challenging cases to provide guidance in their evaluation.
- Provide guidance in using the FOLR1-positive control tissue, normal fallopian tube tissue, which serves as a tissue control when stained with the VENTANA FOLR1 Assay.
- Provide guidance in using the VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001) as a stain intensity reference for evaluation of slides stained as part of the VENTANA FOLR1 Assay.

Clinical Evaluation

Staining Characteristics

In EOC tissue, neoplastic cells labeled with the VENTANA FOLR1 Assay are evaluated for percent tumor cell staining of the diaminobenzidine (DAB) signal. VENTANA FOLR1 Assay staining in EOC tissue follows a cytoplasmic and membranous pattern. The signal is classified as strong, moderate, weak, or negative based on membrane localization only. The VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001) should be used as reference for determination of moderate signal intensity.

- **Negative (0)** signal intensity is characterized by an absence of any detectable signal. Negative cases may still exhibit pale grey cytoplasmic and/or membranous discoloration.

- **Weak (1+)** signal intensity is characterized by a faint gold/light brown hue that may be partial or circumferential.

- **Moderate (2+)** or **Strong (3+)** signal intensity is characterized by a chocolate brown to thickened dark brown, black hue that may be partial or circumferential.

The signal may be distributed heterogeneously having more than one intensity level. The relative percentages of neoplastic cells staining at each of the following signal intensities: strong (3+), moderate (2+), weak (1+), and negative (0), are visually estimated and used to generate a diagnostic score.

Scoring Algorithm

Evaluating VENTANA FOLR1 (FOLR1-2.1) RxDx Assay IN EOC:

For the VENTANA FOLR1 Assay, each case is stained with the VENTANA FOLR1 (FOLR1-2.1) mouse monoclonal primary antibody and a matched negative reagent control (NRC), Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001). Neoplastic cells labeled with the VENTANA FOLR1 Assay are evaluated for presence or absence of the DAB signal. The matched NRC-stained slide is used to assess non-specific background staining and degree of background staining known to occur due to specific tissue elements. Please note: OptiView DAB IHC Detection Kit is the only detection reagent that is recommended for use with the VENTANA FOLR1 Assay.

The scoring algorithm for the VENTANA FOLR1 Assay is provided below in **Table 1**.

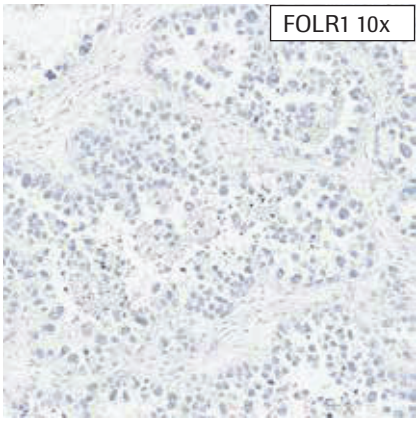
Table 1: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Scoring Algorithm for EOC

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

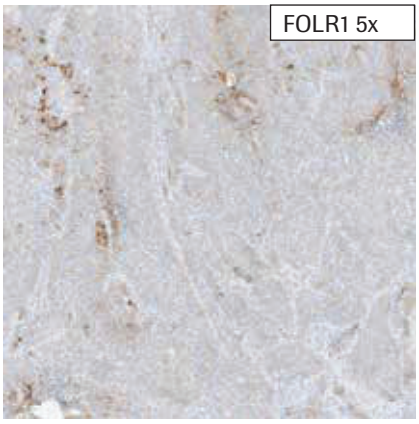
* Re-reading by Additional Pathologists for FOLR1 Scoring

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 Positive should be obtained by either a majority rule or by consensus among the pathologists.

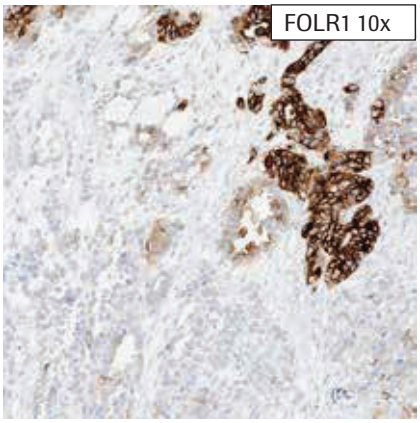
Clinical Diagnosis Negative



Exhibits no moderate or strong tumor cell membrane staining or < 75% moderate and/or strong tumor cell membrane staining

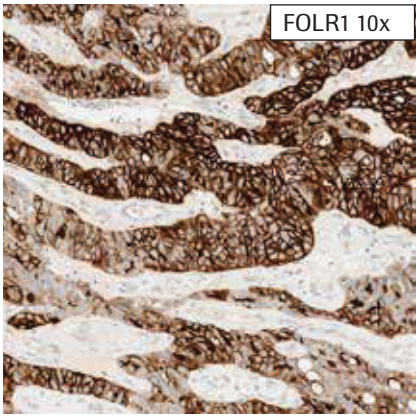


Exhibits 7% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining

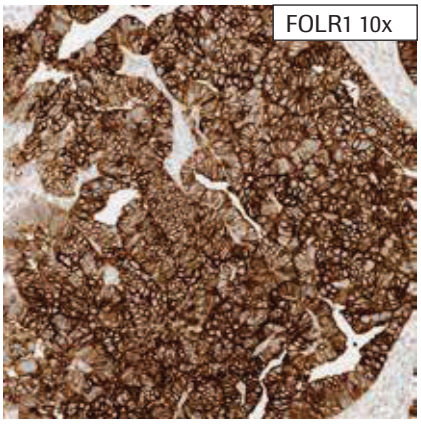


Exhibits 20% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining

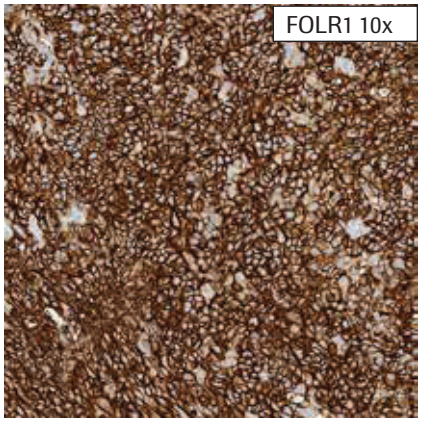
Clinical Diagnosis Positive



Exhibits 95% moderate and strong membrane staining or ≥ 75% moderate or strong membrane staining with complete circumferential pattern*



Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*



Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*

*Note: Partial circumferential membrane staining patterns are also acceptable for FOLR1 positivity. FOLR1 positivity does not require complete circumferential membrane staining.

Scoring Method

VENTANA FOLR1 Assay staining can be observed in tumor cells of EOC tissues, which exhibit a cytoplasmic and membranous staining pattern with varying ranges of stain intensity; only membranous staining is evaluated for the assay. Membrane staining pattern may be apical or circumferential (partial or complete).

Tissue morphology and background acceptability are assessed for each case using the criteria described in **Tables 2** and **3**.

The percentage of tumor cells staining at each stain intensity (negative, weak, moderate, strong) 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. If the H&E evaluation indicates that the patient specimen is inadequate (for example, if less than 100 tumor cells are present), then a new specimen should be obtained and stained with the VENTANA FOLR1 Assay. EOC tissue cases are considered positive for FOLR1 status if ≥ 75% of viable tumor cells demonstrate moderate and/or strong membrane staining. FOLR1 staining percentage at each intensity is determined by a trained pathologist using the stain intensity reference slide as the baseline for moderate stain intensity. Viewing a case at multiple levels of magnification may be useful in visually estimating the relative percentages of tumor cells staining and differentiating tumor cells from normal stained cells. Because the scoring method for EOC utilizes stain intensity, a pre-stained stain intensity reference slides is used as a required adjunct tool in the interpretation of moderate FOLR1 stain intensity. Image on **Page 8** shows an example of moderate stain intensity that users should refer to on the VENTANA FOLR1 Stain Intensity Reference Slide when evaluating FOLR1-stained slides. The VENTANA FOLR1 Assay Scoring Method is described in **Table 1**.

Table 2: Morphology Acceptance Criteria

Interpretation	Microscope Observation
Acceptable	Cellular elements of interest are visualized allowing clinical interpretation of the stain.
Not Acceptable	Cellular elements of interest are not visualized compromising the clinical interpretation of the stain.

Table 3: Background Acceptance Criteria

Interpretation	Microscope Observation
Acceptable	Non-specific staining is not obtrusive to interpretation of specific staining.
Not Acceptable	Non-specific staining is obtrusive to interpretation of specific staining.

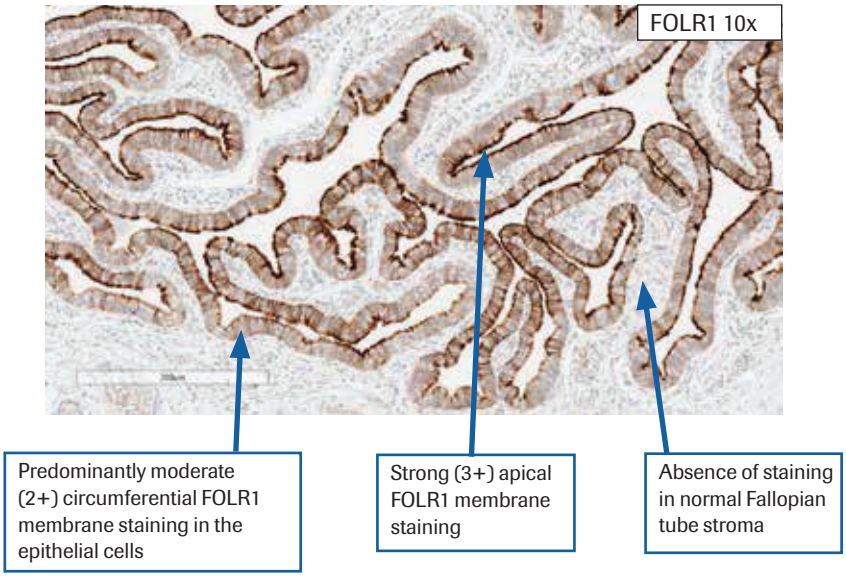
Stain Intensity Reference

VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001)

Pre-stained, formalin-fixed, paraffin-embedded normal human fallopian tube tissue evaluated for FOLR1 stain intensity according to the criteria described in the figure below will be provided for use as a stain intensity reference when evaluating FOLR1-stained slides. Because the scoring method for EOC utilizes stain intensity, a pre-stained stain intensity reference slide is used as a required adjunct tool in the interpretation of moderate FOLR1 stain intensity.

An appropriate and acceptable range of staining on the stain intensity reference slide must be present to proceed with the evaluation of FOLR1-stained slides. An effective stain intensity reference slide must exhibit at least one area with moderate circumferential membrane staining (region needs to contain at least 10 contiguous cells).

Since the scoring method for EOC is based on staining that is of moderate or greater intensity, the stain intensity reference slide is used as an example of moderate staining to aid readers as they evaluate EOC specimens. The moderate (2+) circumferential staining seen in normal fallopian tube tissue serves as a representation of the intensity expected in moderate intensity staining of tumor cell membranes in EOC tissues. While stronger staining components may be present on the stain intensity reference slide, they are not used as a reference when scoring EOC specimen samples. Image below shows an example of moderate stain intensity that users should refer to on the VENTANA FOLR1 Stain Intensity Reference Slide when evaluating FOLR1-stained slides.



FOLR1 Stain Intensity Criteria for Normal Fallopian Tube and EOC Tissues

Controls

Normal fallopian tube tissue with positive and negative staining elements is recommended for use as a run control tissue that can be used to detect out-of-specification issues that might be instrument-related. The luminal membrane of the normal fallopian tube tissue shows specific membranous staining for the FOLR1 protein. When qualifying a normal fallopian tube tissue to serve as a system-level control tissue, the tissue must exhibit predominately moderate circumferential membrane staining and strong apical membrane staining when stained with the VENTANA FOLR1 Assay as described in **Table 4** and depicted on the following page.

When qualifying a normal fallopian tube tissue to serve as a system-level control tissue, the tissue must also exhibit an absence of staining in the stroma.

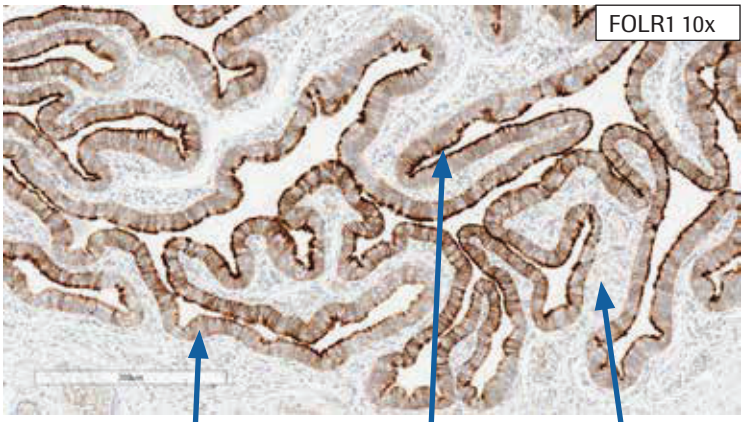
A positive control tissue should be a fresh autopsy/surgical specimen that is fixed and processed in the same manner as the patient specimens and should be run for each set of test conditions with every VENTANA FOLR1 Assay staining procedure performed. This tissue is provided by the end user and may be used to monitor all steps of specimen processing and staining. A tissue section fixed or processed differently from the test specimen can be used as a control for reagents and staining, but not for fixation or tissue preparation. Positive predominately moderate circumferential membrane staining and strong apical membrane staining and absence of specific staining in the stroma of the normal fallopian tube specimen confirms that the VENTANA FOLR1 Assay was applied and the instrument functioned properly. The positive tissue control should only be used to monitor performance and it should not be used to aid the clinical diagnosis of patient samples, as per CLSI I/LA28-A2.

