VENTANA MET (SP44) RxDx Assay

REF 740-7064

08866856001

IVD Σ 50

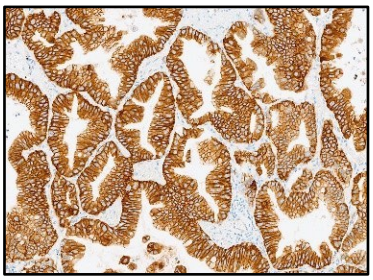

Figure 1. VENTANA MET (SP44) RxDx Assay staining of MET on non-squamous non-small cell lung cancer.

INTENDED USE

VENTANA MET (SP44) RxDx Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-MET, clone SP44, intended for use in the assessment of MET protein in formalin-fixed paraffin embedded (FFPE) non-squamous non-small cell lung cancer (NSCLC) specimens by light microscopy. This assay is for use with OptiView DAB IHC Detection Kit for staining on a BenchMark ULTRA or BenchMark ULTRA PLUS instrument.

MET protein expression clinical cut-off is ≥ 50% of tumor cells exhibiting strong membrane and/or cytoplasmic staining (3+) in non-squamous NSCLC.

VENTANA MET (SP44) RxDx Assay is indicated as an aid in identifying non-squamous NSCLC patients who may be eligible for treatment with EMRELIS™ (telisotuzumab vedotin-tllv).

The results of VENTANA MET (SP44) 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 MET (SP44) RxDx Assay (MET (SP44) Assay) is a rabbit monoclonal antibody, clone SP44, produced against the carboxyl region of the human MET protein. It recognizes MET proto-oncogene, receptor tyrosine kinase (MET), also known as hepatocyte growth receptor and sometimes abbreviated as c-Met.

The MET (SP44) Assay demonstrates membrane and cytoplasmic staining, which are both evaluated for the determination of MET status in non-squamous NSCLC.

PRINCIPLE OF THE PROCEDURE

MET (SP44) Assay binds to the MET protein in sections of formalin-fixed, paraffin-embedded tissue. The specific antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Refer to the OptiView DAB IHC Detection Kit method sheet for further information. Results are interpreted using a light microscope.

Clinical cases must be evaluated with appropriate tissue controls. In addition to staining with VENTANA MET (SP44) RxDx Assay, a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001). This slide must be negative for specific staining to be considered acceptable.

MATERIAL PROVIDED

MET (SP44) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of MET (SP44) Assay contains approximately 17.5 µg of a recombinant rabbit monoclonal MET (SP44) antibody.

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

Specific antibody concentration is approximately 3.5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

MET (SP44) Assay is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet 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 may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
8. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
9. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
10. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
11. General purpose laboratory equipment
12. BenchMark ULTRA or BenchMark ULTRA PLUS instrument
13. Permanent Mounting Medium
14. Cover glass
15. Automated coverslipper

STORAGE AND STABILITY

Upon receipt 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 FFPE non-squamous NSCLC tissues are suitable for use with this primary antibody when used with OptiView DAB IHC Detection Kit and BenchMark ULTRA and ULTRA PLUS instruments. The recommended fixative is 10% neutral buffered formalin (NBF).[1] Fix samples in formalin for 6-72 hours. Fixation times less than 6 hours have demonstrated a loss of specific MET immunoreactivity. Zinc formalin fixative is also acceptable for a fixation time of 6-72 hours.

Fixatives such as AFA, PREFER fixative, Z-fix, Alcohol Formalin and 95% ethanol are not acceptable for use with this assay and have demonstrated a significant loss of specific MET immunoreactivity.

Cut sections at approximately 4-5 µm in thickness and mount on positively charged slides. Stain slides immediately or store at 2-8 °C for no more than 45 days, as antigenicity of cut tissue sections can diminish over time.

It is recommended that positive and negative controls be run simultaneously with every IHC run.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. 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.
5. 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.

6. 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]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark ULTRA or BenchMark ULTRA PLUS instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. 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 and BenchMark ULTRA PLUS instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 for recommended staining protocol.

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 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 740-7064.

Table 2. Recommended staining protocol for MET (SP44) Assay with OptiView DAB IHC Detection Kit on BenchMark ULTRA and BenchMark ULTRA PLUS instruments.

Procedure Type	Method
	ULTRA or ULTRA PLUS
Staining Procedure	U VENTANA MET (SP44) RxDx Assay
Baking*	Optional**
Deparaffinization	4 minutes (default), 72 °C
Cell Conditioning (Antigen Unmasking)	ULTRA Cell Conditioning 1 64 minutes, 100 °C
Pre-antibody peroxidase inhibitor	4 minutes, 36 °C
Antibody (Primary)*	VENTANA MET (SP44) RxDx Assay (16 minutes, 36 °C) Or Rabbit Monoclonal Negative Control Ig (16 minutes, 36 °C)
OptiView HQ Linker	8 minutes (default), 36 °C
OptiView HRP Multimer	8 minutes (default), 36 °C
Counterstain*	Hematoxylin II, 4 minutes
Post Counterstain*	Bluing, 4 minutes

*Selectable by customer

** May be performed on-board the instrument (4-32 minutes, 60 °C) or performed offline (up to 60 minutes, 60 °C)

NEGATIVE REAGENT CONTROL

In addition to staining with MET (SP44) 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 staining run. Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures in 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 positive control tissue for this antibody is non-neoplastic gallbladder tissue, which demonstrates moderate staining of the membrane in epithelial cells and an absence of staining within cells of stromal tissue. Gallbladder tissue contains positive and negative staining elements for the MET protein and is therefore suitable for use as a tissue control. The positive and negative staining elements should be examined to ascertain if all reagents are functioning properly. If these elements fail to demonstrate appropriate

staining, any results with the test specimens included in the same staining run should be considered invalid. Refer to Table 3.

Table 3. System-Level Control Non-neoplastic Gallbladder for VENTANA MET (SP44) RxDx Assay

Acceptable	Unacceptable
Presence of moderate* membrane staining in the epithelium AND Absence of specific MET staining within cells of stromal tissue	Absence of moderate* membrane staining in the epithelium OR Excessive background staining of the stromal tissue that interferes with interpretation

* Moderate staining intensity is characterized by rich brown thickened membranes that are detectable at low magnifications such as 4x or 10x.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for MET (SP44) Assay is membranous and/or cytoplasmic with varying ranges of stain intensity. The stained slide(s) are interpreted using light microscopy. A qualified pathologist experienced in IHC staining interpretation must evaluate tissue controls before interpreting patient results.

Refer to VENTANA MET (SP44) RxDx Assay Interpretation Guide for NSCLC (P/N 1018336US) for specifics and images.

Negative Reagent Control

Non-specific staining, if present, may have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Refer to Table 5 for the acceptability criteria for non-specific staining.

Patient Tissue

Patient specimens should be examined last and must be evaluated according to the VENTANA MET (SP44) RxDx Assay scoring algorithm provided in Table 4 and Table 5. Refer to the VENTANA MET (SP44) RxDx Assay Interpretation Guide for NSCLC (P/N 1018336US) for representative images and instructions for scoring.

The cellular staining pattern for VENTANA MET (SP44) RxDx Assay is membranous and/or cytoplasmic staining of tumor cells in non-squamous NSCLC tissue with varying ranges of stain intensity. 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 strong intensity membranous and/or cytoplasmic staining will contribute to the MET status determination using the scoring method.

Strong (3+) signal intensity is characterized by dark brown to black hue with saturated DAB. Membranes/cytoplasm exhibiting strong signal are easily detectable at low power magnifications such as 2x or 4x. Non-squamous NSCLC tissue cases are considered positive for MET status if ≥ 50% of viable tumor cells (TC) demonstrate strong membranous and/or cytoplasmic staining. Non-squamous NSCLC tissue is evaluated for MET IHC Status according to the VENTANA MET (SP44) scoring algorithms (Table 4) and the non-specific background scoring criteria (Table 5).

Table 4. VENTANA MET (SP44) RxDx Assay Scoring Algorithm for NSCLC for the 50% Strong Tumor Cell Staining Cutoff.

MET IHC Status	Staining Criteria
Positive*	≥ 50% of tumor cells with strong membrane and/or cytoplasmic staining intensity.
Negative*	< 50% of tumor cells with strong membrane and/or cytoplasmic staining intensity
Not Evaluable (N/E)	Interpretation is not possible, e.g., no tissue present, presence of staining artifacts or poor tissue quality

*Re-reading by Additional Pathologists for MET Scoring

Table 5. Non-specific background scoring criteria for VENTANA MET (SP44) RxDx Assay

Interpretation	Staining Description
Acceptable	Non-specific staining that is not obtrusive to interpretation of specific staining.
Unacceptable	Non-specific staining that is obtrusive to interpretation of specific staining.

To decrease variability of MET results for cases with %TC near the threshold of 50% (40% to 60%) or with staining intensity near the strong (3+) versus moderate (2+) threshold, re-reading of the slide by a second pathologist is recommended. A case result with %TC between 40-60% or borderline strong versus moderate intensity by a pathologist should be adjudicated by one or two independent pathologists. The patient's final result with regard to MET staining (positive or negative) should be obtained by consensus among the pathologists. In NSCLC samples, weak to moderate MET staining can also occur in type II pneumocytes and the normal epithelium of bronchi and bronchioles, which may serve as an internal control. If the internal controls show no staining, this can suggest a staining failure.

SPECIFIC LIMITATIONS

This antibody demonstrates the following specific limitations:

1. The VENTANA MET (SP44) RxDx Assay has been optimized on the BenchMark ULTRA and BenchMark ULTRA PLUS instruments in combination with the OptiView DAB IHC Detection Kit at a 16 minute primary antibody incubation time. Incubation times and temperatures other than those specified may give erroneous results.
2. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of slides, and interpretation of staining results.
3. 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 or false negative results. Inconsistent results may arise from variations in fixation and embedding methods.
4. The recommended fixative is 10% NBF. Fix samples in formalin for 6-72 hours. Zinc formalin is also acceptable for fixative time of 6-72 hours. Samples fixed in formalin and zinc formalin for fixation times less than 6 hours have demonstrated a loss of specific MET immunoreactivity and can lead to a false negative result.
5. Fixatives such as AFA, PREFER fixative, Z-fix, Alcohol Formalin and 95% ethanol are not acceptable for use with this assay and have demonstrated a significant loss of specific MET immunoreactivity and can lead to a false negative result. When fixative type and/or fixation duration are unknown or outside of the recommended parameters, and a negative test result is obtained, consider including a statement in the pathology report that re-testing with a new sample collected and prepared according to the recommended device specifications may be warranted.
6. Cytology specimen processing requirements for the use of cytology fixatives such as CytoLyt® and SurePath™ collection media have not been established. Cytology specimens may be collected into and rinsed in 10% NBF and cell blocks may be fixed in 10% NBF.
7. Patient tissue sections should be refrigerated (5 ºC ± 3 ºC) and stained within 45 days of sectioning from the tissue block. Storage outside of recommended conditions have demonstrated a loss of specific MET immunoreactivity and can lead to a false negative result. Please refer to IX.A.5.b Section in SSED for more details.
8. For optimal results, consultation with a second pathologist with reporting on the consensus result is recommended for cases scored near the %TC and staining intensity cutoffs for companion diagnostic indications.
9. This assay has not been validated for use with cytology smears or decalcified specimens.

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.

VENTANA®

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Sensitivity and Specificity

In a commercial cohort of 100 unique NSCLC (resection, biopsy (core needle biopsy (CNB)) and cell block [fine needle aspirate (FNA)]) cases were evaluated for MET staining. Of the 100 cases, 91 were described as having both membrane and cytoplasmic staining and 8 had cytoplasmic staining only. One case had no staining present. MET positive status was observed in 12% (12/100) of cases. MET negative status was observed in 88% (88/100) of cases.

Analytical sensitivity and specificity was evaluated using arrays and tissue resections containing a variety of non-neoplastic and neoplastic tissues stained with VENTANA MET (SP44) RxDx Assay and evaluated for presence of membranous and/or cytoplasmic MET staining as listed in Table 6 and Table 7.

Off-target focal nuclear staining was observed in <1.0% of evaluable samples (4/606), including non-neoplastic fallopian tube and heart and squamous cell carcinoma (esophagus and lung).

Table 6. Sensitivity/Specificity of MET (SP44) Assay was determined by testing FFPE non-neoplastic tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/7	Esophagus[c]	3/4
Cerebellum	0/7	Stomach[g,h]	7/7
Adrenal gland	0/7	Small intestine[g]	5/7
Ovary	0/7	Colon[g]	5/6
Pancreas[a]	1/7	Liver[i]	2/7
Parathyroid gland	0/5	Salivary gland[j]	5/6
Pituitary gland	0/5	Kidney[k]	5/9
Testis	0/7	Prostate[c]	5/7
Thyroid[b]	3/7	Cervix[c]	3/5
Breast[c]	2/5	Skin[c]	2/5
Spleen	0/6	Mesothelium[l]	3/4
Tonsil[d]	5/6	Appendix[g]	3/3
Endometrium[c]	3/6	Bladder[m]	4/5
Skeletal muscle	0/6	Fallopian tube[c]	4/7
Nerve	0/5	Rectum[g]	3/4
Thymus[e]	0/6	Ureter[n]	2/2
Myeloid (bone marrow)	0/5	Lymph node (reactive)	0/1
Lung[f,g]	13/16	Placenta[o]	5/6
Heart	0/6	Spinal cord	0/2

[a] acinar cells (focal staining), [b] follicular cells, [c] epithelial cells (weak staining), [d] germinal center lymphocytes (weak staining), [e] epithelial cells (focal staining), [f] type II pneumocytes, [g] epithelial cells, [h] fundic glands, [i] focal hepatocyte staining, [j] serous glands and striated duct epithelium, [k] renal tubular epithelial cells, [l] mesothelial cells, [m] urothelial cells, [n] urothelial cells (weak staining), [o] cytotrophoblastic and syncytiotrophoblastic cells

Table 7. Sensitivity/Specificity of MET (SP44) Assay was determined by testing FFPE neoplastic tissues.

Pathology	# positive / total cases
Adenoma, cortical (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Urothelial carcinoma (Bladder)	2/2
Fibroadenoma (Breast)	0/1
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Meningioma (Cerebellum)	0/2
Meningioma (Cerebrum)	1/1
Astrocytoma (Cerebrum)	0/1
Squamous cell carcinoma (Esophagus)	2/2
Adenocarcinoma (Stomach)	3/3
Adenoma (Small intestine)	1/1
Adenocarcinoma (Small intestine)	1/1
Adenoma (Colon)	1/1
Adenocarcinoma (Colon)	3/3
Adenocarcinoma (Rectum)	3/3
Clear cell carcinoma (Kidney)	2/2
Hepatocellular carcinoma (Liver)	2/4
Squamous cell carcinoma (Lung)	105/145
Adenocarcinoma (Lung)	96/116
Small cell carcinoma (Lung)	0/1
Adenosquamous carcinoma (Lung)	1/1
Adenocarcinoma in-situ (Lung)	12/13
Mucinous adenocarcinoma (Lung)	8/8
Papillary carcinoma (Lung)	5/6
Neuroendocrine tumor, typical carcinoid (Lung)	3/11
Neuroendocrine carcinoma (Lung)	5/5
Large cell carcinoma (Lung)	8/9
Large cell neuroendocrine carcinoma (Lung)	3/3
Pleomorphic carcinoma (Lung)	5/5
Mucoepidermoid carcinoma (Lung)	1/1
Hodgkin lymphoma (Lymph node)	0/1
B-cell lymphoma, NOS (Lymph node)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Adenocarcinoma (Oral cavity)	0/1
Squamous cell carcinoma (Oral cavity)	1/1
Nasopharyngeal carcinoma (Nasopharynx)	1/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	1/1
Endometrioid adenocarcinoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Adenocarcinoma (Prostate)	0/2
Pleomorphic adenoma (Salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	1/1

Pathology	# positive / total cases
Squamous cell carcinoma (Skin)	1/1
Melanoma (Nasal cavity)	1/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	2/3
Follicular carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Squamous cell carcinoma (Cervix)	2/2
Adenocarcinoma (Endometrium)	2/2
Metastatic Colon adenocarcinoma (Liver)	1/1
Metastatic Breast ductal carcinoma (Lymph Node)	1/1
Metastatic Colon signet ring cell carcinoma (Ovary)	1/1
Metastatic Esophageal squamous cell carcinoma (Lymph Node)	1/1

Precision- NSCLC 50% Strong Tumor Cell Staining

The precision of the VENTANA MET (SP44) RxDx Assay was evaluated in multiple precision studies: Repeatability and Intermediate Precision study, Between Day Precision study and Reader (pathologist) Precision study.

Repeatability and Intermediate Precision- NSCLC 50% Strong Tumor Cell Staining

Twenty-four unique NSCLC resection tissue cases were enrolled (12 MET Positive and 12 MET Negative) in the intermediate precision study. The study design for evaluation of staining precision on NSCLC stained with MET (SP44) Assay included:

- Three lots of MET antibody
- Three lots of OptiView DAB IHC Detection Kits
- Three BenchMark ULTRA instruments
- Three non-consecutive day precision on the BenchMark ULTRA
- Within run repeatability on the BenchMark ULTRA

Twenty-eight unique NSCLC resection tissue cases were enrolled (12 MET Positive and 16 MET Negative) in the intermediate precision study. The study design for evaluation of staining precision on NSCLC stained with MET (SP44) Assay included:

- Three BenchMark ULTRA PLUS instruments
- Three non-consecutive day precision on the BenchMark ULTRA PLUS
- Within run repeatability on the BenchMark ULTRA PLUS

Twenty-eight unique NSCLC resection tissue cases were enrolled (12 MET Positive and 16 MET Negative) in the intermediate precision study. The study design for evaluation of staining precision on NSCLC stained with MET (SP44) Assay included:

- Five non-consecutive day precision on the BenchMark ULTRA
- Five non-consecutive day precision on the BenchMark ULTRA PLUS

All slides were blinded, randomized and then evaluated using the VENTANA MET (SP44) RxDx Assay scoring algorithm for the 50% Strong TC staining cutoff (Table 4).

All studies met their acceptance criteria. Results are summarized in Table 8.

Table 8. Repeatability and intermediate precision of the VENTANA MET (SP44) RxDx Assay staining of NSCLC specimens for the 50% Strong TC Staining cutoff.

Repeatability/Intermediate Precision Parameter	Agreement			
	Type	n/N	%	95% CI
Between antibody lot precision	PPA	76/78	97.4	(91.7, 100.0)
	NPA	64/64	100.0	(94.3, 100.0)
	OPA	140/142	98.6	(95.7, 100.0)
	PPA	75/78	96.2	(90.3, 100.0)
Between detection kit lot precision	NPA	61/65	93.8	(83.3, 100.0)
	OPA	136/143	95.1	(89.5, 99.3)
Between ULTRA instrument precision	PPA	78/78	100.0	(95.3, 100.0)
	NPA	65/65	100.0	(94.4, 100.0)
	OPA	143/143	100.0	(97.4, 100.0)
Between 3 non-consecutive day intermediate precision-ULTRA	PPA	75/78	96.2	(89.7, 100.0)
	NPA	63/65	96.9	(89.8, 100.0)
	OPA	138/143	96.5	(91.7, 100.0)
Within run repeatability-ULTRA	PPA	230/232	99.1	(97.9, 100.0)
	NPA	196/198	99.0	(97.5, 100.0)
	OPA	426/430	99.1	(98.1, 99.8)
Between ULTRA PLUS instrument precision	PPA	54/54	100.0	(93.4, 100.0)
	NPA	114/114	100.0	(96.7, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Between 3 non-consecutive day intermediate precision-ULTRA PLUS	PPA	54/54	100.0	(93.4, 100.0)
	NPA	114/114	100.0	(96.7, 100.0)
	OPA	168/168	100.0	(97.8, 100.0)
Within run repeatability-ULTRA PLUS	PPA	45/45	100.0	(92.1, 100.0)
	NPA	95/95	100.0	(96.1, 100.0)
	OPA	140/140	100.0	(97.3, 100.0)
Between 5 non-consecutive day intermediate precision-ULTRA	PPA	120/130	92.3	(85.5, 98.0)
	NPA	144/147	98.0	(94.7, 100.0)
	OPA	264/277	95.3	(91.8, 98.2)
Between 5 non-consecutive day intermediate precision-ULTRA PLUS	PPA	129/130	99.2	(97.7, 100.0)
	NPA	148/149	99.3	(97.8, 100.0)
	OPA	277/279	99.3	(98.2, 100.0)

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

Between Day Intermediate Precision Biopsies- NSCLC 50% Strong Tumor Cell Staining

Twenty unique NSCLC biopsy cases were enrolled (9 MET Positive and 11 MET Negative) in the between day precision study on the BenchMark ULTRA. The study evaluated the staining precision on NSCLC biopsy cases stained with MET (SP44) Assay across three non-consecutive days. All slides were blinded, randomized and then evaluated using the VENTANA MET (SP44) RxDx Assay scoring algorithm for the 50% Strong TC staining cutoff (Table 4).

All studies met their acceptance criteria. Results are summarized in Table 9.

Table 9. Between day intermediate precision of the VENTANA MET (SP44) RxDx Assay staining of NSCLC biopsy specimens for the 50% Strong TC staining cutoff.

Intermediate Precision Parameter	Agreement			
	Type	n/N	%	95% CI
Between day intermediate precision biopsies	PPA	40/42	95.2	(85.2, 100.0)
	NPA	77/78	98.7	(95.8, 100.0)
	OPA	117/120	97.5	(93.3, 100.0)

Intermediate Precision Parameter	Agreement			
	Type	n/N	%	95% CI

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

Between Day Intermediate Precision Cell Blocks- NSCLC 50% Strong Tumor Cell Staining

Twenty-one unique NSCLC cell block (FNA) cases were enrolled (10 MET Positive and 11 MET Negative) in the between day precision study on the BenchMark ULTRA. The study evaluated the staining precision on NSCLC FNA cell block cases stained with MET (SP44) Assay across three non-consecutive days.

Sixteen unique NSCLC cell block (pleural effusion) cases were enrolled (2 MET Positive and 14 MET Negative) in the between day precision study on the BenchMark ULTRA. The study evaluated the staining precision on NSCLC pleural effusion cell block cases stained with MET (SP44) Assay across three non-consecutive days.

All slides were blinded, randomized and then evaluated using the VENTANA MET (SP44) RxDx Assay scoring algorithm for the 50% Strong TC staining cutoff (Table 4).

All studies met their acceptance criteria. Results are summarized in Table 10.

Table 10. Between day intermediate precision of the VENTANA MET (SP44) RxDx Assay staining of NSCLC cell blocks specimens for the 50% Strong TC staining cutoff.

Intermediate Precision Parameter	Agreement			
	Type	n/N	%	95% CI
Between day intermediate precision - FNAs	PPA	54/54	100.0	(93.4, 100.0)
	NPA	72/72	100.0	(94.9, 100.0)
	OPA	126/126	100.0	(97.0, 100.0)
Between day intermediate precision - pleural effusions	PPA	11/11	100.0	(74.1, 100.0)
	NPA	84/84	100.0	(95.6, 100.0)
	OPA	95/95	100.0	(96.1, 100.0)

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

Reader Precision- NSCLC 50% Strong Tumor Cell Staining

In the Reader Precision study for the MET (SP44) Assay, Within-Reader and Between-Reader components of precision for NSCLC tissue reads were evaluated. The study included 100 unique NSCLC (resection and biopsy) specimens (50 MET Positive and 50 MET Negative) that were stained with MET (SP44) Assay. Specimens were blinded and randomized prior to evaluation for MET status using the VENTANA MET (SP44) RxDx Assay scoring algorithm for the 50% Strong TC staining cutoff (Table 4). 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 the three readers). The following precision components were calculated: within-reader and between-reader. Results are summarized in Table 11 below.

Table 11. Within-Reader and Between-Reader Precision of the VENTANA MET (SP44) RxDx Assay for NSCLC specimens for the 50% Strong TC Staining cutoff.

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	288/293	98.3	(96.6, 99.6)
	ANA	302/307	98.4	(96.6, 99.7)
	OPA	295/300	98.3	(96.7, 99.7)
Between-Reader	APA	278/292	95.2	(91.3, 98.2)
	ANA	294/308	95.5	(91.9, 98.4)
	OPA	286/300	95.3	(92.0, 98.0)

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

Reader Precision Cell Blocks- NSCLC 50% Strong Tumor Cell Staining

In the Reader Precision study for the MET (SP44) Assay, Within-Reader and Between-Reader components of precision for NSCLC tissue reads were evaluated. The study included 40 unique NSCLC cell block (FNA and pleural effusion) specimens (20 MET Positive and 20 MET Negative) that were stained with MET (SP44) Assay. Specimens were blinded and randomized prior to evaluation for MET status using the VENTANA MET (SP44) RxDx Assay scoring algorithm for the 50% Strong TC staining cutoff (Table 4). The study included four readers (pathologists). Readers scored all specimens twice, with a minimum of two weeks between reads. Each case had 8 reads (2 reads by each of the four readers). The following precision components were calculated: within-reader and between-reader. Results are summarized in Table 12 below.

Table 12. Within-Reader and Between-Reader Precision of the VENTANA MET (SP44) RxDx Assay for NSCLC specimens for the 50% Strong TC Staining Cutoff.

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	APA	164/165	99.4	(98.1, 100.0)
	ANA	154/155	99.4	(98.0, 100.0)
	OPA	159/160	99.4	(98.1, 100.0)
Between-Reader	APA	234/246	95.1	(90.5, 98.8)
	ANA	222/234	94.9	(89.5, 98.8)
	OPA	228/240	95.0	(90.0, 98.8)

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

Inter-laboratory Reproducibility and Method Comparison Study- NSCLC 50% Strong Tumor Cell Staining

The Inter-laboratory Reproducibility (ILR) and Method Comparison (MC) study for the VENTANA MET (SP44) RxDx Assay was conducted to evaluate the reproducibility of the VENTANA MET (SP44) RxDx Assay on the BenchMark ULTRA and the BenchMark ULTRA PLUS platforms independently. Additionally, a method comparison between the BenchMark ULTRA and BenchMark ULTRA PLUS was performed to determine if the performance of the VENTANA MET (SP44) RxDx Assay is equivalent on both BenchMark platforms. The study included 60 NSCLC specimens (30 MET Positive and 30 MET Negative, including several cases which exhibited borderline/challenging staining relative to the diagnostic cutoff) run across three BenchMark ULTRA and three BenchMark ULTRA PLUS instruments on 3 non-consecutive days at three external laboratories. For each BenchMark platform a set of 3 stained slides per sample per staining day was randomized and evaluated by a total of 6 readers (2 readers per site). Each case had 6 results per site per BenchMark platform (18 results per platform, 36 results in total). Performance was evaluated and the following precision components were calculated: between-reader, between-site and total. Results are presented in the tables below.