Before use as a tissue control, the normal fallopian tube specimens should be qualified by the end user for appropriate staining, according to the interpretation criteria described in **Table 4**, using the VENTANA FOLR1 Assay with the OptiView DAB IHC Detection Kit and recommended staining protocol. Multiple fallopian tube specimens may be required to select an appropriate system-level control candidate.

Table 4: Acceptance Criteria for FOLR1 Staining in Normal Fallopian Tube Tissue

System-level Control Tissue Interpretation	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 predominately 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.	

Acceptable Staining of Control Normal Fallopian Tube Tissue

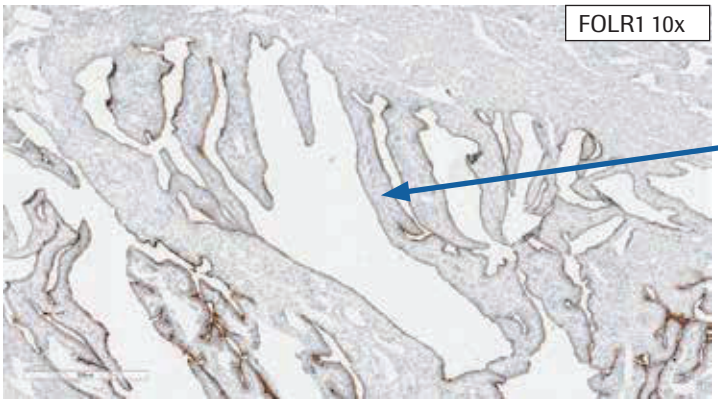


Predominantly moderate (2+) circumferential FOLR1 membrane staining in the epithelial cells

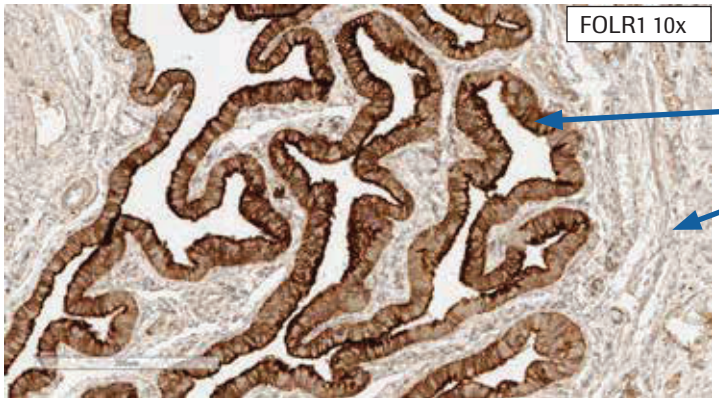
Strong (3+) apical FOLR1 membrane staining

Absence of staining in normal Fallopian tube stroma

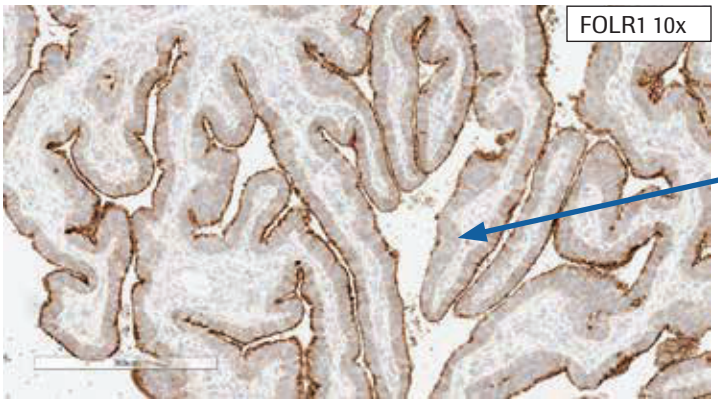
Not Acceptable Staining of Control Normal Fallopian Tube Tissue



Not acceptable normal fallopian tube system level control case exhibits absence of moderate circumferential staining in the epithelium of normal fallopian tube tissue.



Not acceptable normal fallopian tube system-level control case exhibits strong circumferential staining in the epithelium of normal fallopian tube tissue and exhibits background staining that interferes with interpretation.



Not acceptable normal fallopian tube system-level control case exhibits weak membrane staining in the epithelium of normal fallopian tube tissue.

Specimen Workflow

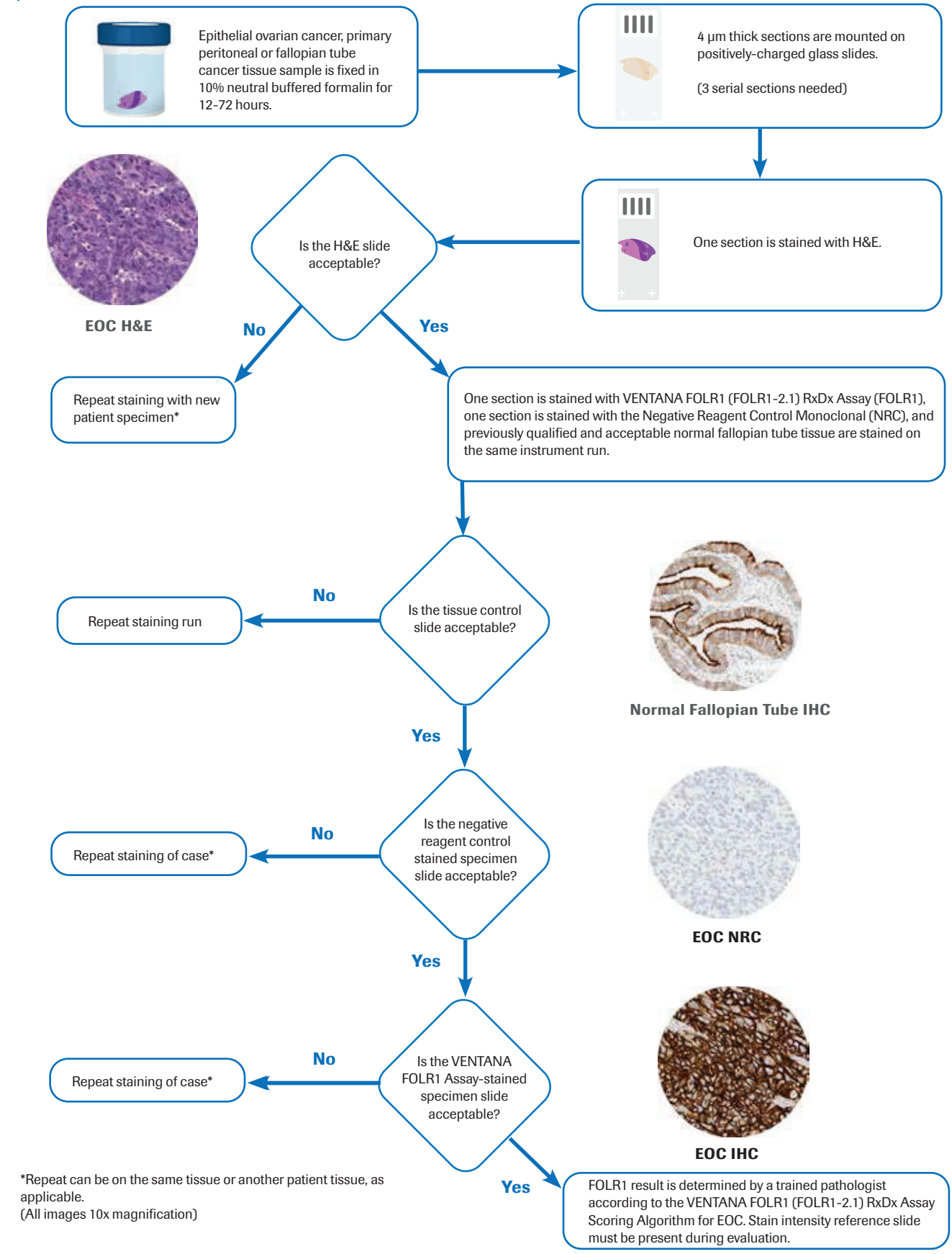
Staining requires three sections from each case, one serial tissue section for hematoxylin and eosin (H&E) staining, a second serial tissue section for NRC staining, and a third serial tissue section for VENTANA FOLR1 Assay staining. As with any IHC assay, it is recommended that specific staining is evaluated on viable tumor cells. Ideally, patient specimens will have a minimum of 100 viable tumor cells identified on the H&E in order to determine FOLR1 status. A normal fallopian tube tissue with moderate stain intensity is to be used as the stain intensity reference slide (VENTANA FOLR1 Stain Intensity Reference Slide (Cat. No. 09382780001)) and is used when evaluating clinical samples. If the H&E evaluation indicates that the patient specimen is inadequate (for example, if less than 100 tumor cells are present), then a new specimen should be obtained and stained with the VENTANA FOLR1 Assay.

A user-supplied normal fallopian tube tissue is recommended as a system-level control for the assay to monitor the proper functioning of the reagents and staining run. Both positive and negative elements must be stained appropriately as defined by the acceptance criteria for normal fallopian tube tissue (**Table 4**) on each run for the run to be considered valid.

A matched NRC slide must be run for every specimen to evaluate nonspecific staining and aid in the interpretation of results.

The FOLR1-stained specimen slides should be assessed by a trained pathologist using the described scoring criteria. If either the FOLR1-stained tissue control slide or the NRC-stained specimen slide is not acceptable, staining of patient samples should be repeated. Repeat may be on the same tissue or another patient tissue, as applicable. A non-evaluable VENTANA FOLR1 Assay-stained slide would mean that determination of reactivity is not possible due to necrosis, absent tissue, or artifacts and the slide cannot be used for clinical evaluation.

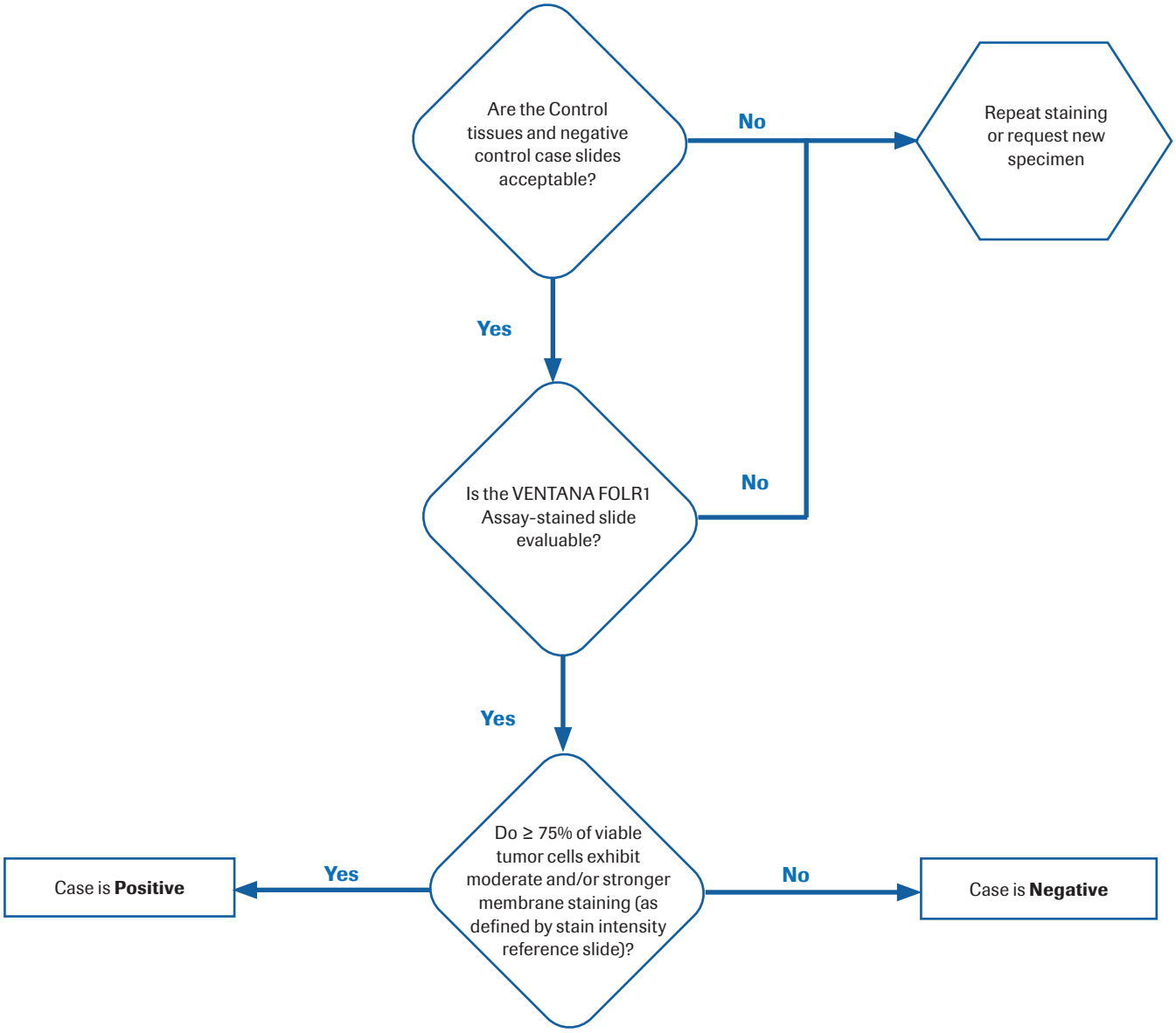
Specimen Flow



*Repeat can be on the same tissue or another patient tissue, as applicable.
(All images 10x magnification)