Table 13. Results of the Inter-Laboratory Reproducibility Study (ULTRA)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
1	Negative	0	0, 0	0	0	0	0	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
2	Negative	0	0, 1	0	0	0	0.24	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
3	Negative	0	0, 2	0.41	0.33	0.10	0.63	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
4	Negative	2.5	0, 15	0.58	0.85	2.72	3.97	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
5	Negative	2.5	0, 5	1.33	0	1.65	2.26	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
6	Negative	2.5	0, 20	1.84	2.27	2.34	5.74	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
7	Negative	3.5	0, 15	2.67	1.67	2.61	4.79	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
8	Negative	4.5	0, 35	5.33	3.81	7.44	10.97	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
9	Negative	5.0	0, 15	1.08	0	4.08	5.14	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
10	Negative	5.0	0, 15	2.01	0	3.06	5.42	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
11	Negative	7.5	0, 20	1.99	2.79	5.32	6.80	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
12	Negative	7.5	0, 20	3.19	2.79	5.84	8.00	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
13	Negative	7.5	0, 35	4.58	6.00	6.21	10.20	0/4 (0.0)	0/6 (0.0)	0/6 (0.0)	0/16 (0.0)
14	Negative	7.5	0, 65	20.24	4.65	8.98	23.24	2/6 (33.3)	0/6 (0.0)	0/4 (0.0)	2/16 (12.5)
15	Negative	10.0	1, 20	3.55	3.70	3.42	7.02	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
16	Negative	10.0	0, 20	0	0.00	7.34	9.07	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
17	Negative	12.5	0, 40	6.28	3.73	12.46	15.41	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
18	Negative	17.5	5, 25	2.47	0	6.25	8.11	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
19	Negative	17.5	0, 50	9.94	0	12.73	18.06	0/6 (0.0)	0/6 (0.0)	1/6 (16.7)	1/18 (5.6)
20	Negative	20.0	0, 40	7.83	0	14.16	16.40	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
21	Negative	22.5	0, 45	6.00	5.41	9.67	15.66	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
22	Negative	25.0	0, 50	12.08	5.77	0	16.16	1/6 (16.7)	1/6 (16.7)	0/6 (0.0)	2/18 (11.1)
23	Negative	27.5	10, 50	8.58	0	5.00	12.25	1/6 (16.7)	0/6 (0.0)	0/6 (0.0)	1/18 (5.6)
24	Negative	27.5	5, 60	15.94	5.53	0	18.18	0/6 (0.0)	0/6 (0.0)	3/6 (50.0)	3/18 (16.7)
25	Negative	27.5	1, 60	0	0	7.68	22.28	2/6 (33.3)	1/6 (16.7)	2/6 (33.3)	5/18 (27.8)
26	Negative	30.0	5, 60	6.12	6.67	10.65	17.02	1/6 (16.7)	0/6 (0.0)	1/6 (16.7)	2/18 (11.1)
27	Negative	40.0	20, 55	7.99	5.53	0	11.96	2/6 (33.3)	0/6 (0.0)	1/6 (16.7)	3/18 (16.7)
28	Negative	40.0	20, 50	12.58	4.25	0	13.99	1/6 (16.7)	1/6 (16.7)	2/6 (33.3)	4/18 (22.2)
29	Negative	40.0	5, 70	12.47	7.55	14.73	22.70	4/6 (66.7)	0/6 (0.0)	3/6 (50.0)	7/18 (38.9)
30	Negative	40.0	5, 70	15.86	0	0	19.86	3/6 (50.0)	3/6 (50.0)	2/6 (33.3)	8/18 (44.4)
31	Negative	45.0	5, 75	12.75	8.25	13.39	22.64	3/6 (50.0)	1/6 (16.7)	3/6 (50.0)	7/18 (38.9)
32	Positive	50.0	10, 70	4.08	6.87	10.18	15.17	6/6 (100.0)	2/6 (33.3)	3/6 (50.0)	11/18 (61.1)
33	Positive	50.0	5, 90	9.43	14.85	11.67	25.33	6/6 (100.0)	3/6 (50.0)	5/6 (83.3)	14/18 (77.8)
34	Positive	50.0	0, 70	9.99	15.85	0	20.27	5/6 (83.3)	3/6 (50.0)	2/6 (33.3)	10/18 (55.6)
35	Positive	50.0	10, 70	8.38	0	9.57	20.94	5/6 (83.3)	2/6 (33.3)	2/5 (40.0)	9/17 (52.9)
36	Positive	50.0	25, 90	22.82	6.97	0	25.90	4/6 (66.7)	3/6 (50.0)	4/6 (66.7)	11/18 (61.1)
37	Positive	50.0	1, 90	17.91	0	0	35.25	4/6 (66.7)	2/6 (33.3)	4/6 (66.7)	10/18 (55.6)
38	Positive	50.0	25, 75	9.35	6.01	4.71	13.39	6/6 (100.0)	1/6 (16.7)	4/6 (66.7)	11/18 (61.1)
39	Positive	50.0	1, 90	20.13	0	18.73	29.47	5/6 (83.3)	1/6 (16.7)	5/6 (83.3)	11/18 (61.1)
40	Positive	50.0	5, 80	22.42	4.08	0	23.66	5/6 (83.3)	3/6 (50.0)	5/6 (83.3)	13/18 (72.2)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
41	Positive	57.5	20, 80	12.19	9.28	6.40	19.98	6/6 (100.0)	3/6 (50.0)	3/6 (50.0)	12/18 (66.7)
42	Positive	62.5	10, 90	17.32	0	6.22	23.27	6/6 (100.0)	3/6 (50.0)	5/6 (83.3)	14/18 (77.8)
43	Positive	65.0	25, 98	13.47	0	3.55	20.84	6/6 (100.0)	4/6 (66.7)	5/6 (83.3)	15/18 (83.3)
44	Positive	72.5	1, 95	11.53	9.40	17.36	28.86	6/6 (100.0)	3/6 (50.0)	6/6 (100.0)	15/18 (83.3)
45	Positive	82.5	20, 100	20.96	7.17	8.04	25.28	6/6 (100.0)	4/6 (66.7)	6/6 (100.0)	16/18 (88.9)
46	Positive	85.0	5, 99	15.65	11.90	0	23.25	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	17/18 (94.4)
47	Positive	90.0	10, 100	0	5.11	11.94	22.38	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	17/18 (94.4)
48	Positive	92.5	5, 100	15.86	6.95	6.90	24.98	6/6 (100.0)	4/6 (66.7)	6/6 (100.0)	16/18 (88.9)
49	Positive	95.0	70, 100	3.81	3.33	2.06	7.22	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
50	Positive	95.0	65, 100	8.23	2.79	0	10.13	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
51	Positive	95.0	80, 100	5.68	0	0	8.80	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
52	Positive	95.0	70, 100	8.64	0.00	0	9.92	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
53	Positive	97.5	90, 100	4.56	1.67	0	5.40	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
54	Positive	97.5	85, 100	6.01	0	0	6.67	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
55	Positive	97.5	80, 100	5.89	0.00	0	6.87	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
56	Positive	100.0	90, 100	2.53	0.75	0	3.04	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
57	Positive	100.0	90, 100	2.64	0	0	3.73	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
58	Positive	100.0	90, 100	3.91	0.00	0	4.56	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
59	Positive	100.0	90, 100	2.64	0	0	3.33	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
60	Positive	100.0	65, 100	5.89	6.24	0	10.74	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)

Table 14. Results of the Inter-Laboratory Reproducibility Study (ULTRA PLUS)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
1	Negative	0	0, 0	0	0	0	0	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
2	Negative	0	0, 1	0	0	0	0.24	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
3	Negative	0	0, 5	0.67	0.58	0.00	1.22	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
4	Negative	1.0	0, 15	3.82	1.91	0	4.66	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
5	Negative	1.0	0, 15	4.62	0.53	0	4.92	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
6	Negative	1.5	0, 15	3.82	1.13	1.32	4.81	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
7	Negative	5.0	0, 15	4.49	1.05	4.53	7.01	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
8	Negative	5.0	0, 25	6.77	1.05	0	7.63	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
9	Negative	5.0	0, 25	6.14	0	3.69	7.86	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
10	Negative	5.0	0, 40	9.96	0	4.31	12.00	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
11	Negative	5.0	0, 40	10.55	3.16	3.43	13.05	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
12	Negative	5.0	0, 80	25.30	8.38	11.64	29.86	2/5 (40.0)	0/6 (0.0)	2/5 (40.0)	4/16 (25.0)
13	Negative	6.5	0, 25	4.81	2.53	5.43	8.17	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
14	Negative	7.0	2, 25	2.01	5.15	0	7.50	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
15	Negative	7.5	0, 40	8.42	4.25	2.68	11.36	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
16	Negative	10.0	1, 40	8.57	5.73	5.65	11.97	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
17	Negative	10.0	2, 25	5.71	2.84	3.11	7.39	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
18	Negative	10.0	0, 70	21.52	0	7.91	23.43	0/6 (0.0)	0/6 (0.0)	3/6 (50.0)	3/18 (16.7)
19	Negative	15.0	0, 40	8.03	0	12.30	15.98	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
20	Negative	19.0	5, 30	3.69	2.98	4.30	7.38	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/18 (0.0)
21	Negative	20.0	5, 50	7.26	0	9.35	15.28	0/6 (0.0)	0/6 (0.0)	2/6 (33.3)	2/18 (11.1)
22	Negative	20.0	1, 50	12.45	3.42	11.42	18.03	1/6 (16.7)	0/6 (0.0)	1/6 (16.7)	2/18 (11.1)
23	Negative	20.0	5, 75	17.54	5.75	0	22.98	1/6 (16.7)	1/6 (16.7)	3/6 (50.0)	5/18 (27.8)
24	Negative	24.5	1, 70	14.11	0	11.56	20.68	3/6 (50.0)	0/6 (0.0)	1/6 (16.7)	4/18 (22.2)
25	Negative	25.0	1, 90	22.66	8.37	0	28.30	3/6 (50.0)	1/6 (16.7)	2/6 (33.3)	6/18 (33.3)
26	Negative	25.0	5, 65	14.45	8.08	14.92	23.91	4/6 (66.7)	0/6 (0.0)	3/6 (50.0)	7/18 (38.9)
27	Negative	25.0	5, 70	17.95	3.12	0	19.97	3/6 (50.0)	0/6 (0.0)	3/6 (50.0)	6/18 (33.3)
28	Negative	30.0	5, 50	9.72	9.57	0	16.29	0/6 (0.0)	2/6 (33.3)	2/6 (33.3)	4/18 (22.2)
29	Negative	32.5	20, 55	5.89	5.65	7.67	11.98	3/6 (50.0)	0/6 (0.0)	0/6 (0.0)	3/18 (16.7)
30	Negative	37.5	5, 70	19.97	7.45	0	23.57	2/6 (33.3)	3/6 (50.0)	2/6 (33.3)	7/18 (38.9)
31	Negative	40.0	15, 60	1.88	0	9.82	13.91	4/5 (80.0)	0/6 (0.0)	0/6 (0.0)	4/17 (23.5)
32	Negative	40.0	10, 90	19.22	2.89	7.98	24.88	3/6 (50.0)	1/6 (16.7)	3/6 (50.0)	7/18 (38.9)
33	Negative	42.5	20, 50	11.06	4.56	0	13.74	1/6 (16.7)	0/6 (0.0)	1/6 (16.7)	2/18 (11.1)
34	Positive	42.5	1, 98	30.70	9.50	11.60	35.69	4/6 (66.7)	1/6 (16.7)	4/6 (66.7)	9/18 (50.0)
35	Positive	50.0	5, 98	26.67	9.31	0	29.60	4/6 (66.7)	2/6 (33.3)	4/6 (66.7)	10/18 (55.6)
36	Positive	50.0	10, 95	13.89	10.80	5.11	24.35	5/6 (83.3)	3/6 (50.0)	4/6 (66.7)	12/18 (66.7)
37	Positive	50.0	15, 90	13.02	10.27	8.18	19.37	6/6 (100.0)	3/6 (50.0)	3/6 (50.0)	12/18 (66.7)
38	Positive	50.0	35, 70	6.35	5.00	5.38	10.46	6/6 (100.0)	4/6 (66.7)	3/6 (50.0)	13/18 (72.2)
39	Positive	50.0	15, 98	25.39	1.29	0	27.11	4/6 (66.7)	3/6 (50.0)	3/6 (50.0)	10/18 (55.6)
40	Positive	50.0	5, 80	17.04	0	0	28.48	3/6 (50.0)	4/6 (66.7)	3/6 (50.0)	10/18 (55.6)
41	Positive	52.5	25, 70	6.24	0	1.73	10.35	6/6 (100.0)	5/6 (83.3)	5/6 (83.3)	16/18 (88.9)
42	Positive	55.0	25, 90	19.40	0	0	21.21	6/6 (100.0)	5/6 (83.3)	3/6 (50.0)	14/18 (77.8)
43	Positive	65.0	8, 99	24.03	13.11	0	29.60	5/6 (83.3)	6/6 (100.0)	4/6 (66.7)	15/18 (83.3)
44	Positive	80.0	50, 95	13.64	7.07	0	17.83	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
45	Positive	85.0	50, 100	19.87	5.06	0	21.49	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
46	Positive	90.0	60, 100	12.50	3.50	0	13.52	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
47	Positive	90.0	70, 100	9.79	0	0	10.14	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
48	Positive	90.0	50, 100	12.22	5.27	1.38	15.18	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
49	Positive	92.5	30, 100	7.36	1.18	4.00	16.69	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	17/18 (94.4)
50	Positive	92.5	25, 100	13.33	4.56	0	20.55	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	17/18 (94.4)
51	Positive	95.0	80, 100	6.45	0.00	0	6.87	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
52	Positive	95.0	70, 100	8.47	0	0	9.30	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
53	Positive	96.5	85, 100	5.92	1.49	0	6.35	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
54	Positive	97.0	10, 100	15.95	2.74	0	22.80	6/6 (100.0)	6/6 (100.0)	5/6 (83.3)	17/18 (94.4)
55	Positive	98.0	65, 100	5.57	1.49	0	9.02	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
56	Positive	100.0	80, 100	4.71	1.67	0	6.24	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
57	Positive	100.0	75, 100	7.73	0.00	0	8.42	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
58	Positive	100.0	90, 100	4.25	0	0	4.56	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
59	Positive	100.0	90, 100	2.64	1.67	0	4.08	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)
60	Positive	100.0	85, 100	5.89	0.00	0	6.24	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	18/18 (100.0)

Table 15. Results of the Inter-Laboratory Reproducibility Study for VENTANA MET (SP44) RxDx Assay on BenchMark ULTRA and ULTRA PLUS Instruments.