Decision Tree

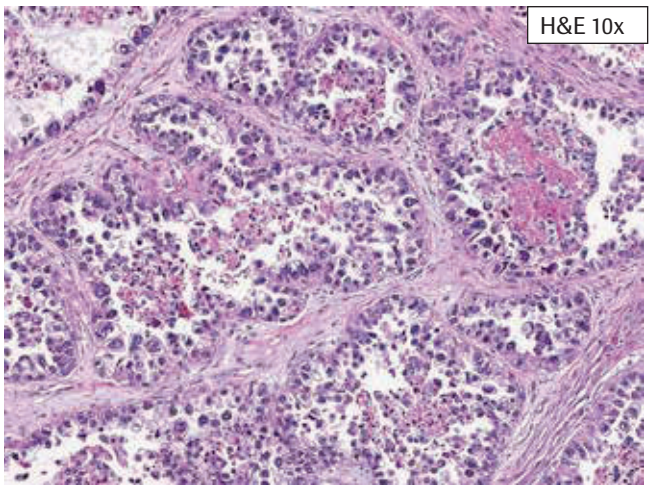
Slides stained with the VENTANA FOLR1 Assay should be evaluated using the approach noted in the figure below

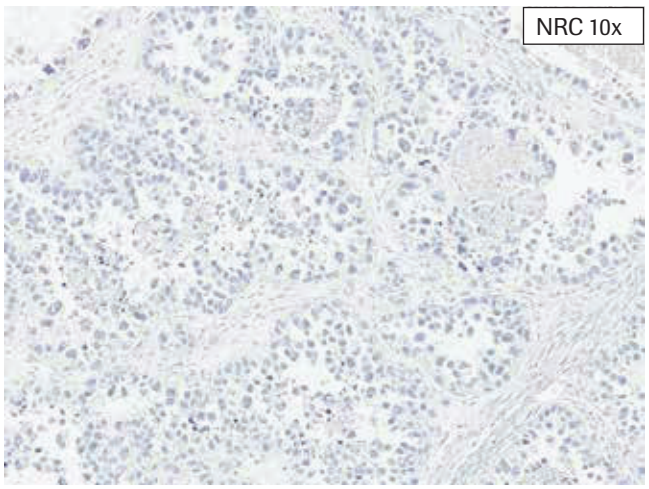


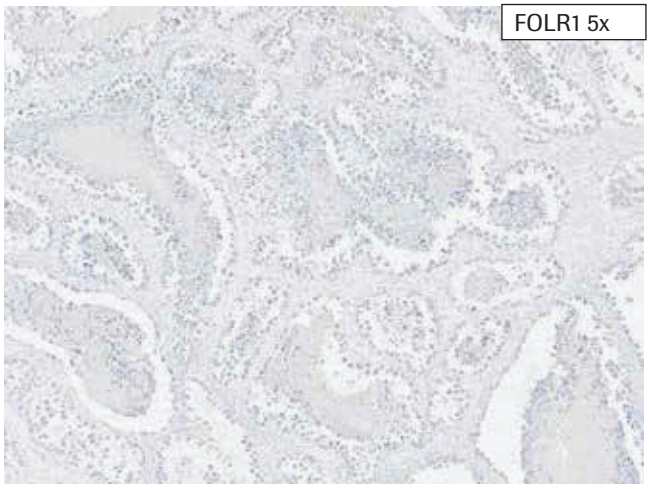
Reference Images

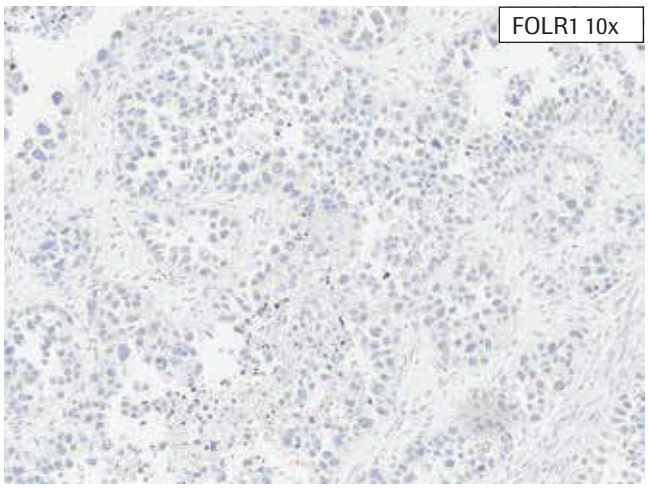
Negative Cases

A case assigned negative for FOLR1 status is characterized by an absence of any detectable signal or less than 75% of neoplastic cells exhibiting moderate and/or strong membrane staining. Scores for FOLR1-stained images were determined using the lower magnification image.

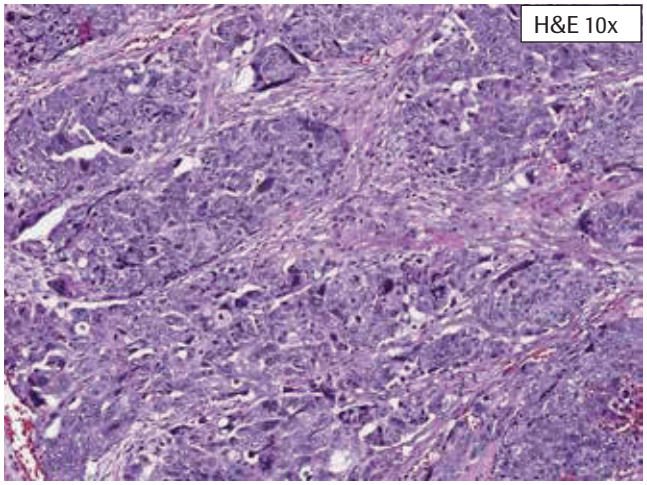


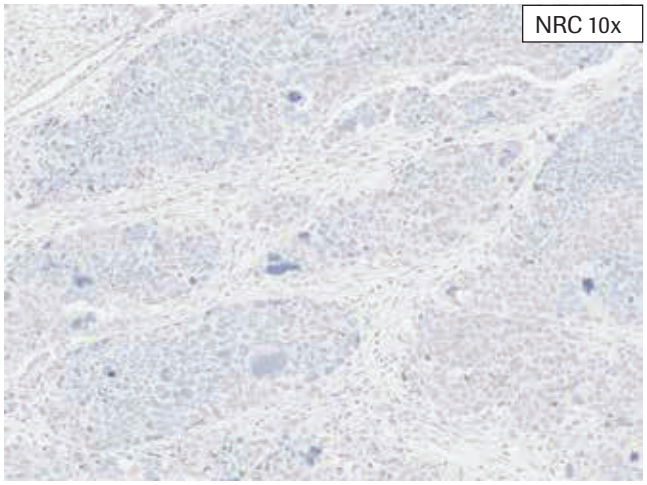


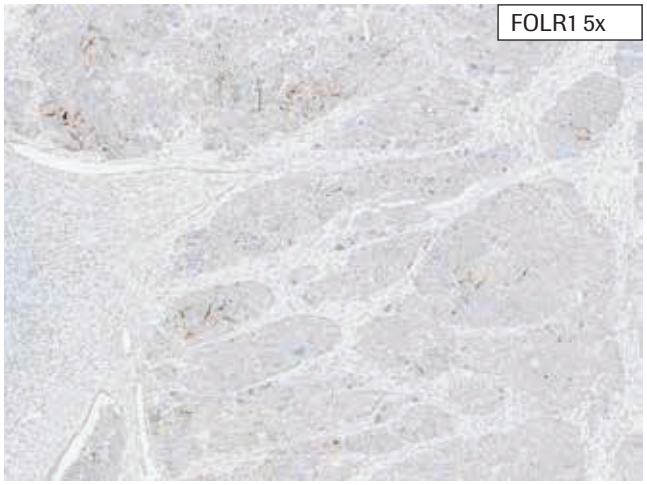


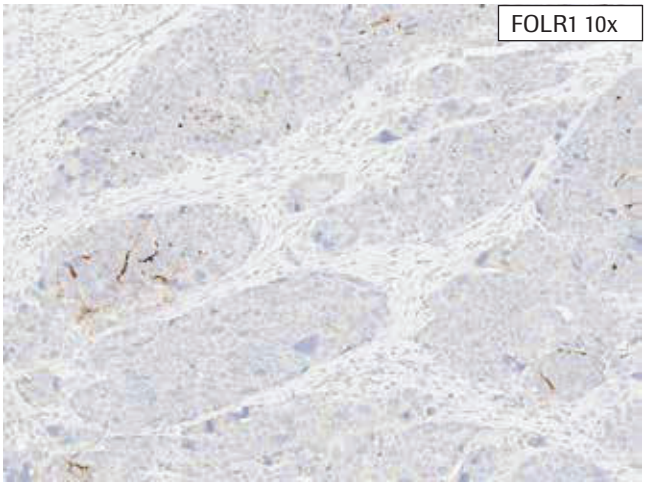


Negative EOC Case 1 exhibits 0% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

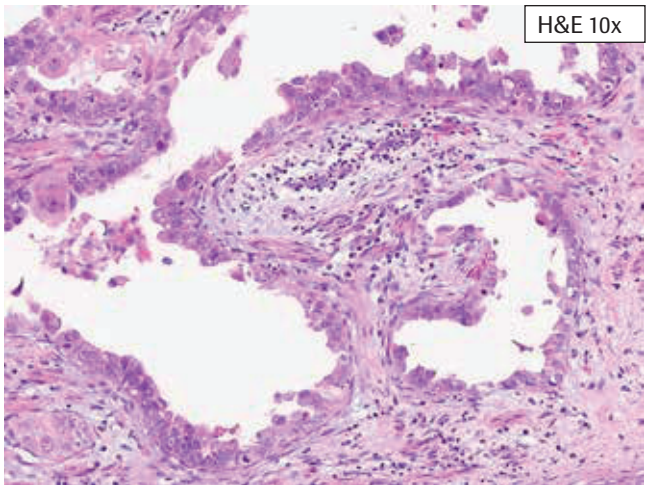


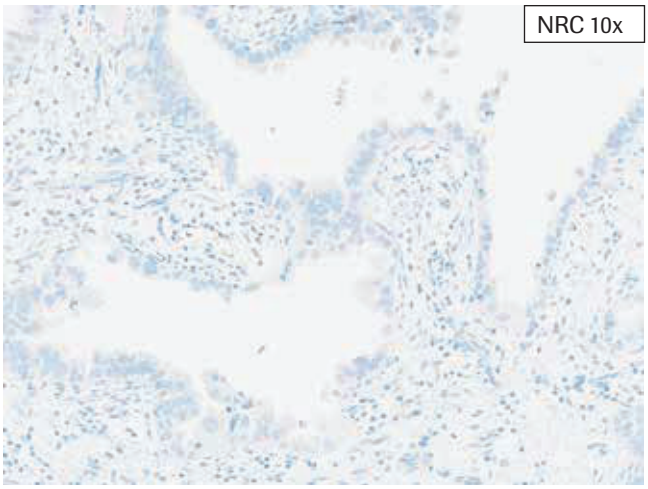


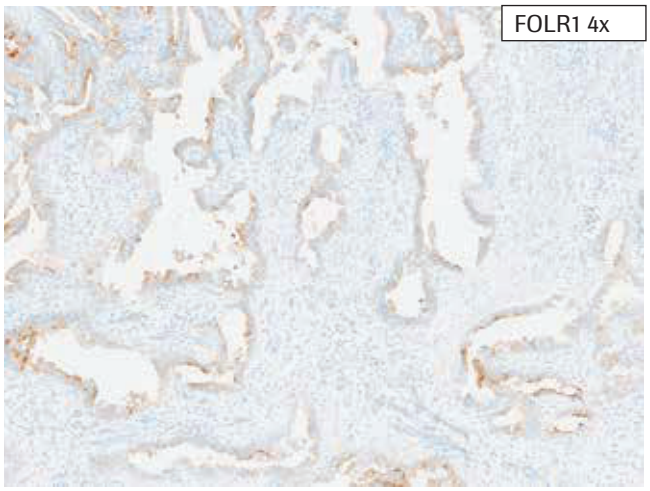


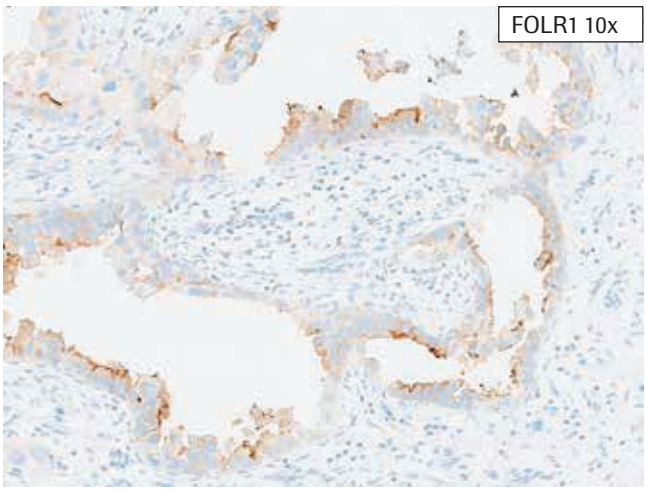


Negative EOC Case 2 exhibits 5% moderate, and strong apical membrane tumor cell staining, or < 75% of tumor cells with moderate and/or strong apical membrane staining relative to the negative control slide. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Negative FOLR1 status.

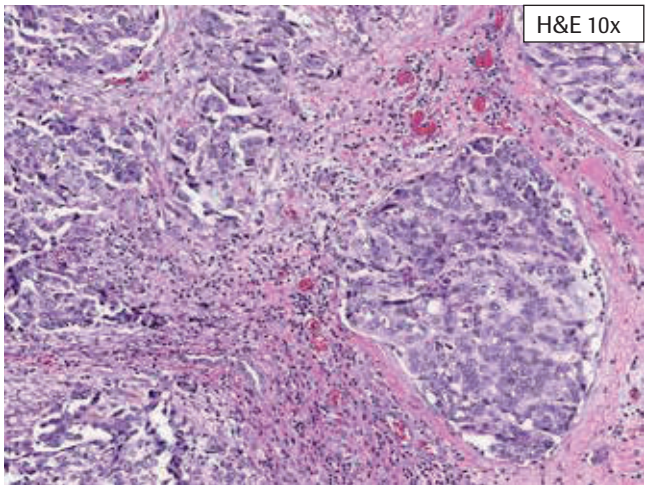


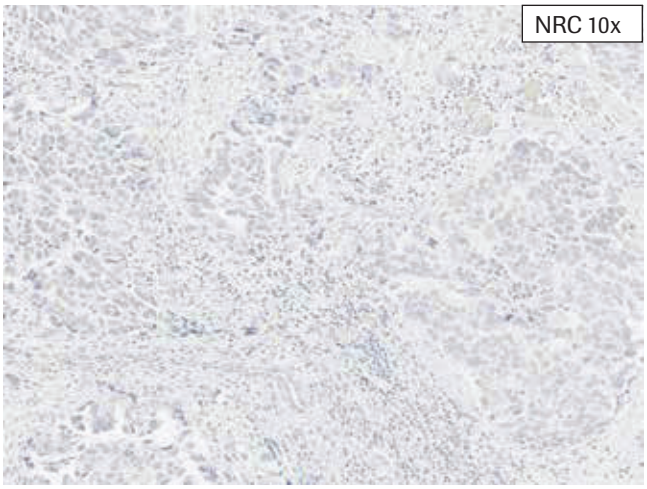


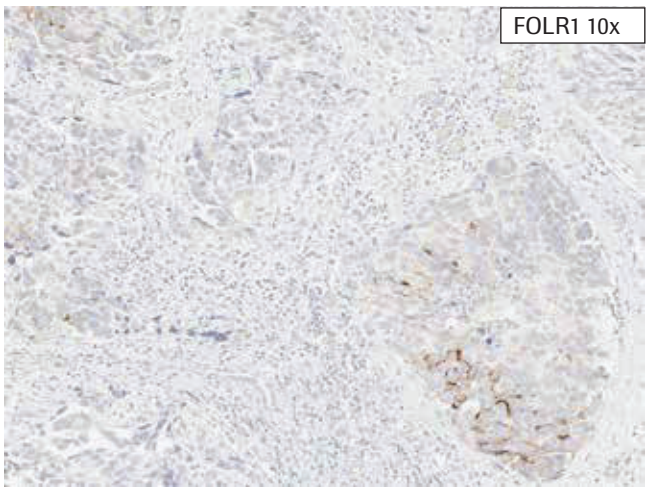




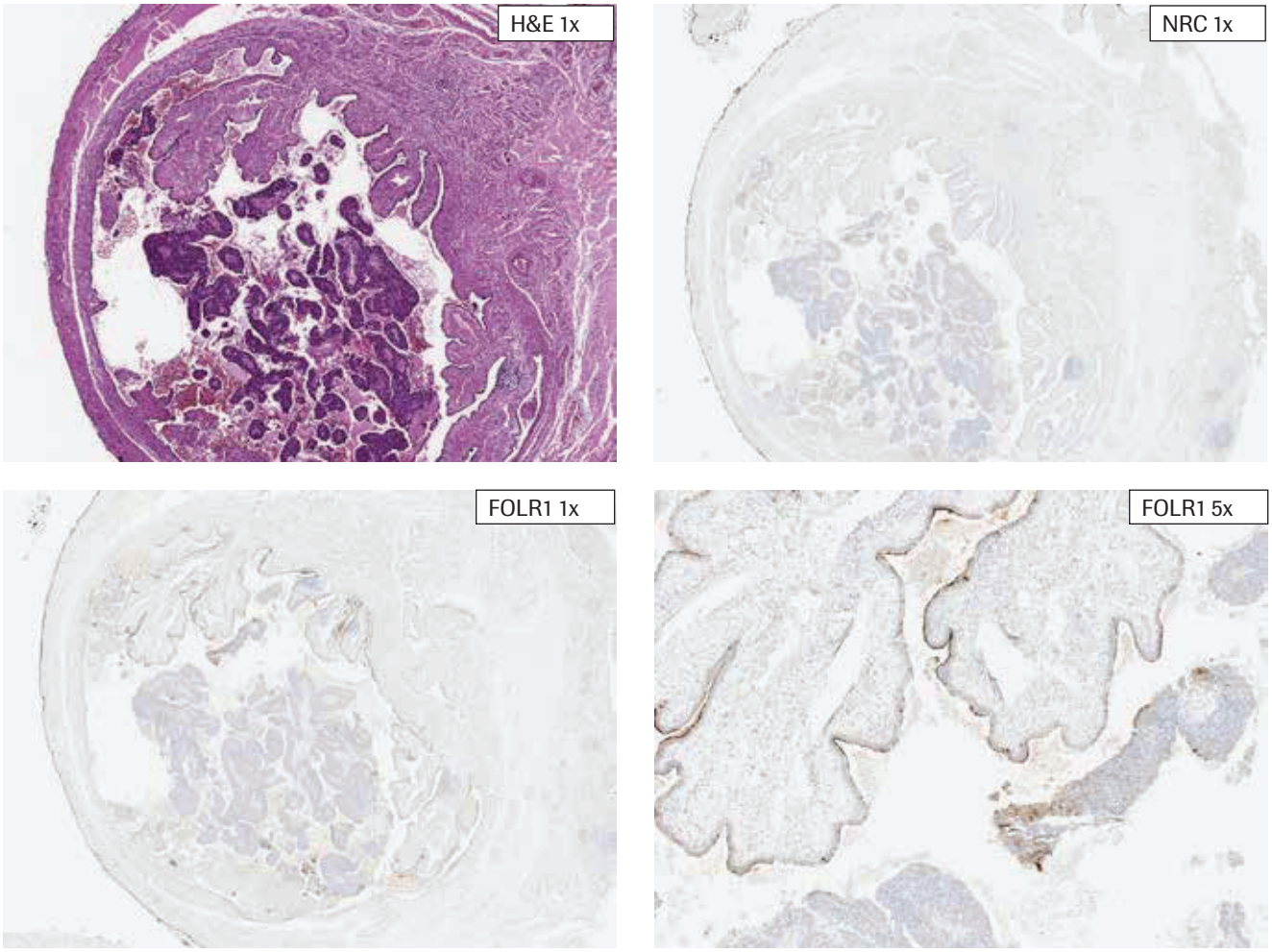
Negative EOC Case 3 exhibits 40% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.







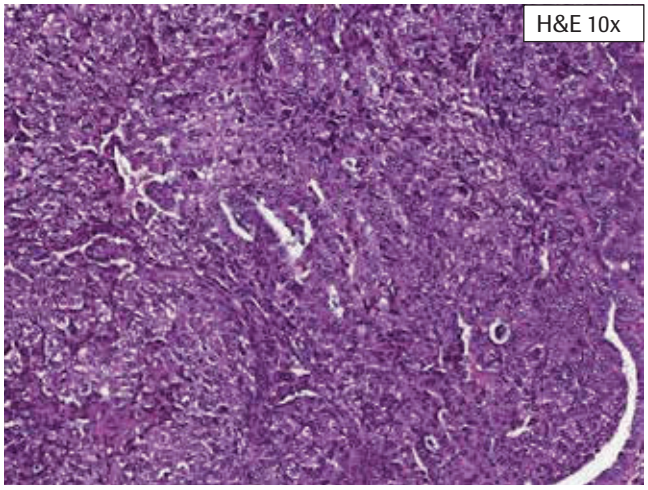
Negative EOC Case 4 exhibits 5% moderate and strong tumor cell membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

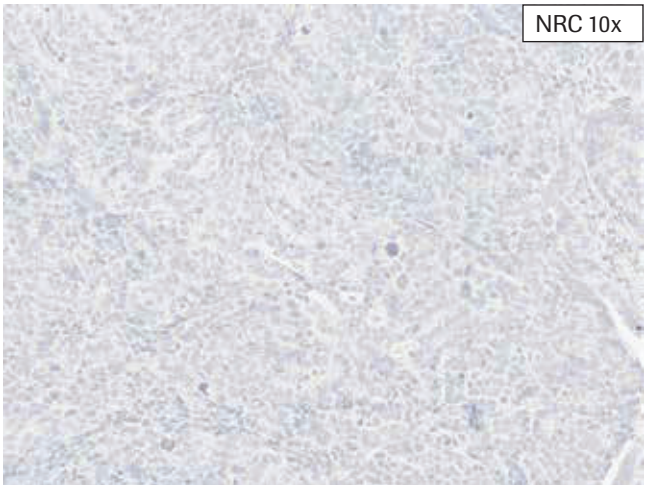


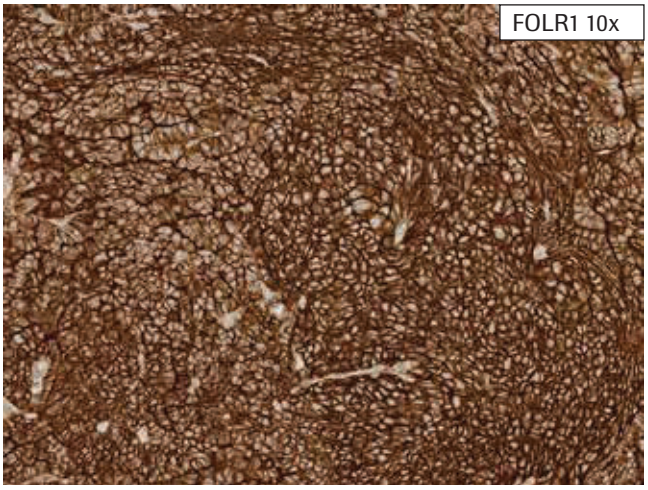
Negative Fallopian Tube Carcinoma Case 5 exhibits 5% moderate and strong fallopian tube staining or < 75% of fallopian tube tumor cells with moderate and/or strong fallopian tube staining relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Positive Cases