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
1	Negative	0	0, 0	0	0	0	0	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
2	Negative	0	0, 1	0.10	0	0	0.24	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
3	Negative	0	0, 5	0.56	0.51	0	0.98	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
4	Negative	1.5	0, 20	2.89	0	2.28	5.33	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
5	Negative	2.0	0, 15	1.77	0	2.06	4.21	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
6	Negative	2.0	0, 15	2.52	0	1.67	3.85	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
7	Negative	4.5	0, 15	3.64	0.51	3.65	6.07	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
8	Negative	5.0	0, 25	2.65	0	4.75	6.89	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
9	Negative	5.0	0, 25	4.49	0	1.33	6.38	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
10	Negative	5.0	0, 25	4.64	1.62	5.03	7.87	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
11	Negative	5.0	0, 40	7.23	0	6.40	11.42	0/10 (0.0)	0/12 (0.0)	0/12 (0.0)	0/34 (0.0)
12	Negative	5.0	0, 40	7.99	3.59	4.42	11.85	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
13	Negative	5.0	0, 80	22.74	7.57	12.86	28.25	4/11 (36.4)	0/12 (0.0)	2/9 (22.2)	6/32 (18.8)
14	Negative	9.5	1, 25	2.75	2.25	0	6.70	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
15	Negative	10.0	0, 40	7.22	3.78	8.58	13.51	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
16	Negative	10.0	0, 25	3.48	1.24	4.85	7.31	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
17	Negative	10.0	0, 40	4.42	2.41	6.22	10.19	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
18	Negative	12.5	0, 70	16.00	0	10.91	20.85	0/12 (0.0)	0/12 (0.0)	4/12 (33.3)	4/36 (11.1)
19	Negative	19.0	5, 30	2.81	0.60	5.55	7.61	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
20	Negative	20.0	0, 40	4.81	0	13.97	16.25	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)	0/36 (0.0)
21	Negative	20.0	0, 50	7.60	0	11.21	16.94	1/12 (8.3)	0/12 (0.0)	1/12 (8.3)	2/36 (5.6)
22	Negative	21.5	1, 75	11.29	0	5.42	21.20	3/12 (25.0)	2/12 (16.7)	5/12 (41.7)	10/36 (27.8)
23	Negative	22.5	0, 50	8.47	2.26	4.85	15.05	1/12 (8.3)	1/12 (8.3)	2/12 (16.7)	4/36 (11.1)
24	Negative	25.0	1, 70	14.38	0	7.43	19.19	3/12 (25.0)	0/12 (0.0)	4/12 (33.3)	7/36 (19.4)
25	Negative	30.0	5, 60	6.88	0	4.38	15.43	1/12 (8.3)	2/12 (16.7)	3/12 (25.0)	6/36 (16.7)
26	Negative	35.0	10, 60	6.24	0.48	7.81	13.02	5/11 (45.5)	0/12 (0.0)	0/12 (0.0)	5/35 (14.3)
27	Negative	35.0	5, 70	13.57	3.64	15.44	23.43	8/12 (66.7)	0/12 (0.0)	6/12 (50.0)	14/36 (38.9)
28	Negative	35.0	5, 75	14.31	3.44	10.30	21.54	6/12 (50.0)	1/12 (8.3)	6/12 (50.0)	13/36 (36.1)
29	Negative	37.5	20, 55	6.12	0	3.83	11.33	5/12 (41.7)	0/12 (0.0)	1/12 (8.3)	6/36 (16.7)
30	Negative	40.0	0, 90	15.18	4.70	0	23.49	8/12 (66.7)	4/12 (33.3)	4/12 (33.3)	16/36 (44.4)
31	Negative	40.0	20, 50	11.29	2.80	0	13.51	2/12 (16.7)	1/12 (8.3)	3/12 (25.0)	6/36 (16.7)

Case ID	Majority MET (SP44) Status	Median % Strong TC	Standard Deviation					Percent Positive Results n/N (%)			
			Range of % Strong TC (Min., Max.)	Between Reader	Between Day	Between Site	Total	Site A	Site B	Site C	Total
32	Negative	40.0	5, 70	17.63	3.83	0	21.32	5/12 (41.7)	6/12 (50.0)	4/12 (33.3)	15/36 (41.7)
33	Negative	45.0	10, 90	16.88	4.95	5.82	22.71	8/12 (66.7)	3/12 (25.0)	5/11 (45.5)	16/35 (45.7)
34	Positive	50.0	10, 70	5.49	0	6.85	13.14	12/12 (100.0)	7/12 (58.3)	8/12 (66.7)	27/36 (75.0)
35	Positive	50.0	5, 98	17.58	8.13	8.79	26.52	10/12 (83.3)	5/12 (41.7)	9/12 (75.0)	24/36 (66.7)
36	Positive	50.0	10, 95	18.40	0	0	24.75	9/12 (75.0)	6/12 (50.0)	8/12 (66.7)	23/36 (63.9)
37	Positive	50.0	1, 98	26.39	0	0	33.93	8/12 (66.7)	3/12 (25.0)	8/12 (66.7)	19/36 (52.8)
38	Positive	50.0	25, 75	7.52	0	5.41	12.20	12/12 (100.0)	5/12 (41.7)	7/12 (58.3)	24/36 (66.7)
39	Positive	50.0	1, 98	22.88	0	8.83	27.33	9/12 (75.0)	4/12 (33.3)	8/12 (66.7)	21/36 (58.3)
40	Positive	50.0	5, 80	19.95	0	0	25.67	8/12 (66.7)	7/12 (58.3)	8/12 (66.7)	23/36 (63.9)
41	Positive	52.5	15, 90	12.43	0	9.33	19.90	12/12 (100.0)	6/12 (50.0)	6/12 (50.0)	24/36 (66.7)
42	Positive	60.0	10, 90	17.96	0	0	21.10	12/12 (100.0)	8/12 (66.7)	8/12 (66.7)	28/36 (77.8)
43	Positive	65.0	8, 99	19.02	7.19	0	25.19	11/12 (91.7)	10/12 (83.3)	9/12 (75.0)	30/36 (83.3)
44	Positive	75.0	1, 95	12.38	2.03	4.44	22.76	12/12 (100.0)	9/12 (75.0)	12/12 (100.0)	33/36 (91.7)
45	Positive	85.0	20, 100	18.68	0	0	23.44	12/12 (100.0)	10/12 (83.3)	12/12 (100.0)	34/36 (94.4)
46	Positive	90.0	10, 100	10.27	6.05	5.65	17.97	12/12 (100.0)	11/12 (91.7)	12/12 (100.0)	35/36 (97.2)
47	Positive	90.0	5, 100	14.66	0	0	22.45	12/12 (100.0)	10/12 (83.3)	12/12 (100.0)	34/36 (94.4)
48	Positive	90.0	5, 100	14.08	0	6.41	20.94	12/12 (100.0)	10/12 (83.3)	12/12 (100.0)	34/36 (94.4)
49	Positive	95.0	80, 100	6.33	0.36	0	6.77	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
50	Positive	95.0	30, 100	6.41	0	3.52	13.30	12/12 (100.0)	11/12 (91.7)	12/12 (100.0)	35/36 (97.2)
51	Positive	95.0	65, 100	9.16	2.43	0	10.16	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
52	Positive	95.0	70, 100	7.48	0	0	8.81	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
53	Positive	95.0	10, 100	11.90	0	0	17.62	12/12 (100.0)	12/12 (100.0)	11/12 (91.7)	35/36 (97.2)
54	Positive	96.5	80, 100	6.02	0	0	6.61	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
55	Positive	100.0	80, 100	3.40	0	0	5.10	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
56	Positive	100.0	75, 100	6.19	0.55	0	7.01	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
57	Positive	100.0	90, 100	3.50	0.36	0	4.17	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
58	Positive	100.0	65, 100	5.04	0.26	0	7.17	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
59	Positive	100.0	90, 100	2.80	1.41	0	3.78	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)
60	Positive	100.0	65, 100	5.80	2.98	0	8.71	12/12 (100.0)	12/12 (100.0)	12/12 (100.0)	36/36 (100.0)

The data were analyzed for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) across all evaluable observations, and a summary is presented in the tables below.

Table 16. Overall, Site-Stratified, and Reader-Stratified Reproducibility Analyses of MET Status vs. Mode for BenchMark ULTRA Instrument for 50% Strong TC Staining Cutoff.

ULTRA External Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	438/521	84.1	(81.4, 86.8)
	NPA	509/554	91.9	(88.5, 95.1)
	OPA	947/1075	88.1	(86.3, 89.9)
Within Site	PPA	436/498	87.6	(85.3, 89.8)
	NPA	530/577	91.9	(89.6, 94.1)
	OPA	966/1075	89.9	(88.2, 91.5)
Within Reader	PPA	445/473	94.1	(92.7, 95.7)
	NPA	564/602	93.7	(92.2, 95.2)
	OPA	1009/1075	93.9	(92.9, 94.8)

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

Table 17. Overall, Site-Stratified, and Reader-Stratified Reproducibility Analyses of MET Status vs. Mode for BenchMark ULTRA PLUS Instrument for 50% Strong TC Staining Cutoff.

ULTRA PLUS External Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	424/468	87.2	(83.9, 91.3)
	NPA	525/591	88.8	(85.8, 91.9)
	OPA	949/1077	88.1	(86.2, 90.1)
Within Site	PPA	441/509	86.6	(83.9, 89.6)
	NPA	519/568	91.4	(88.9, 93.9)
	OPA	960/1077	89.1	(87.5, 91.0)
Within Reader	PPA	465/491	94.7	(93.2, 96.2)
	NPA	561/586	95.7	(94.5, 97.0)
	OPA	1026/1077	95.3	(94.4, 96.1)

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

The performance equivalence analysis (method comparison) of the VENTANA MET (SP44) RxDx Assay on the BenchMark ULTRA and BenchMark ULTRA PLUS was evaluated twice using each instrument platform as a reference. Each reader at each site produced 180 reads (1080 total reads). Results of the method comparison study are presented in Table 18 and Table 19 below. Please note that, due to an observed decrease in precision for cases with borderline expression, consultation with a second pathologist is recommended per standard medical practice for cases with borderline expression and/or intensity levels. Please refer to the limitation in the Scoring Algorithm and the Specific Limitations section.

Table 18. Overall Method Comparison Study Results

Rate	ULTRA as Reference		ULTRA PLUS as Reference	
	% (n/N)	95% CI	% (n/N)	95% CI
PPA	87.2 (421/783)	(84.7, 89.8)	86.3 (421/488)	(83.8, 88.6)
NPA	88.6 (523/590)	(86.3, 90.7)	89.4 (523/585)	(87.4, 91.6)
OPA	88.0 (944/1073)	(86.3, 89.5)	-	-

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

Table 19. Overall Method Comparison Study Results (Reader 1, Day 1)

Rate	ULTRA as Reference		ULTRA PLUS as Reference	
	% (n/N)	95% CI	% (n/N)	95% CI
PPA	81.3 (65/80)	75.3, 87.2	85.5 (65/76)	78.7, 92.0
NPA	88.9 (88/99)	83.2, 94.0	85.4 (88/103)	80.6, 90.2
OPA	85.5 (153/179)	81.5, 89.4	-	-

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

CLINICAL PERFORMANCE

LUMINOSITY: Previously Treated EGFR Wild-Type Non-squamous NSCLC with MET Protein Overexpression

The efficacy of EMRELIS™ (telisotuzumab vedotin-tllv) as monotherapy was evaluated in the LUMINOSITY study (NCT03539536), a multicenter, open-label, single-arm study. Eligible patients were required to have locally advanced or metastatic non-squamous NSCLC with MET protein overexpression and treatment with prior systemic therapy (including no more than one line of prior chemotherapy). The major efficacy outcome measure was overall response rate (ORR) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 as assessed by a blinded independent central review (BICR). An additional efficacy outcome measure was duration of response (DOR) by BICR. The primary endpoint of ORR was calculated based on the Primary Efficacy population (n=84).

As part of enrollment screening, archived or post-progression/recent NSCLC tumor samples from prospective study participants were assessed for the presence or absence of MET protein overexpression at a central laboratory using an IHC clinical trial assay (CTA) based on the MET-directed monoclonal antibody clone SP44 [MET (SP44)]. For patients with non-squamous NSCLC, subjects were enrolled on the basis of MET protein expression CTA result demonstrating strong IHC staining (3+ intensity) in ≥25% of tumor cells (a "CTA^{+}_{25}" result). Tumor specimens from enrolled subjects were also assessed for high MET expression, defined as strong CTA IHC staining (3+ intensity) in ≥50% of tumor cells (designated "CTA^{+}_{50}"). Efficacy was evaluated in 84 EGFR wild-type non-squamous NSCLC patients with CTA result indicating high MET expression (≥50% tumor cells with strong (3+) membrane staining) who received telisotuzumab vedotin at 1.9 mg/kg intravenously every two weeks until disease progression or unacceptable toxicity (the primary efficacy population).