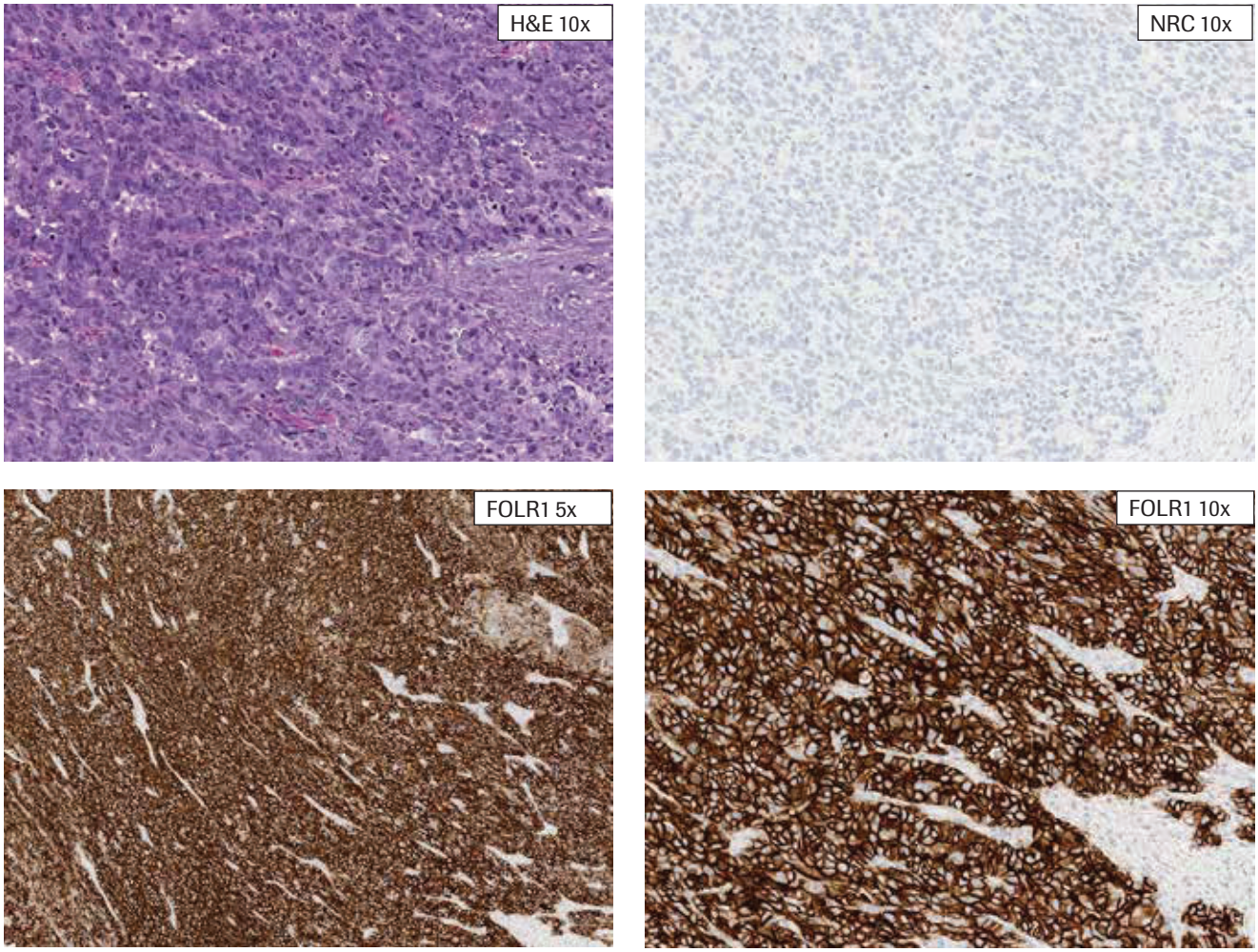
Staining with the VENTANA FOLR1 Assay can be observed in the membrane of tumor cells. A case is deemed positive if 75% or more of the neoplastic cells exhibit staining at a moderate and/or strong intensity. Scores for FOLR1-stained images were determined using the lower magnification image



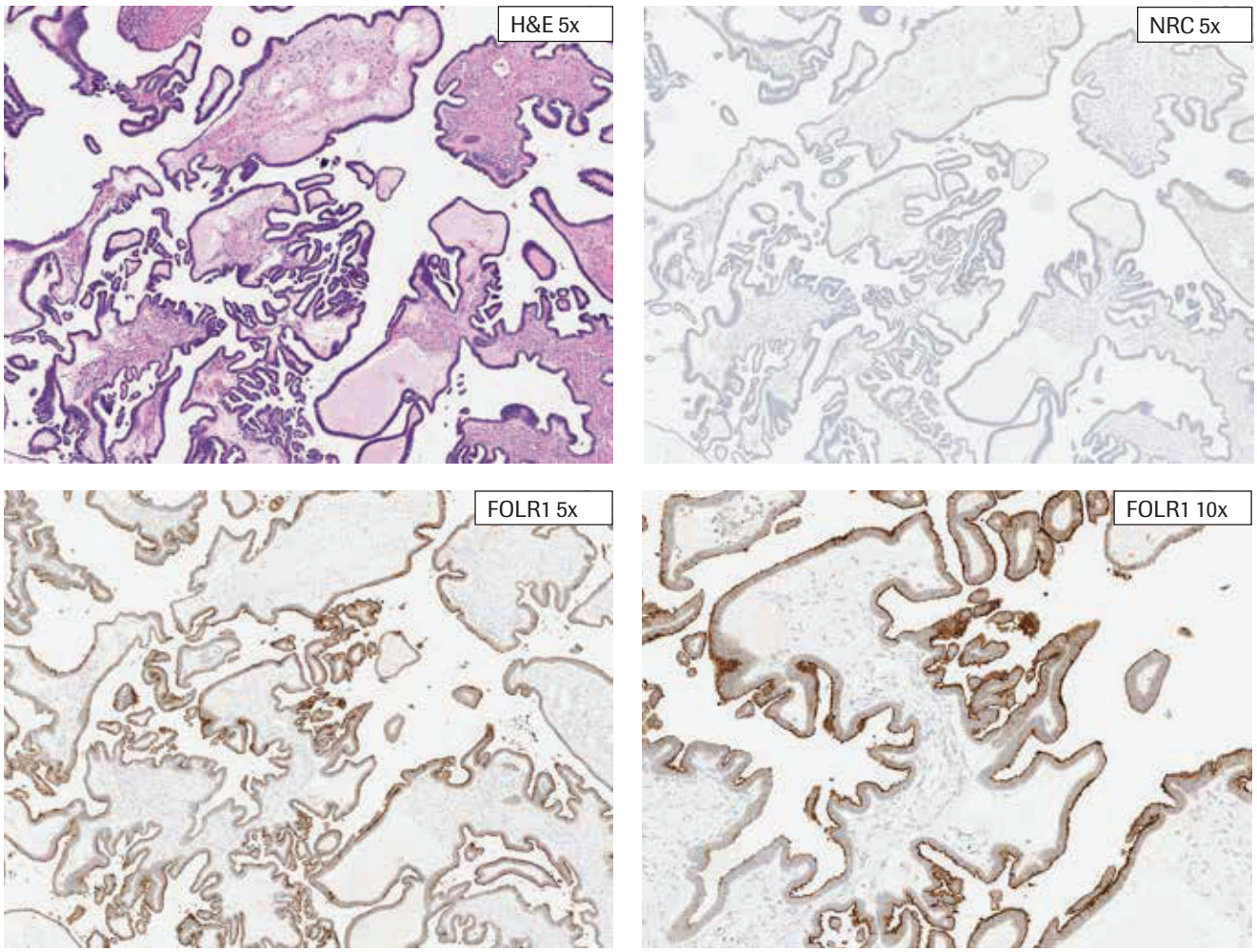




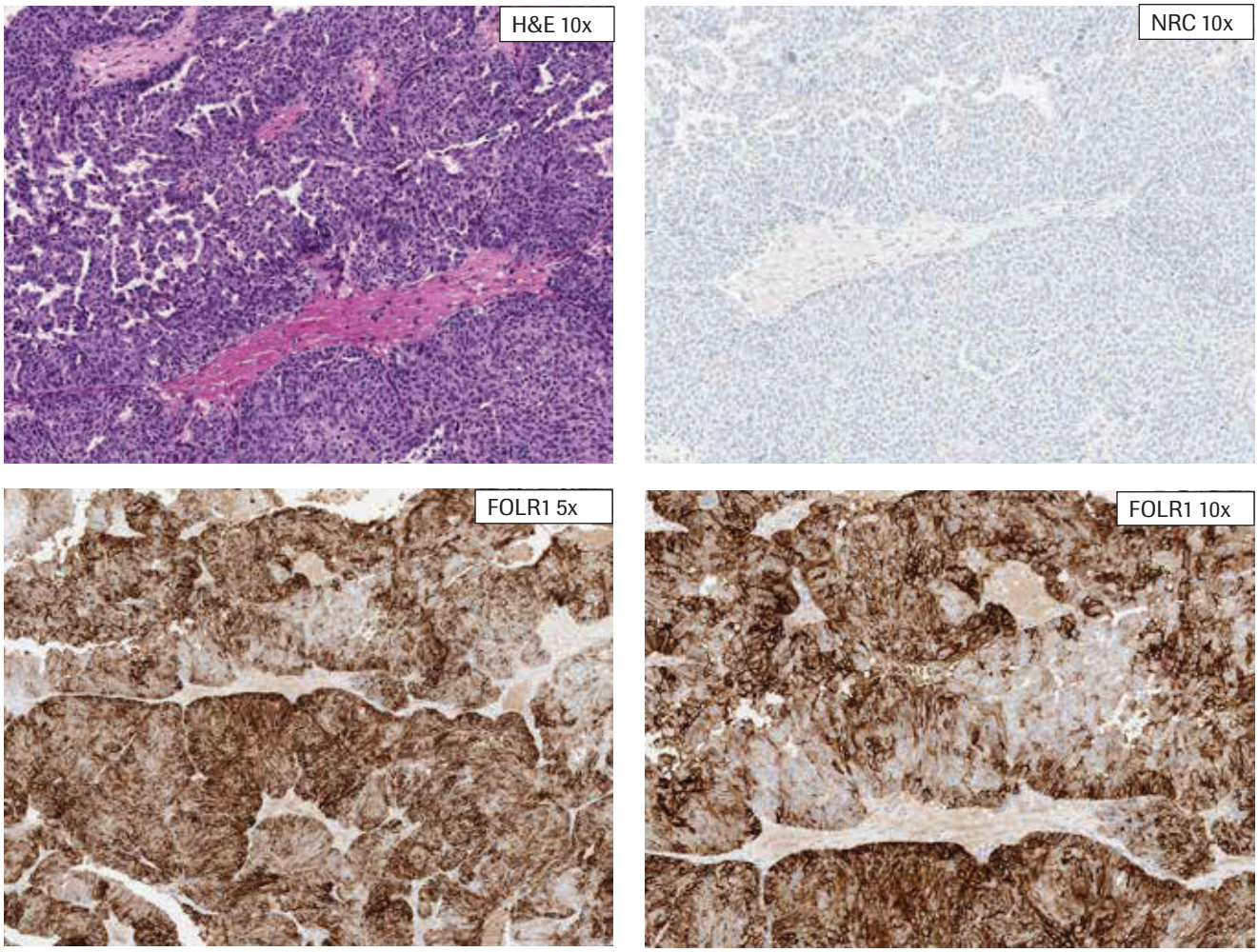
Positive EOC Case 1 exhibits 100% moderate and strong membrane staining with complete circumferential pattern, or ≥ 75% of the tumor cells with moderate and/or strong membranous staining. This case is assigned a Positive FOLR1 status.



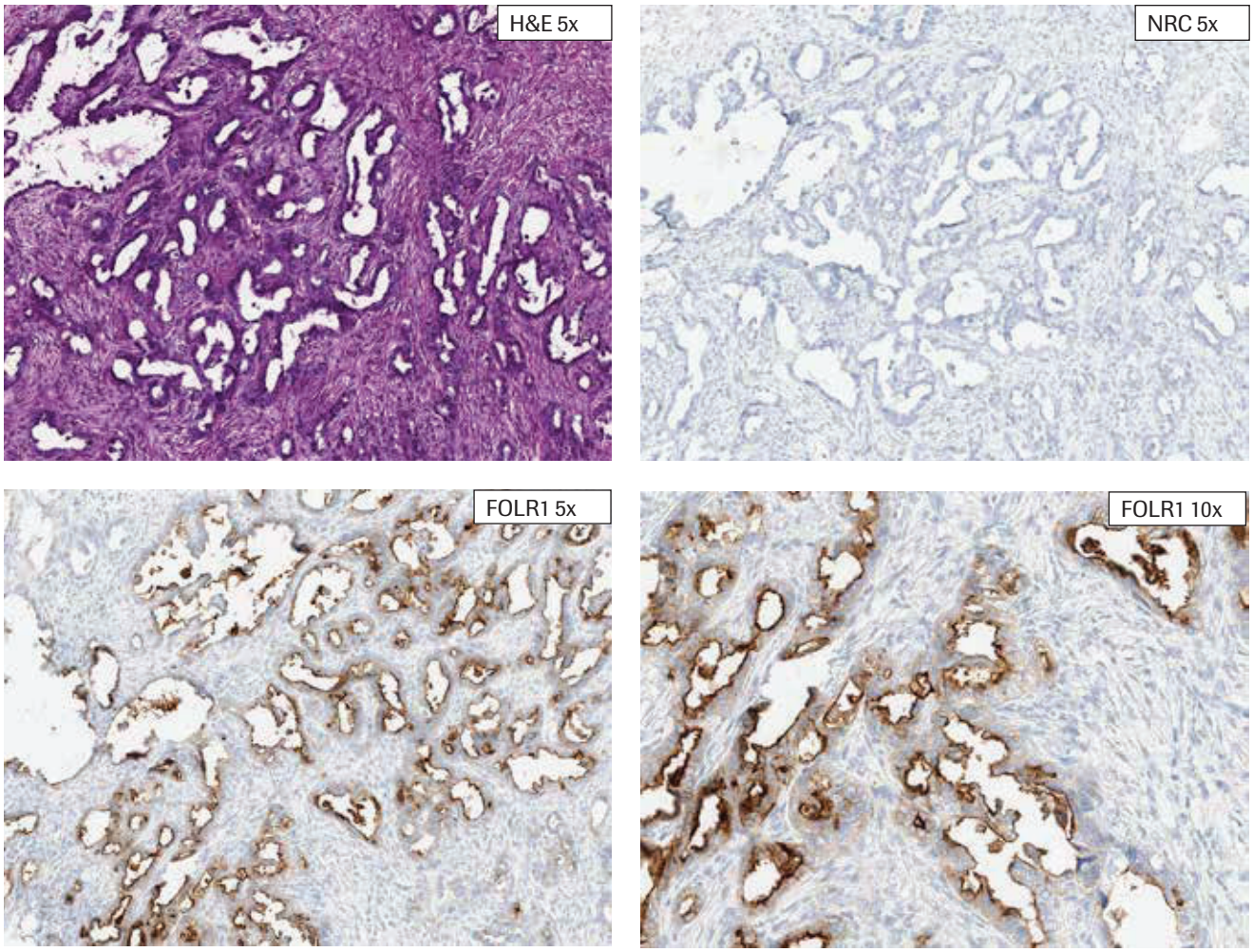
Positive EOC Case 2 exhibits 95% moderate and strong membrane staining with complete circumferential pattern or ≥ 75% of the tumor cells with moderate and/or strong membranous staining. This case is assigned a Positive FOLR1 status.



Positive EOC Case 3 exhibits 80% moderate and strong apical membrane staining in tumor cells or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a positive FOLR1 status.



Positive EOC Case 4 exhibits 85% moderate and strong membrane staining with complete circumferential pattern or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. This case is assigned a Positive FOLR1 status.



Positive EOC Case 5 exhibits 85% moderate and strong apical membranous staining or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Positive FOLR1 status.

Challenging Cases

While the vast majority of cases stained with the VENTANA FOLR1 Assay are clearly positive or negative in their staining results, a few cases have been observed that present a challenge in interpretation. Cases are placed into FOLR1 clinical categories according to the percentage of cells staining. The staining can be heterogeneous. Percent cell staining is determined by noting the number of tumor cells showing membranous staining. Viewing a case at multiple levels of magnification may be useful in visually estimating the relative percentages of tumor cells staining and differentiating tumor cells from normal stained cells.

Some cases may be particularly challenging due to the following issues:

- **Heterogeneous Expression**

Heterogeneity presents a challenge in case interpretation because it contributes to variable membrane staining. Variable staining requires closer examination to determine percentage of staining.

- **Cytoplasmic Staining**

Some cases may exhibit cytoplasmic staining. This may present a challenge in interpretation of presence or absence of membrane staining. Evaluation of the FOLR1 slide must include examination at higher magnification. Cytoplasmic staining is not included in scoring.

- **Dot-Like Staining**

Some cases may exhibit dark brown secretions filling the gland lumens. This pattern should be included in scoring.

- **Tissue or Staining Artifact**

Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of FOLR1 Clinical Score. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

- **Borderline Category**

Some cases near the cut off are at the border between a positive and negative FOLR1 status (± 10% of cutoff). These cases are particularly challenging to estimate the number of tumor cells staining. For these borderline cases it may be helpful to view the case at a magnification that enables the entire tumor area to be assessed.

- **Apical Staining**

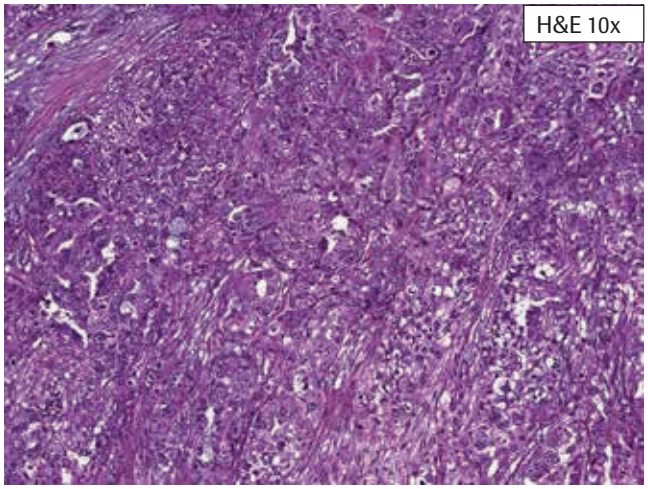
Some cases may exhibit apical, or luminal, staining. In tissues with glandular or tubular morphology, apical or luminal staining is defined as staining in the portion of the cells oriented towards the lumen of a gland or tubule, in contrast to basolateral staining, which describes staining in the portion of cells oriented away from the lumen (i.e., at the base). This staining pattern should be included in scoring.

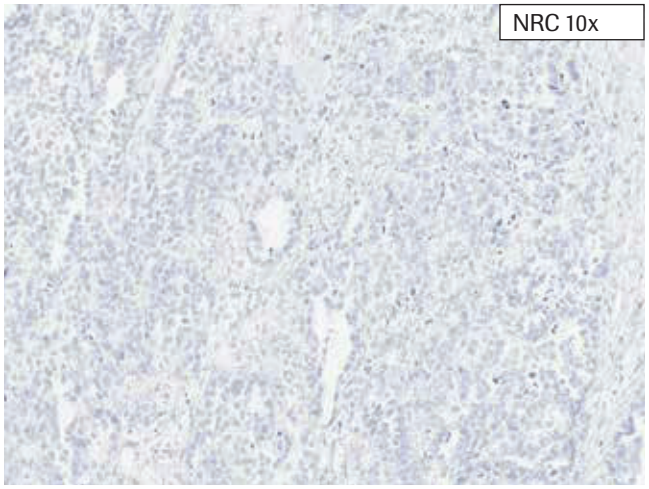
- **Non-Specific Background**

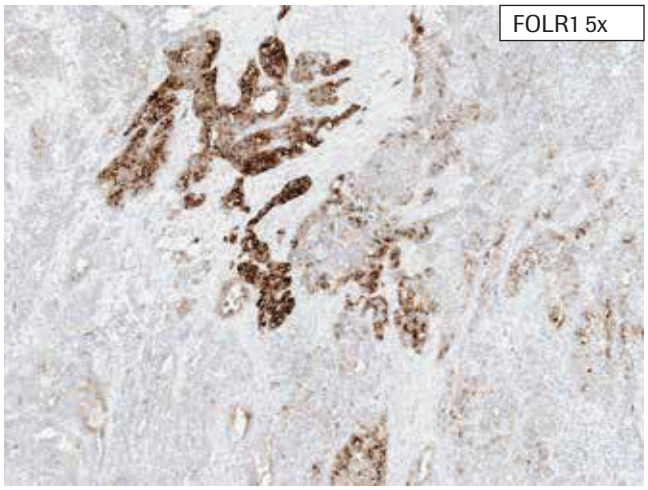
Some specimens may exhibit non-specific background staining for reasons that are not well understood. For this reason, evaluation of the FOLR1 IHC slide must include a comparison of the slide to the negative control slide to determine the level of non-specific background staining.

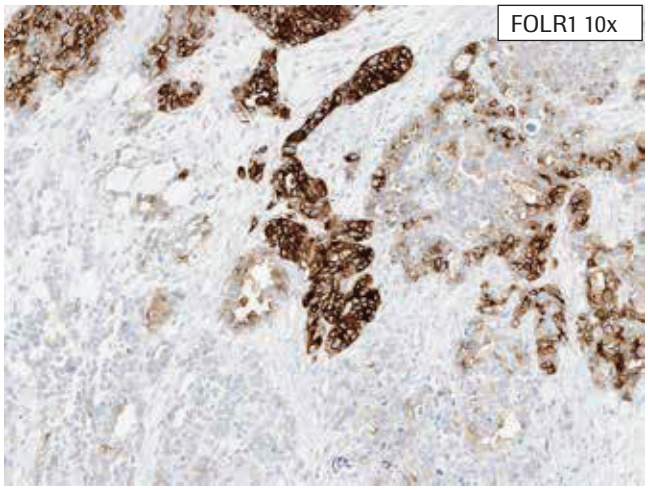
Examples of challenging cases are shown on the following pages. Scores for FOLR1-stained images were determined using the lower magnification image.

Heterogenous FOLR1 expression



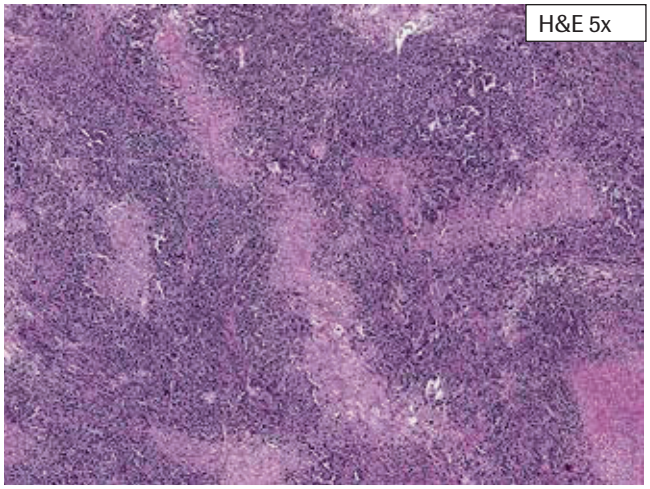


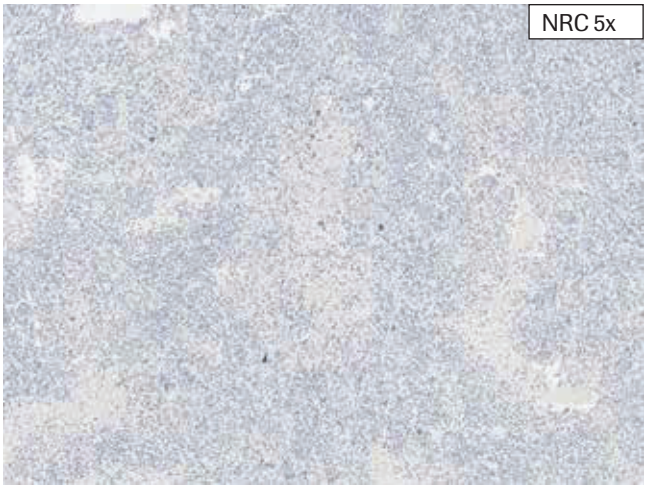


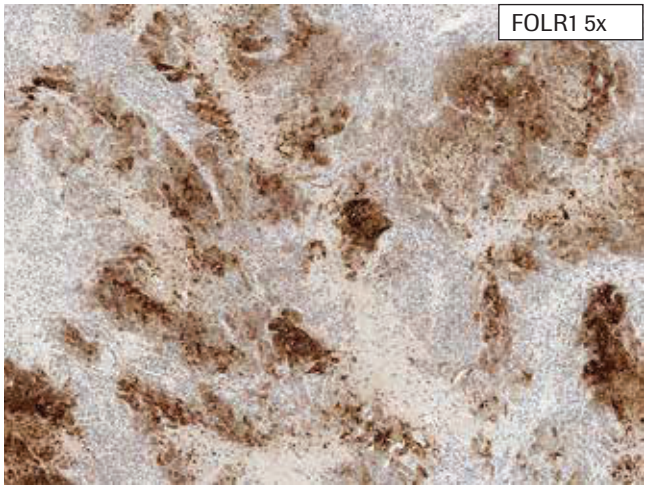


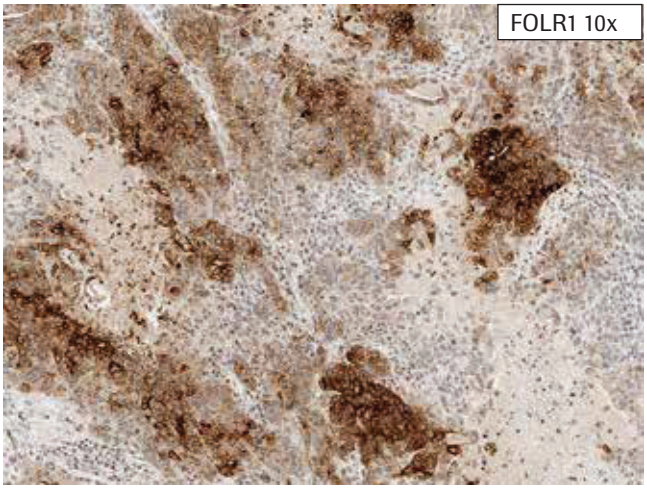
Challenging EOC Case 1 exhibits 40% moderate and strong membrane staining in tumor cells or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Cytoplasmic FOLR1 expression