The median age was 64 years (range: 38 to 83 years); 75% were male; 61% were White, 1.2% were Black or African American, 38% were Asian; none were Hispanic or Latino. Twenty-five percent had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 and 74% had ECOG PS of 1; 19% were never smokers, 68% were former smokers, and 13% were current smokers; 99% had Stage IV disease; and 19% of patients had previously treated brain metastases. The median number of lines of prior therapies was 1 (range 1 - 3); 73% of patients had one line, 24% had two lines, and 3.6% had three lines of prior systemic therapy; 96% of patients had prior platinum therapy, 82% had prior immunotherapy (anti-PD-1/PD-

L1), 6% had prior targeted therapy, and 3.6% had prior MET tyrosine kinase inhibitor therapy. Efficacy results for Study LUMINOSITY are summarized below.

The primary efficacy endpoint was overall response rate (ORR) determined by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 as assessed by Independent Central Review (ICR). Efficacy results, for patients selected based on the CTA, are summarized in Table 20.

Table 20. Clinical Benefit of MET Patient Population in LUMINOSITY

	CTA+ (n=84)	CTA+/CDx+ (n=32)	CTA+/CDx- (n=5)	CTA+/CDx unevaluable (n=47)
ORR (95% CI)	34.5 (24.5, 45.7)	34.4 (18.6, 53.2)	20 (0.5, 71.6)	36.2 (22.7, 51.5)
Complete response (CR), n (%)	0	0	0	0
Partial response (PR), n (%)	29 (34.5)	11 (34.4)	1 (20)	17 (36.2)
Duration of Response (DOR)				
Median DoR, months (95% CI)	7.2 (4.2, 12.0)	7.2 (2.8, 13.0)	4.2 (-,-)	10.0 (3.8, 18.9)
% with DoR ≥6 months, n (%)	17 (58.6)	7 (63.6)	0	10 (58.8)
% with DoR ≥12 months, n (%)	6 (20.7)	2 (18.2)	0	4 (23.5)

The clinical performance of the VENTANA MET (SP44) RxDx Assay as a companion diagnostic (CDx) device to aid in the identification of patients with non-squamous non-small cell lung cancer (NSCLC) who are likely to benefit from EMRELIS™ (telisotuzumab vedotin-tllv) treatment was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the LUMINOSITY study using the CTA. Remnant specimens from patients previously screened for LUMINOSITY were retrospectively assessed for MET protein expression using VENTANA MET (SP44) RxDx Assay (978 patients) at central laboratories. The re-tested patient set included 37 of 84 patients with outcome data available from the LUMINOSITY trial and 830 individuals representative of LUMINOSITY screen-failed subjects.

The agreement of MET status between the CTA results and VENTANA MET (SP44) RxDx Assay (CDx) was calculated at the 50% strong tumor cell staining cutoff using the CTA results as the reference. The concordance analysis results for the patients included in the bridging efficacy analysis are shown in Table 21.

Table 21. MET status concordance at the 50% strong tumor cell staining cutoff between LUMINOSITY study CTA and the CDx in the bridging intended use population.

LUMINOSITY Study		MET CTA Status		
		Positive	Negative	Total
MET CDx Status	Positive	32	42	74
	Negative	5	461	466
	Unknown[1]	47	327	374
	Total	84	830	914
	PPA: 86.5% (32/37) [95% CI: 71.23, 95.46]			
	NPA: 91.7% (461/503) [95% CI: 88.88, 93.92]			
	OPA: 91.3% (493/540) [95% CI: 88.59, 93.53]			

[1]CDx unknown includes: 1) protocol deviations, incident events, and/or screen failures for reasons other than CTA result, 2) CDx not evaluable.

CDx = VENTANA MET (SP44) assay; CI = confidence interval; CTA = clinical trial assay; NPA = negative percent agreement; PPA = positive percent agreement; OPA = overall percent agreement

Note: Concordance was estimated in only those patients who had an evaluable CDx result.

Sensitivity analysis with regard to the missing CDx test results were conducted to evaluate the robustness of the ORR estimates considering VENTANA MET (SP44) RxDx Assay (CDx) unevaluable patients enrolled in the LUMINOSITY trial. Samples's CDx results were considered missing if the samples were not tested by the CDx, or if they were tested but returned an invalid result, or if they were excluded from the analysis due to protocol deviations, incident events, and/or because they were screen failures for reasons other than CTA result. Under the reasonable assumption of missing at random (MAR), the missing CDx results were imputed. A multiple imputation approach was utilized to impute the missing CDx MET status. The CTA MET result, treatment outcome variable, patient characteristics variables, and sample characteristics variables were considered. Amongst all CTA+ patients, 56% did not have VENTANA MET (SP44) RxDx Assay result (47/84).

The imputed ORR was estimated to be 32.4% (95% CI: 24.23, 40.51), which is comparable to the ORR for the CTA-positive population [34.5% (24.5, 45.7)]. Thus, the sensitivity analysis demonstrated the robustness of the clinical efficacy estimate to the missing VENTANA MET (SP44) RxDx Assay results.

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 22. Troubleshooting guidance for VENTANA MET (SP44) RxDx Assay.

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that U VENTANA MET (SP44) RxDx Assay procedure was used.
		Verify that VENTANA MET (SP44) RxDx Assay 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 method sheet associated with P/N 740-7064 located at navifyportal.roche.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 VENTANA MET (SP44) RxDx Assay procedure was used.
	Incorrect of missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detachment from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

REFERENCES

1. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
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.

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.

INTELLECTUAL PROPERTY

For USA: Rx only

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VENTANA®

Roche

VENTANA MET (SP44) RxDx Assay Interpretation Guide for Non-Squamous Non-Small Cell Lung Cancer

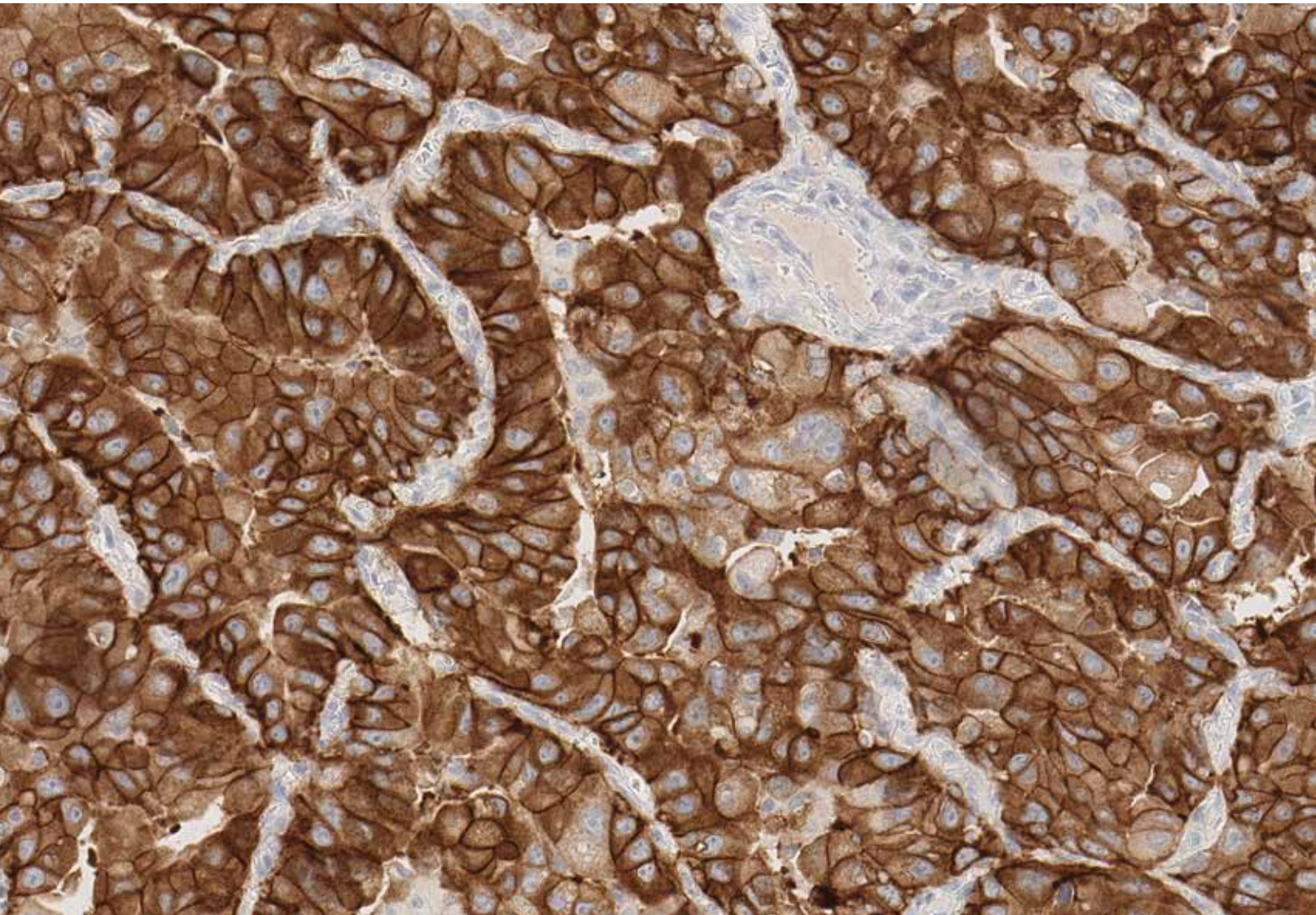


Table of Contents

Introduction

MET (also called tyrosine-protein kinase MET or c-Met) is a heterodimeric receptor tyrosine kinase encoded by the MET proto-oncogene located on chromosome 7q21-q31 and is expressed on the surface of various epithelial cells.[1] MET is the receptor for hepatocyte growth factor/scatter factor (HGF/SF), a large polypeptide growth factor discovered as a protein causing dispersion of epithelial colonies and cell migration.[2]

MET is expressed on the surface of hepatocytes, neurons, hematopoietic, endothelial, and various epithelial cells.[3,4] HGF is primarily produced by mesenchymal cells and acts in a paracrine fashion on MET-expressing epithelial cells.[5] Binding of HGF ligand to the MET receptor induces phosphorylation of tyrosine residues, which initiates several downstream signaling pathways including the ERK/MAPK, PI3K/Akt, Wnt/beta-catenin, NF-κβ, and STAT pathways.[1] In normal cells, these complex signaling pathways act together to maintain tissue homeostasis and play crucial roles during tissue regeneration, wound healing, and embryonic development.[3,4,6,7]

Lung cancer is one of the most frequently diagnosed cancers and is the leading cause of cancer-related death worldwide in both men and women.[8] Non-small-cell lung cancer (non-squamous NSCLC) represents approximately 80-85% of all new lung cancer cases.[8,9] Patients diagnosed with advanced non-squamous NSCLC often face a poor prognosis, with median survival of less than one year. The MET pathway is frequently dysregulated in a number of tumor types including non-squamous NSCLC .[3] MET dysregulation occurs through a variety of mechanisms including mutations, amplification (de novo or acquired), and protein overexpression of MET and/or its ligand HGF.[3] In non-squamous NSCLC , acquired secondary MET amplification is a common mechanism of resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), with MET amplification reported in up to 20% of cases.[4] Targeted therapy that blocks MET signaling activity may be an effective treatment for patients with non-squamous NSCLC .[10-12]

The VENTANA MET (SP44) RxDx Assay binds to MET protein in sections of formalin-fixed, paraffin-embedded non-squamous NSCLC tissue and cell block sections. The specific antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product.

Intended Use

Intended Use of product

Refer to the corresponding VENTANA MET (SP44) RxDx Assay method sheet (package insert) (P/N 1018335US Cat. No. 740-7064) for the detailed intended use of this product.

Caution: Federal law restricts this device to sale by or on the order of a physician.

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) non-squamous NSCLC sections, including resections, biopsies, and cell blocks stained with VENTANA MET (SP44) RxDx Assay in accordance with the proposed product labeling.
- Provide photographic images that illustrate the staining patterns that may result from staining of non-squamous NSCLC tissue and cell blocks with the VENTANA MET (SP44) RxDx Assay.
- Provide example images of challenging cases to provide guidance in their evaluation.
- Provide guidance in using a MET-expressing control tissue (e.g., gallbladder), which serves as a system level control when stained with the VENTANA MET (SP44) RxDx Assay.

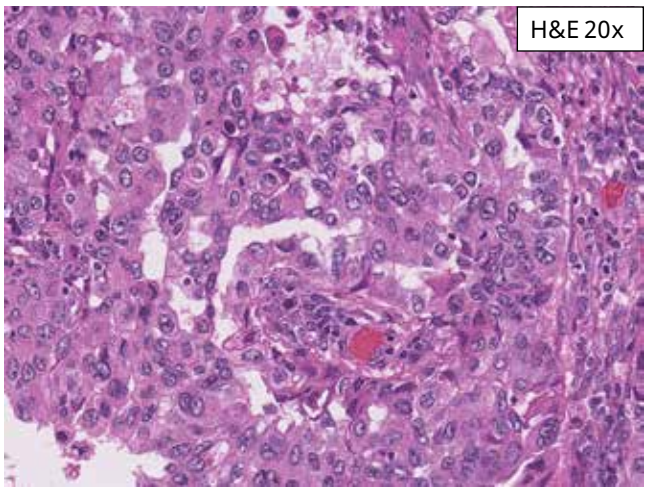
Clinical Evaluation

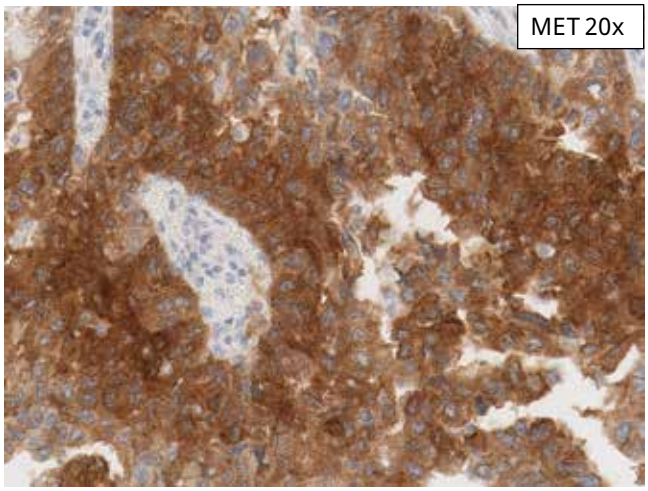
Staining Characteristics

In non-squamous NSCLC, tumor cells stained with VENTANA MET (SP44) RxDx Assay are evaluated for percentage of tumor cells exhibiting strong intensity of the diaminobenzidine (DAB) signal. VENTANA MET (SP44) RxDx Assay staining in non-squamous NSCLC exhibits a membranous and/or cytoplasmic pattern. The staining signal intensity is classified as strong, moderate, weak, or negative based on membranous and/or cytoplasmic localization. Any pattern of strong membrane staining is included (complete, partial, basolateral, etc.) when determining percent cells staining.

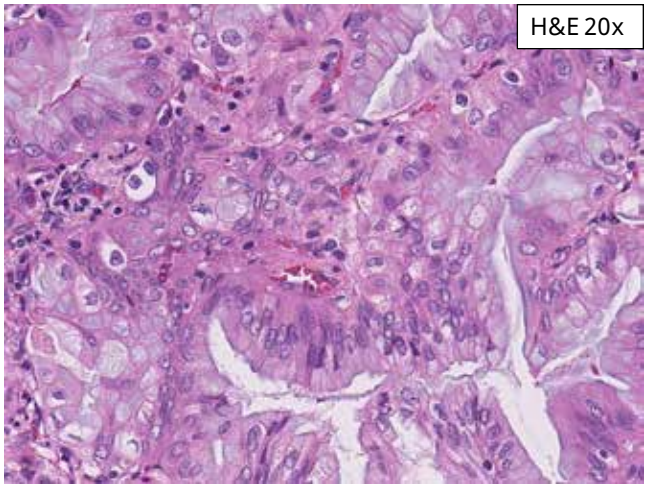
- **Strong** signal intensity is characterized by dark brown to black hue with saturated DAB. Membranes/cytoplasm exhibiting strong signal are easily detectable at low power magnifications such as 2x or 4x.
- **Moderate** signal intensity is characterized by rich brown stained membranes/cytoplasm that are more easily detected at magnifications such as 4x or 10x. The moderate signal shows thinner staining membranes and lighter cytoplasm, which are not as easily detected at lower magnification.
- **Weak** signal intensity is characterized by pale tan to light brown membranes/cytoplasm that require detection at higher magnifications such as 10x or 20x. The weak signal lacks the rich brown color seen in moderate staining intensity and may not be detectable at lower magnification.
- **Negative** signal intensity is characterized by an absence of any detectable signal. Negative cases may still exhibit pale grey membranous discoloration.

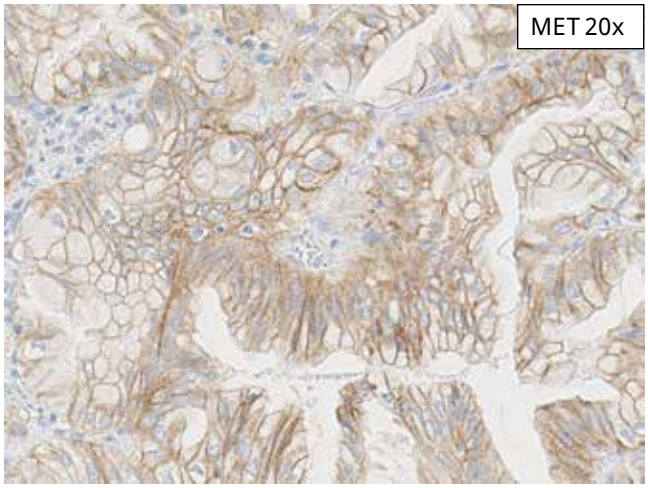
The signal may be distributed homogeneously, having a uniform level of intensity throughout the neoplastic portions of the sample, or distributed heterogeneously having more than one intensity level. The relative percentage of strong signal intensity is visually estimated and used to generate a diagnostic score. In non-squamous NSCLC samples, weak to moderate MET staining can also occur in type II pneumocytes and the normal epithelium of bronchi and bronchioles. The isotype matched Rabbit Monoclonal Negative Control Ig (790-4795/ 06683380001) is used as a negative reagent control (NRC) to evaluate the presence of background in test samples and establish a staining intensity baseline.



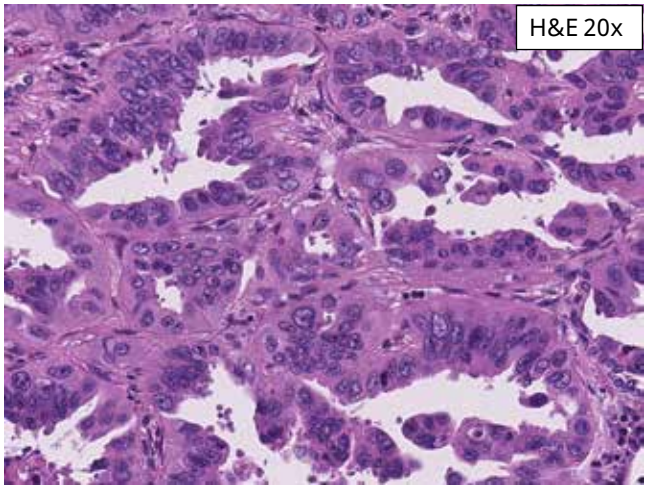


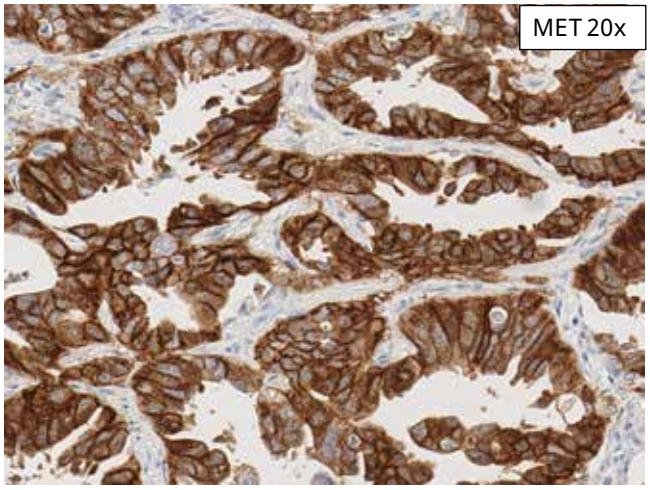
Staining Characteristics Photoset 1: This case exhibits staining that is primarily strong cytoplasmic (20x).





Staining Characteristics Photoset 2: This case exhibits staining that is primarily weak membranous (20x).





Staining Characteristics Photoset 3: This case exhibits staining that has both strong cytoplasmic and membranous components (20x).

Sample Types

The VENTANA MET (SP44) RxDx Assay is for use in the detection of the MET protein in FFPE non-squamous NSCLC samples, including resections, biopsies, or cell blocks.

Cell blocks: include fine needle aspirates (FNAs), as well as other types of lung cytology samples, such as those collected via bronchoalveolar lavage (BAL), bronchial brush and others, that could be spun down and made into paraffin embedded cell blocks provided sufficient viable tumor cells (>100 cells) are present. Tumor cells that are crushed, necrotic, or going through apoptosis are not considered viable. The final decision to accept the sample is at the discretion of the pathologist. Cytology smears from any source are excluded. Representative cell block cases are discussed in the **Reference Images** section.

Note: Cytology specimen processing requirements for the use of cytology fixatives such as CytoLyt® and SurePath™ collection media have not been established. Cytology specimens may be collected into and rinsed in 10% neutral buffered formalin (NBF) and cell blocks may be fixed in 10% NBF for 6-72 hours (see the **Impact of Pre-analytical Conditions** section). Ethanol collection and fixation is not recommended for use with the VENTANA MET (SP44) RxDx Assay.

Scoring Algorithm

Evaluating VENTANA MET (SP44) RxDx Assay in non-squamous NSCLC:

For the VENTANA MET (SP44) RxDx Assay, each case is stained with the VENTANA MET (SP44) Rabbit Monoclonal Primary Antibody and a matched negative reagent control (NRC), Rabbit Monoclonal Negative Control Ig. Neoplastic cells stained with VENTANA MET (SP44) RxDx 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 MET (SP44) RxDx Assay.

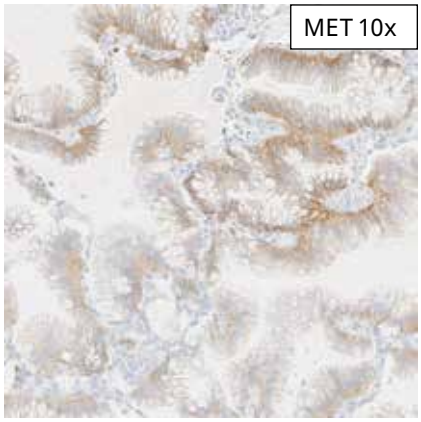
The scoring algorithms for VENTANA MET (SP44) RxDx Assay are provided below in **Table 1**. Representative cases are discussed in the **Reference Images** section.

Table 1: VENTANA MET (SP44) RxDx Assay Scoring Algorithm for Non-squamous NSCLC

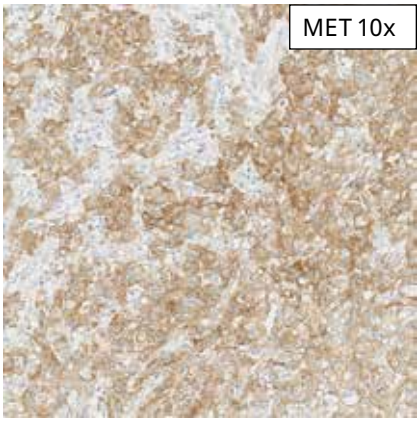
MET IHC Status	Staining Criteria/Characteristics
Positive	≥ 50% of tumor cells with strong membrane and/or cytoplasmic staining intensity
Negative	< 50% of tumor cells with strong membrane and/or cytoplasmic staining intensity
Not Evaluable (N/E)	Interpretation is not possible, e.g., no tissue present, presence of staining artifacts, or poor tissue quality

MET IHC Dynamic Staining Range

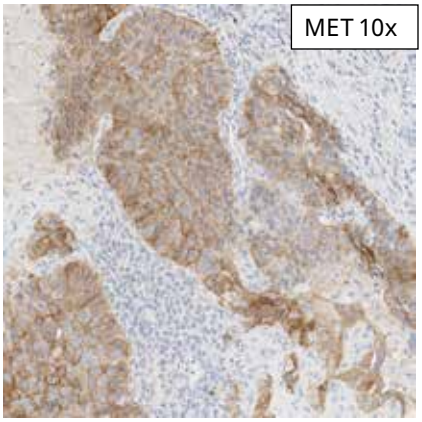
Clinical Diagnosis Negative



Weak to moderate membrane and cytoplasmic staining intensity

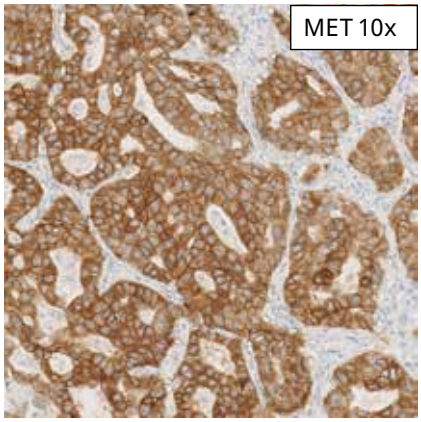


Moderate membrane staining intensity with weak cytoplasmic staining intensity

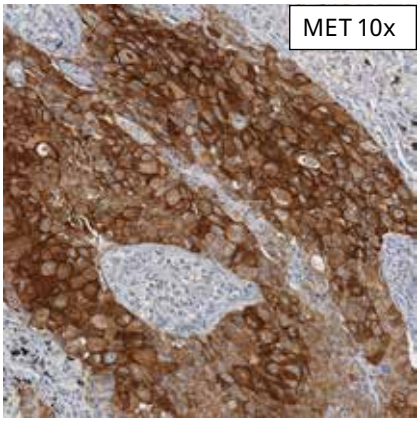


Moderate to strong membrane staining intensity with focal strong membrane staining intensity and weak cytoplasmic staining intensity

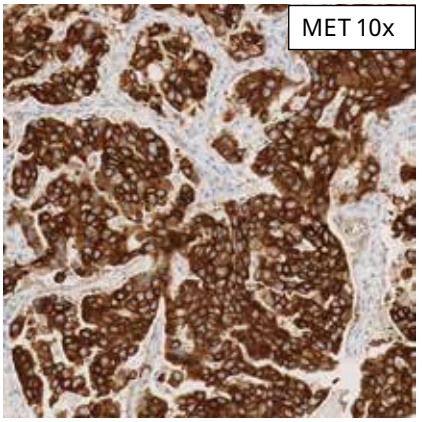
Clinical Diagnosis Positive



Predominately strong membrane staining intensity and moderate to strong cytoplasmic staining intensity



Predominately strong membrane and cytoplasmic staining intensity



Strong membrane and cytoplasmic staining intensity

The MET positive images depict the range of strong staining intensity, extending from the lower end in the leftmost image to the higher end in the rightmost image.