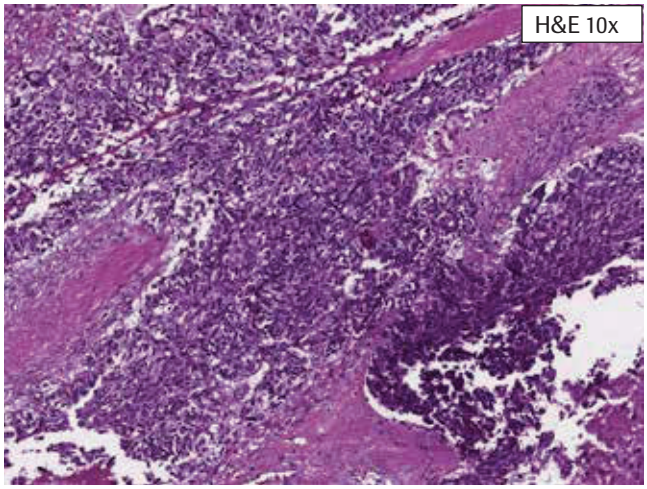


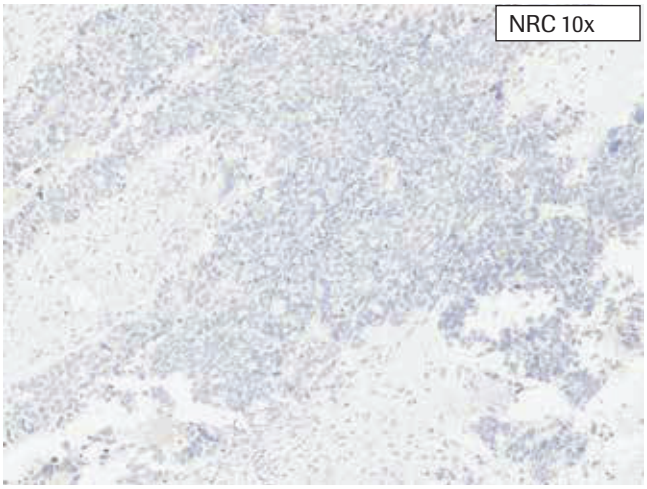


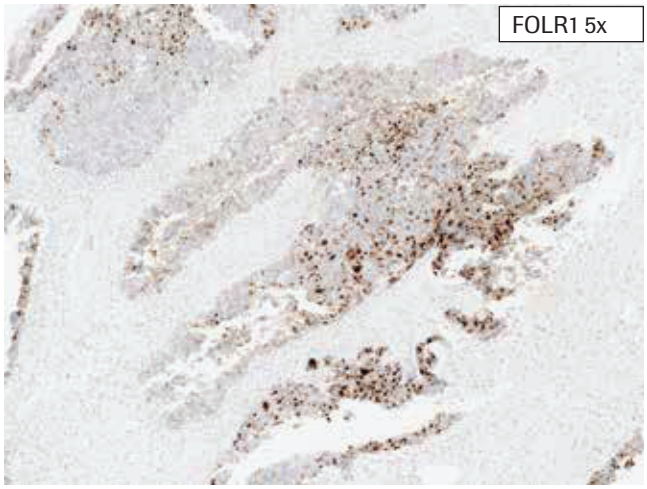


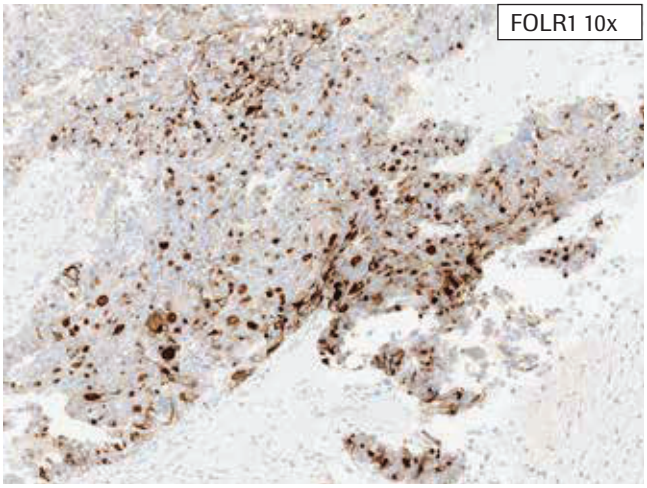
Challenging EOC Case 2 exhibits 55% moderate and strong membrane staining in tumor cells but also exhibits cytoplasmic staining which is not included as part of the FOLR1 scoring method. Cytoplasmic staining can interfere with membrane assessment. In this case, although cytoplasmic staining is present, moderate and/or strong membrane staining is not present in more than 75% of tumor cells. This case is assigned a Negative FOLR1 status.

Dot-like staining



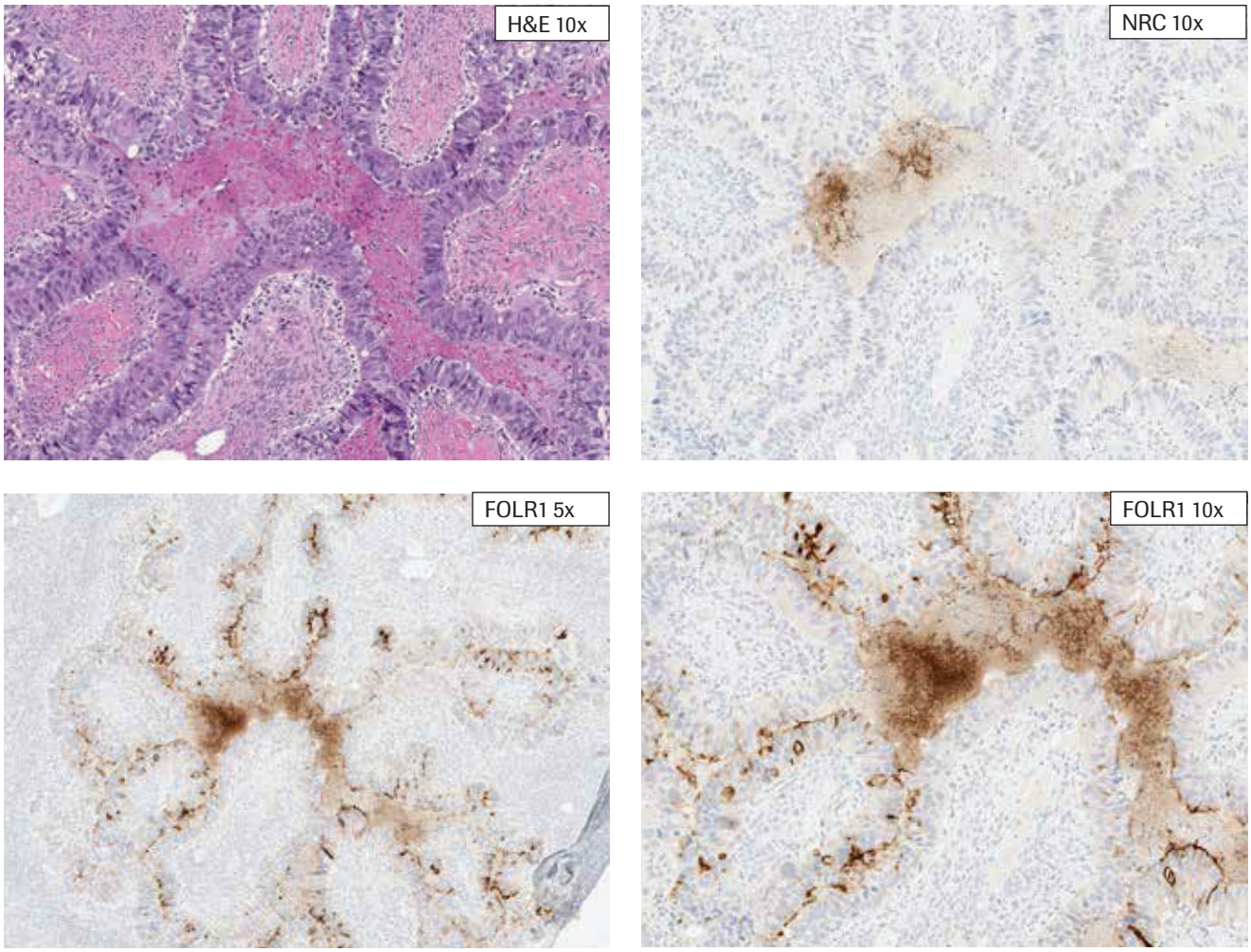






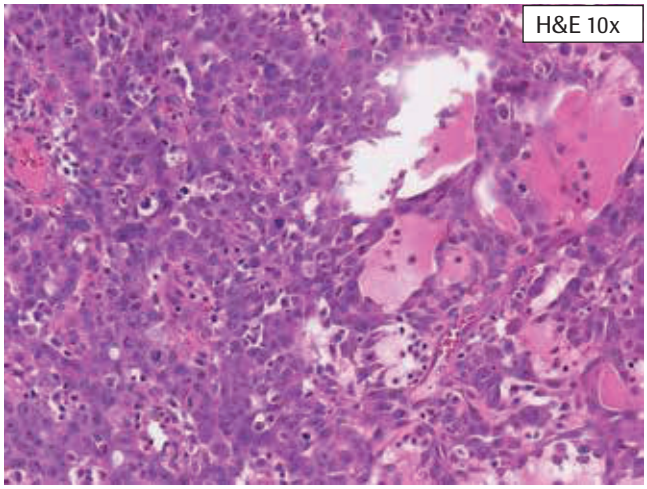
Challenging EOC Case 3 exhibits mostly dot-like staining, which is included as part of the FOLR1 scoring method. This image exhibits 70% moderate and strong membranous tumor cell staining or < 75% of the tumor cells with moderate and/or strong apical membrane staining. This case is assigned a Negative FOLR1 status.

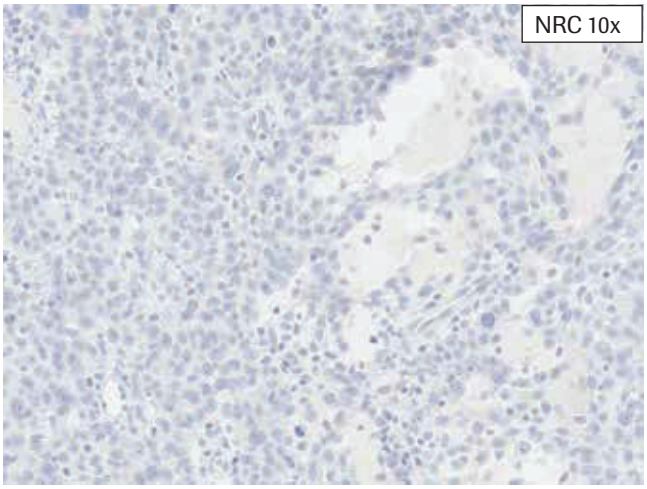
Staining artifact

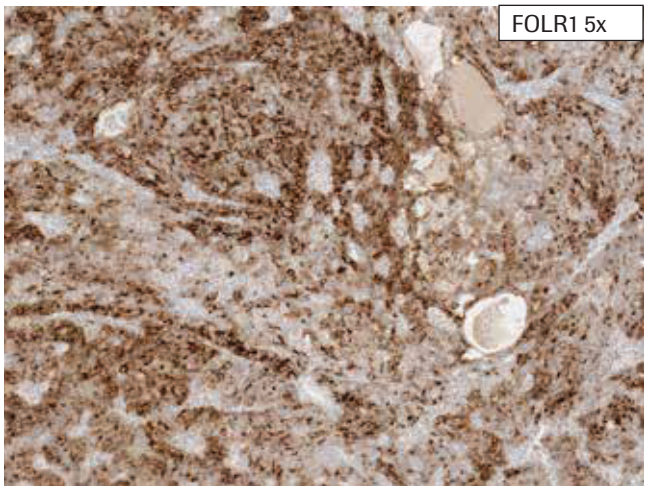


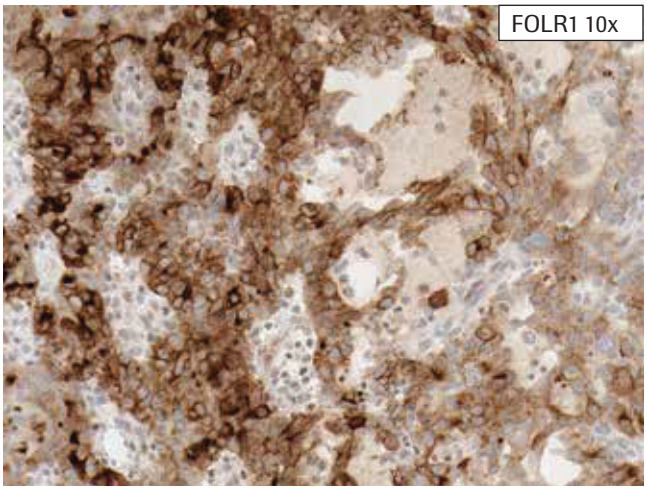
Challenging EOC Case 4 exhibits a staining artifact: DAB trapping. This is not included as part of the FOLR1 scoring method. Moderate and/or strong membrane staining, primarily apical, is present in < 75% of tumor cells. This image exhibits 35% moderate and strong primarily apical membrane staining or < 75% of tumor cells with moderate and/or strong tumor cell membrane relative to the negative control slide. This case is assigned a Negative FOLR1 status.

Borderline category



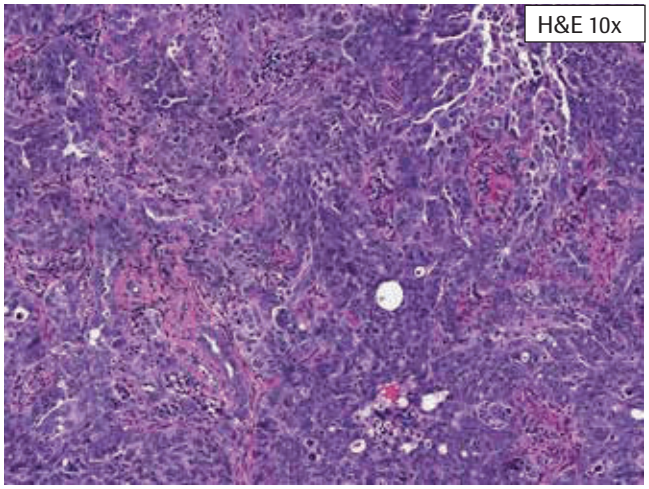


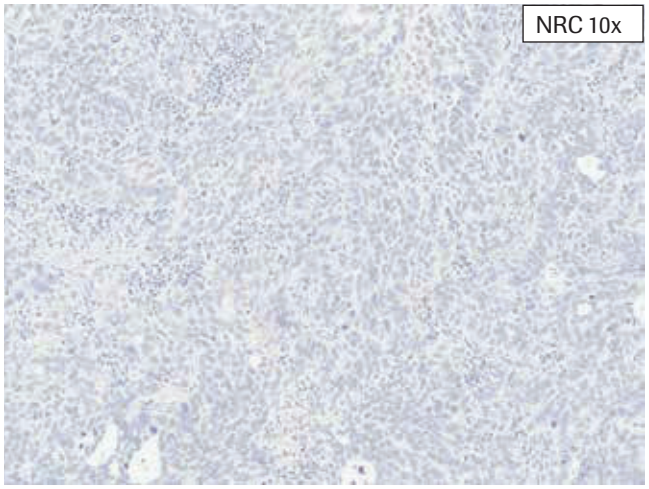


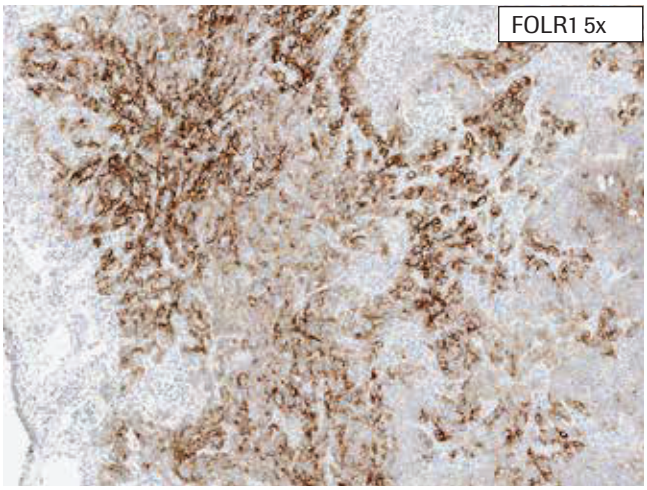


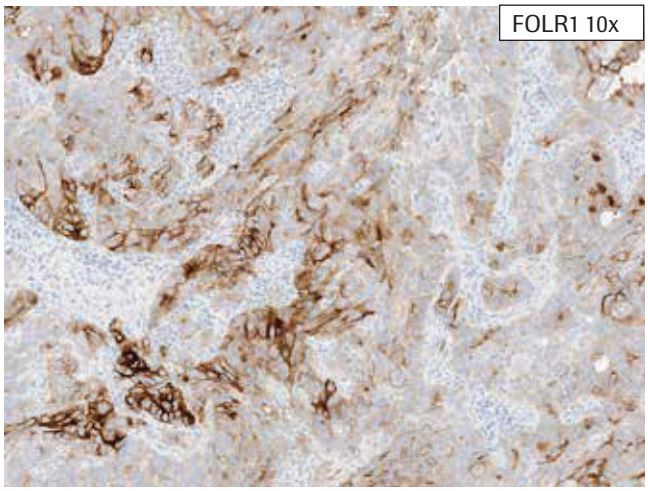
Challenging EOC Case 5 exhibits moderate and strong membrane staining that is at the borderline for the 75% cut off. This image exhibits 75% moderate and strong membrane staining in tumor cells. This borderline case is assigned Positive FOLR1 status when the 75% cut off is used.

Borderline category



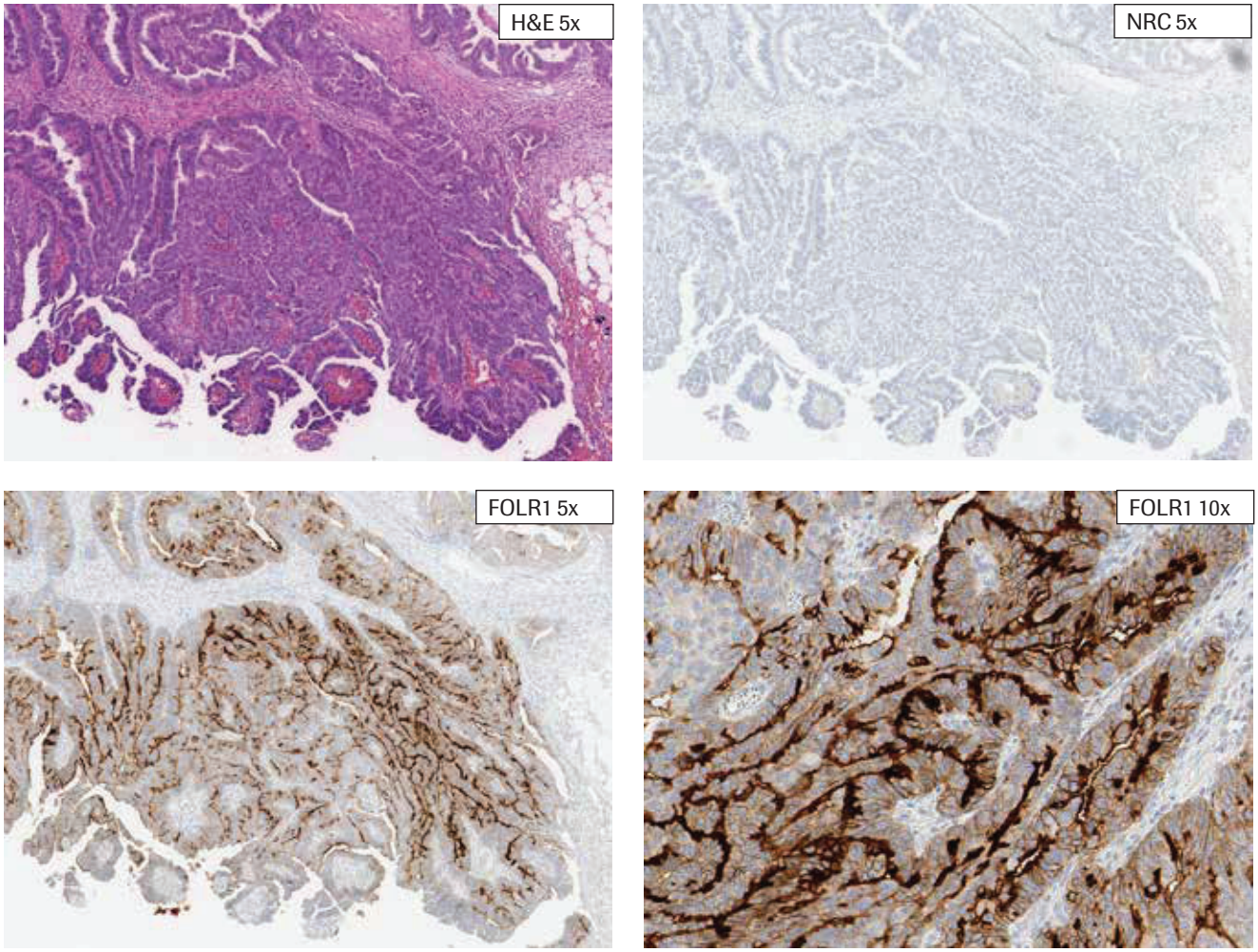






Challenging EOC Case 6 exhibits moderate and strong membrane staining that is at the borderline for the 75% cut off. This image exhibits 70% moderate and strong tumor cell membrane staining. This borderline case is assigned a Negative FOLR1 status when the 75% cut off is used.

Apical staining



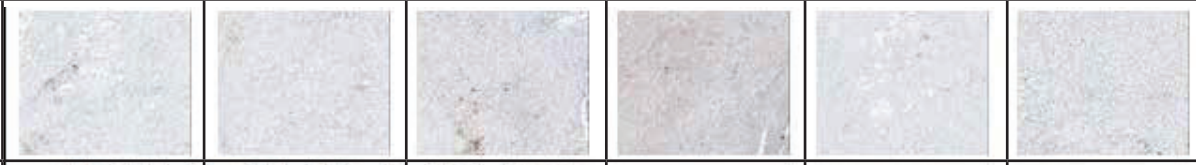
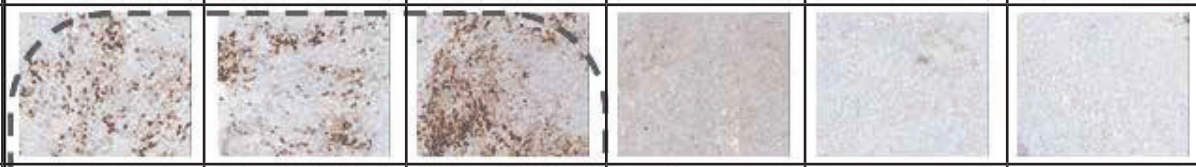
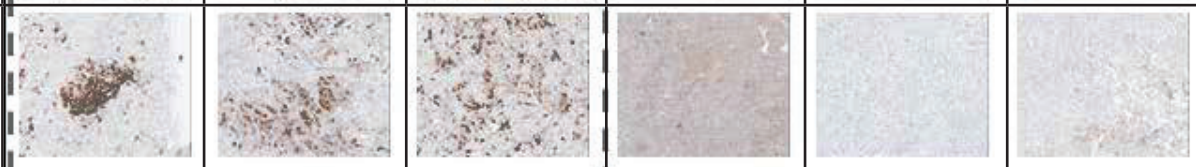
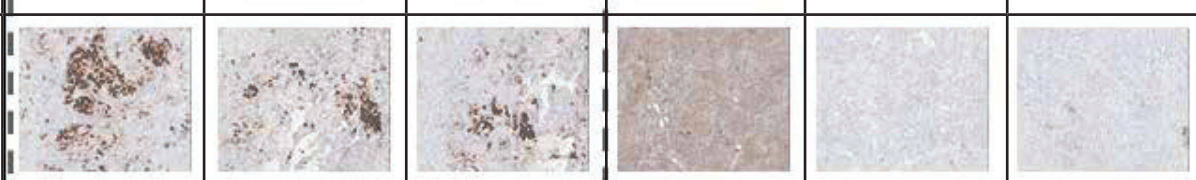
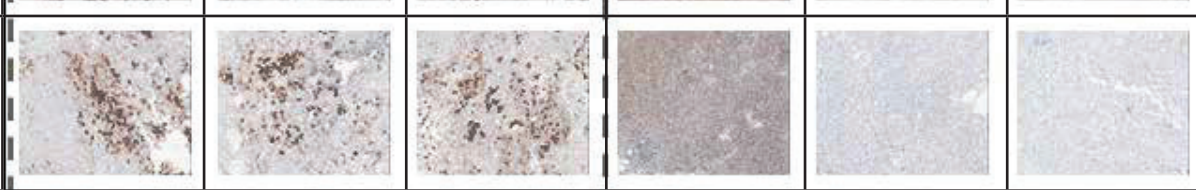
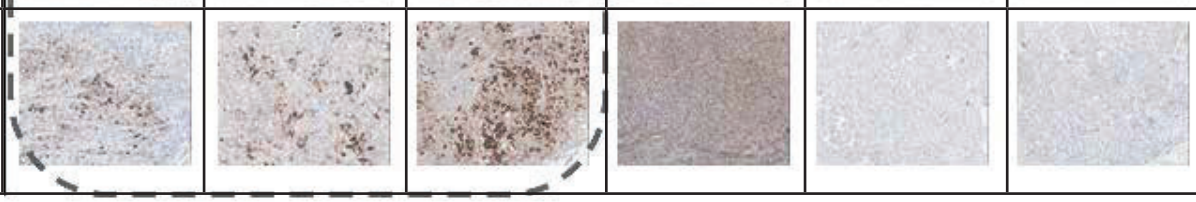
Challenging EOC Case 7 exhibits 80% moderate and strong primarily apical membrane staining or ≥ 75% of the tumor cells with moderate and/or strong apical membrane staining. Apical staining is defined as staining in the portion of the cells oriented towards the lumen of the gland/tubule. This case is assigned a Positive FOLR1 status.

Impact of Pre-analytical Conditions on VENTANA FOLR1 (FOLR1-2.1) RxDx Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results

- **Ventana recommends fixation in 10% NBF for 12-72 hours.** See acceptable fixatives and fixation times in rectangular box below.

Table 5: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Staining of Prostate Cancer Xenografts Across Fixatives and Fixation Time

Fixation Time (hrs)	Fixative					
	10% NBF	Zinc Formalin	Z-5	Prefer**	AFA**	95% Ethanol**
1*						
6*						
12						
24						
48						
72						

The following fixatives and fixation times are **not recommended**:

*Less than 12 hour fixation is not recommended.

**Use of Prefer or AFA or alcoholic fixatives (weaker staining) is not recommended. Use of Zinc Formalin or Z-5 are not recommended due to variability in percent tumor cell staining and potential for change in FOLR1 status.

Cut Slide Stability

Ventana has determined that the VENTANA FOLR1 Assay should not be performed on cut slides that have been stored longer than 45 days. Ventana has not tested the impact of cut slide stability combined with different fixatives, and 45 days may not be the optimal stability for fixatives other than NBF.

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