Scoring Method

In non-squamous NSCLC, tumor cells stained with VENTANA MET (SP44) RxDx Assay are evaluated for percentage of tumor cells exhibiting strong intensity of the diaminobenzidine (DAB) signal. The staining signal intensity can be strong, moderate, weak, or negative based on membranous and/or cytoplasmic localization.

As with any IHC assay, specific staining should be evaluated on viable tumor cells only. Ideally, patient specimens will have a minimum of approximately 100 viable tumor cells identified on the H&E in order to determine MET status. 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 MET (SP44) RxDx Assay.

The criteria detailing case morphology and background acceptability is found below in **Table 2**.

Table 2: VENTANA MET (SP44) RxDx Assay Morphology and Background Acceptability

	Acceptable	Unacceptable
Morphology	Cellular elements of interest are visualized allowing interpretation of the stain	Cellular elements of interest are not visualized compromising interpretation of the stain
Background	Non-specific staining that is not obtrusive to interpretation of specific staining	Non-specific staining that is obtrusive to interpretation of specific staining

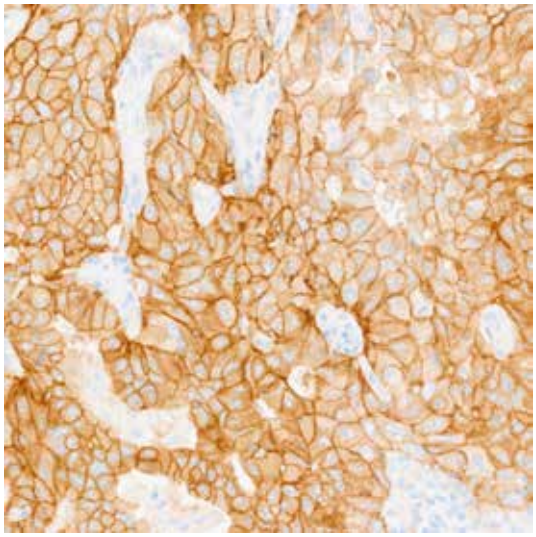
Stain Intensity Challenges

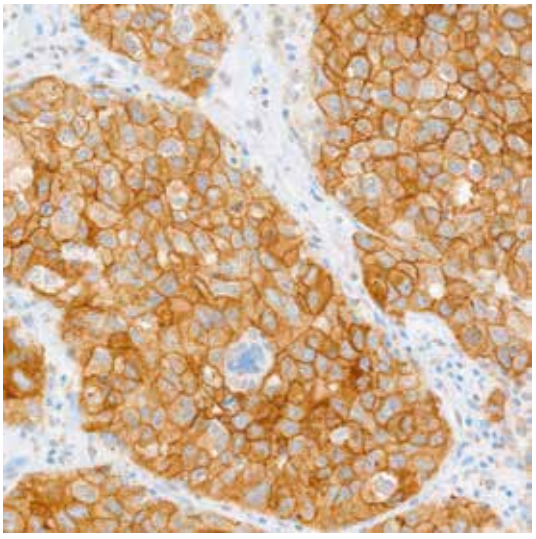
Differentiation between strong and moderate signal intensity may be challenging. The images below identify the slight differences between Strong and moderate signal intensity.

- **MET IHC Negative (moderate)** staining is characterized by light to rich brown staining of the membranes and/or cytoplasm. Membranes are thinner and there is lighter staining cytoplasm than in cells with strong intensity.
- **MET IHC Positive (strong)** staining is characterized by dark brown or black staining of the membranes and/or cytoplasm. Strong staining membranes are easily detectable at low power magnifications such as 2x or 4x.

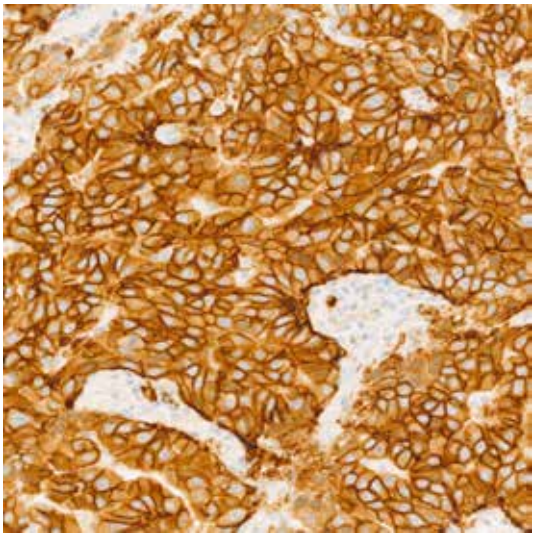
It is important to view cases at both low and high power magnifications to evaluate intensity. Some cases, particularly cell blocks prepared from cytology samples, may appear to be staining strongly at low magnification but upon review at higher magnifications are revealed to be staining moderately.

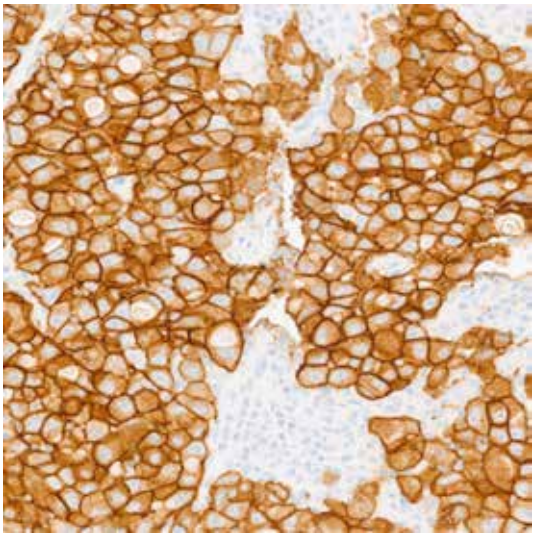
MET IHC Negative (moderate)





MET IHC Positive (strong)





Controls

Non-neoplastic gallbladder tissue with positive and negative staining elements is recommended for use as a system-level control tissue. Non-neoplastic gallbladder tissue stained with the VENTANA MET (SP44) RxDx Assay exhibits moderate membranous staining in the epithelium and absence of MET staining within stromal tissue. When qualifying non-neoplastic gallbladder tissue to serve as a system-level control tissue, the tissue must exhibit predominantly moderate membranous staining and negative staining in the stromal tissue when stained with the VENTANA MET (SP44) Assay as described in **Table 3** and depicted on the following page.

A control tissue should be a fresh autopsy, biopsy, or 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 MET (SP44) RxDx Assay 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. Moderate intensity membrane staining of epithelial cells and absence of staining in the stroma within the non-neoplastic gallbladder specimen confirms that VENTANA MET (SP44) RxDx Assay was applied and the instrument functioned properly (**Table 3**). The 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. Gallbladder specimens must be qualified before use as a control tissue to ensure appropriate staining of the tissue elements.

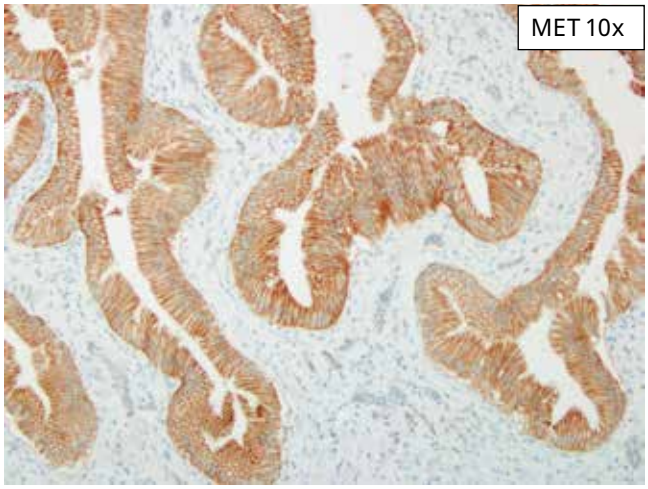
Before use as a tissue control, the non-neoplastic gallbladder specimens should be qualified by the end user for appropriate staining, according to the interpretation criteria described in **Table 3**, using the VENTANA MET (SP44) Assay with the OptiView DAB IHC Detection Kit and recommended staining protocol. Multiple non-neoplastic gallbladder specimens may be required to select an appropriate system-level control candidate.

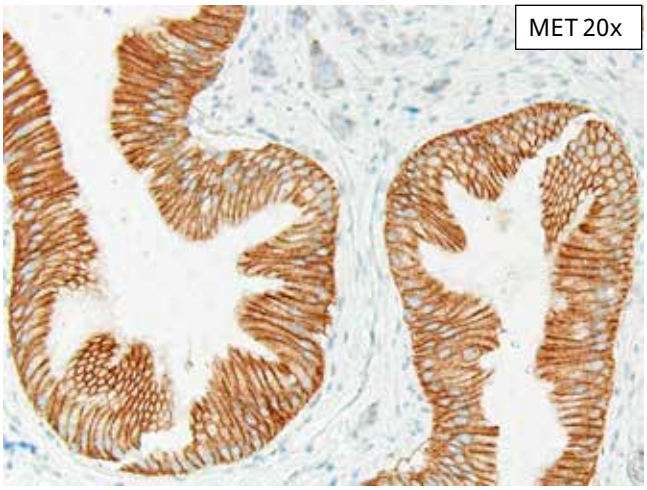
Table 3: Acceptance Criteria for MET Staining in Non-Neoplastic Gallbladder Tissue

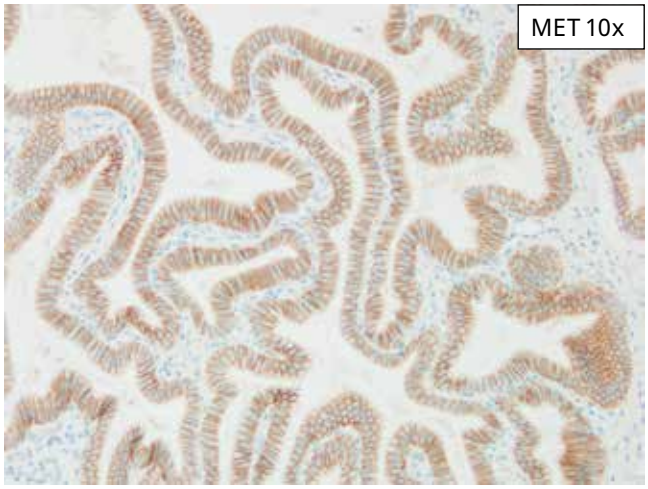
Acceptable	Unacceptable
Presence of moderate* membrane staining in epithelium AND Absence of specific MET staining within cells of stromal tissue	Absence of moderate* membrane staining in epithelium OR Excessive background staining of stromal tissue that interferes with interpretation

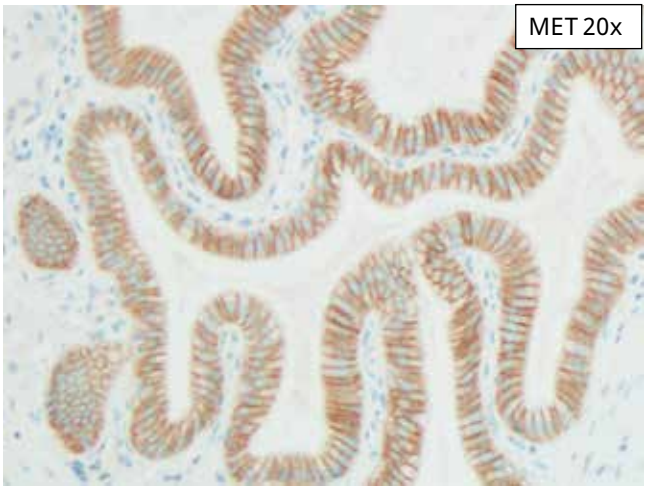
*Moderate staining intensity is characterized by rich brown thickened membranes that are detectable at low magnifications such as 4x or 10x

Acceptable Staining of Gallbladder Control Tissue



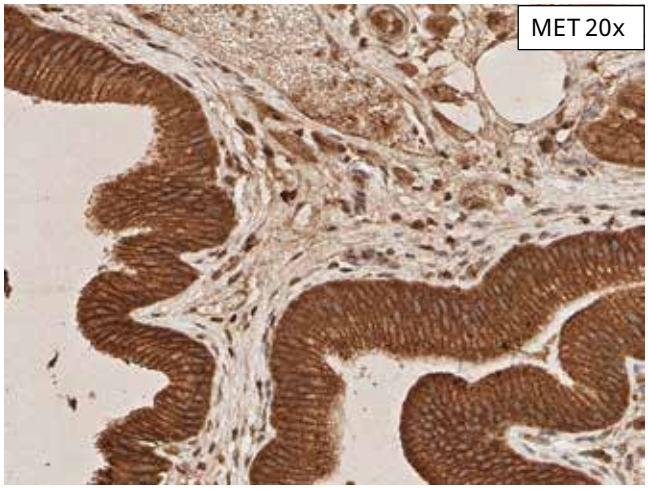


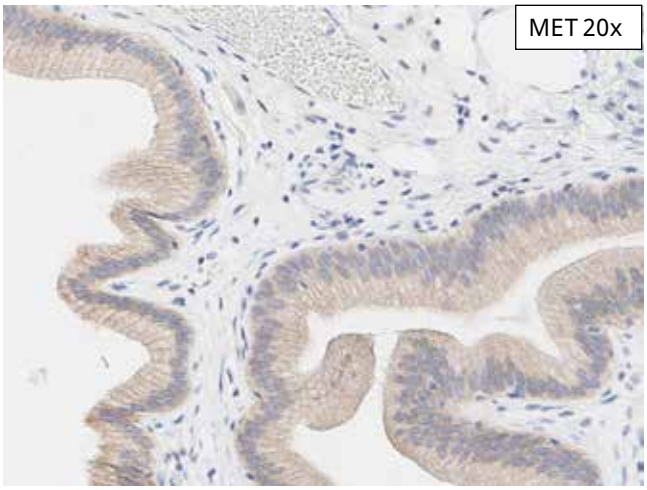




Acceptable System Level Control Staining: These gallbladder cases exhibit MET staining of moderate intensity in the epithelium and an absence of staining in cells of the stroma (10x and 20x). The cases demonstrate the range of moderate staining intensity that can be seen, with areas occasionally approaching strong intensity as shown in the upper right image.

Unacceptable Staining of Gallbladder Control Tissue

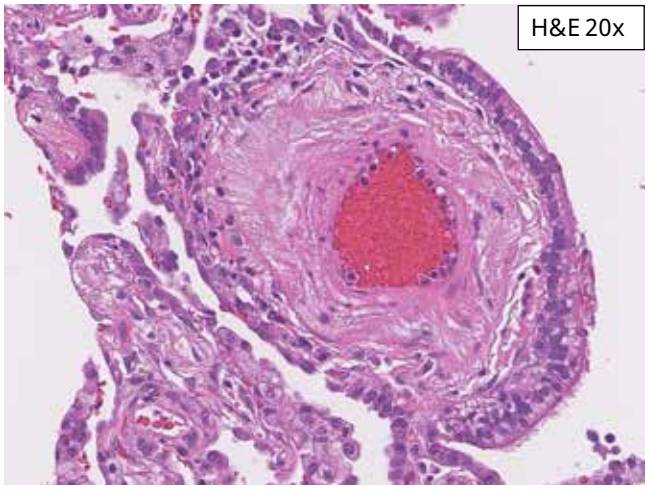


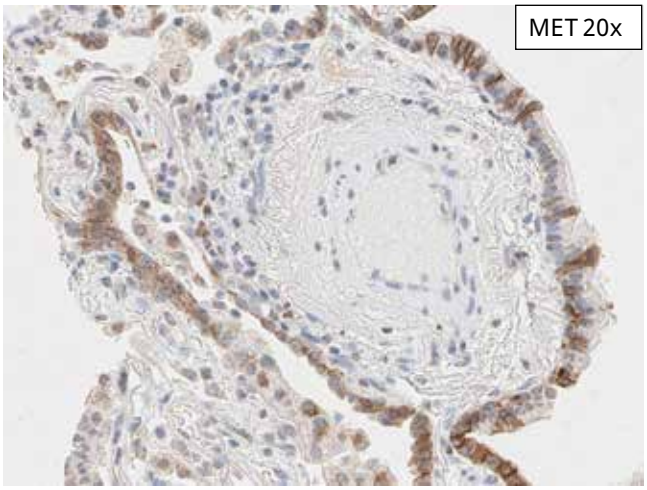


Unacceptable System Level Control Staining: These gallbladder cases exhibit MET staining unacceptable for a system level control sample. Left image: Epithelial cells exhibit strong intensity staining and the stroma exhibits staining. Right image: Epithelial cells exhibit staining that does not reach moderate intensity (20x).

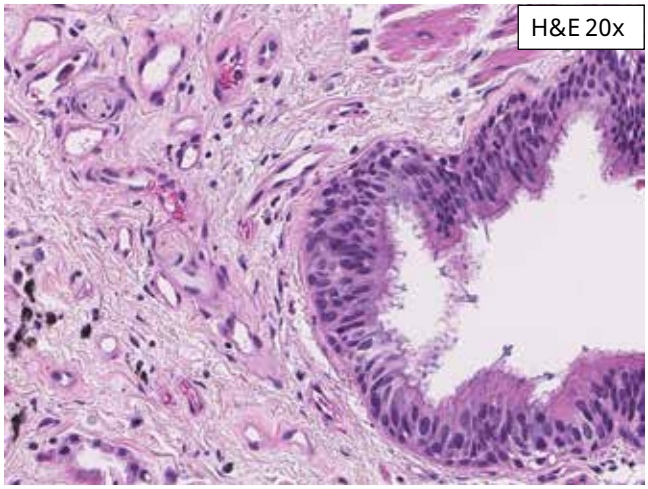
Internal Positive Controls

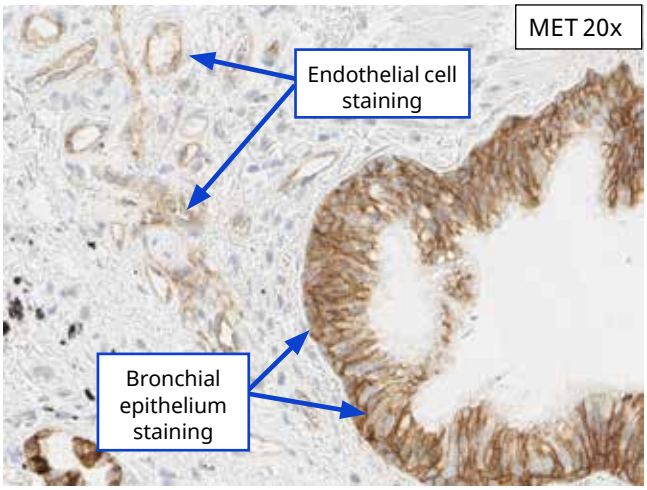
Normal bronchial epithelium and type II pneumocytes will commonly exhibit moderate to strong membranous staining following application of the VENTANA MET (SP44) RxDx Assay. Furthermore, endothelial cells will often exhibit weak cytoplasmic staining. These tissue elements, however, may not stain in all patient specimens and are not required for case evaluation.



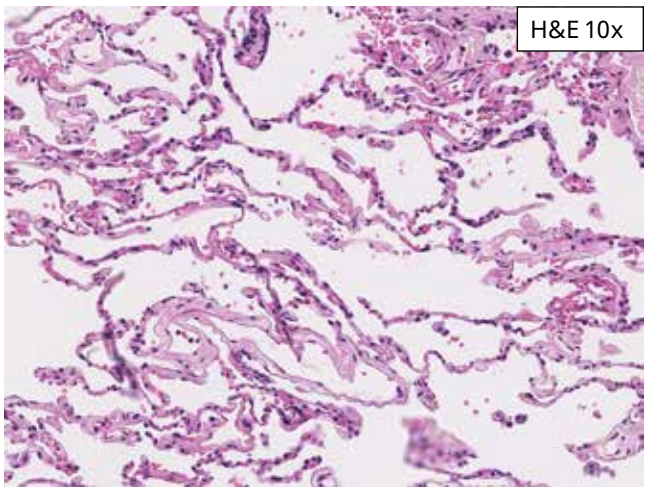


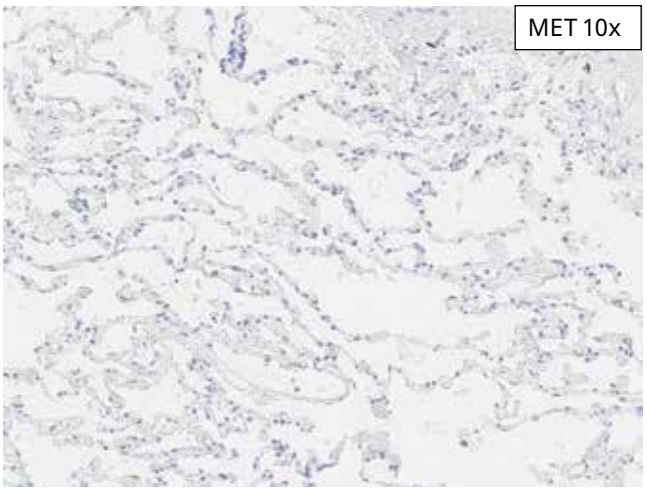
Bronchial Epithelium: This case exhibits non-neoplastic bronchial epithelial moderate to strong membranous and cytoplasmic staining (20x).





Bronchial Epithelium: This case exhibits bronchial epithelial moderate to strong membranous and cytoplasmic staining, as well as weak cytoplasmic endothelial cell staining (20x).





Pneumocytes: This case exhibits type II pneumocyte staining (10x).

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 Rabbit Monoclonal Negative Control lg staining, and a third serial tissue section for VENTANA MET (SP44) RxDx Assay staining. If the H&E evaluation indicates that the patient specimen is inadequate, then a new specimen should be obtained and stained with VENTANA MET (SP44) RxDx Assay.

Pre-qualified gallbladder tissue exhibiting moderate intensity MET staining of epithelial cells and absence of staining in cells of the gallbladder stromal tissue may be used as run controls. Both positive and negative elements must be stained appropriately as defined by the criteria for gallbladder (**Table 3**) on each run for the run to be considered valid.

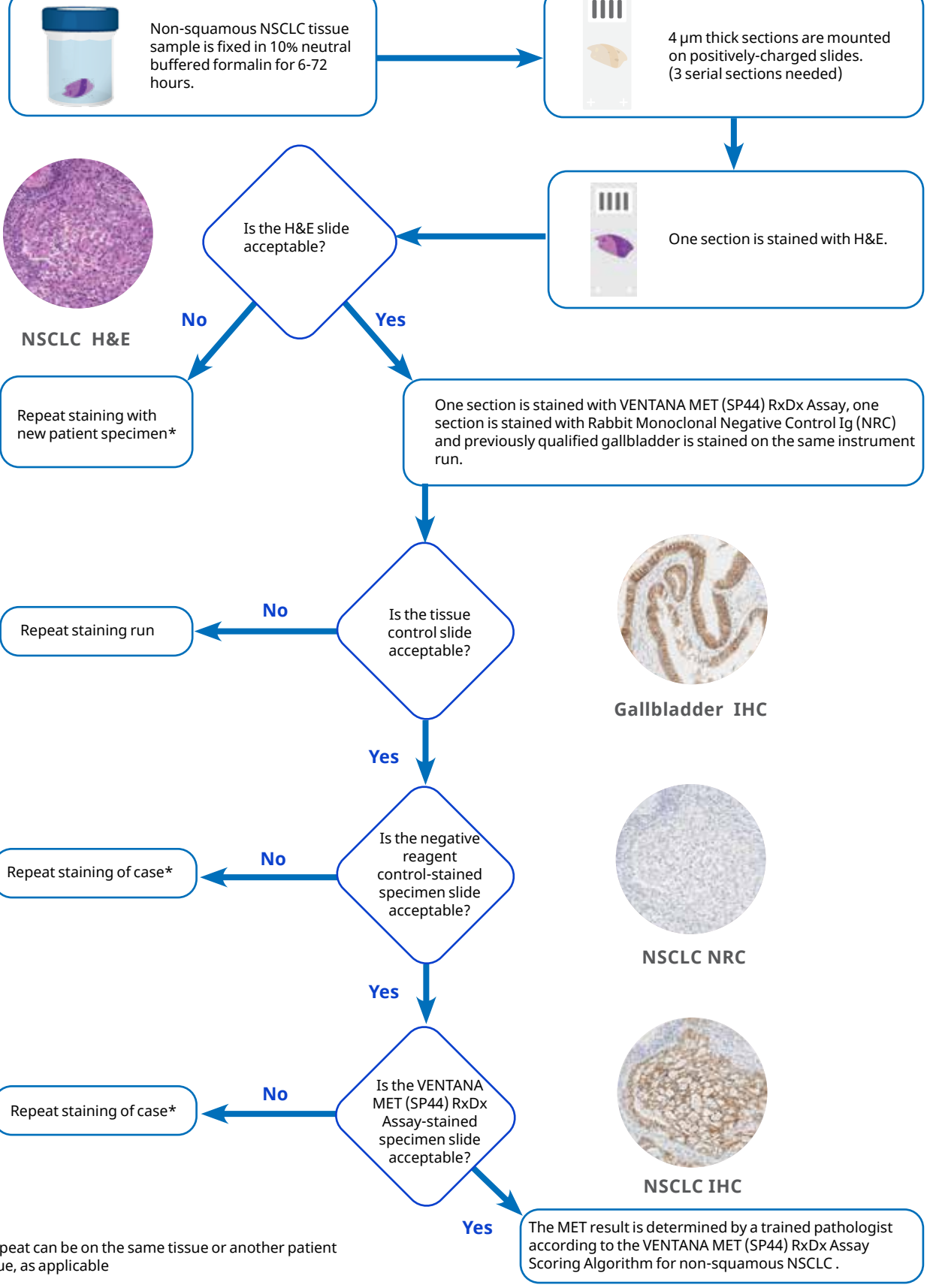
A matched NRC slide must be run for every specimen to evaluate nonspecific staining and aid in the interpretation of results.

The MET-stained specimen slides should be assessed by a trained pathologist. If either the MET-stained tissue control slide or the NRC-stained specimen slide is not acceptable, staining of patient samples should be repeated. Repeat staining may be on the same tissue or another patient tissue, as applicable. A non-evaluable VENTANA MET (SP44) RxDx Assay-stained slide would mean that determination of reactivity is not possible due to necrosis, absent tumor, or artifacts.

50% strong TC Cut-off

To decrease variability of MET results for cases with %TC near the threshold of 50% (40% to 60%) or with staining intensity near the strong versus moderate threshold, re-reading of slides that fall into these categories by a second pathologist is recommended. The case result with %TC between 40-60% or borderline strong versus moderate intensity by a pathologist should be adjudicated by one or two independent pathologists. The patient's final result with regard to MET staining (positive and negative) should be obtained by consensus among the pathologists.

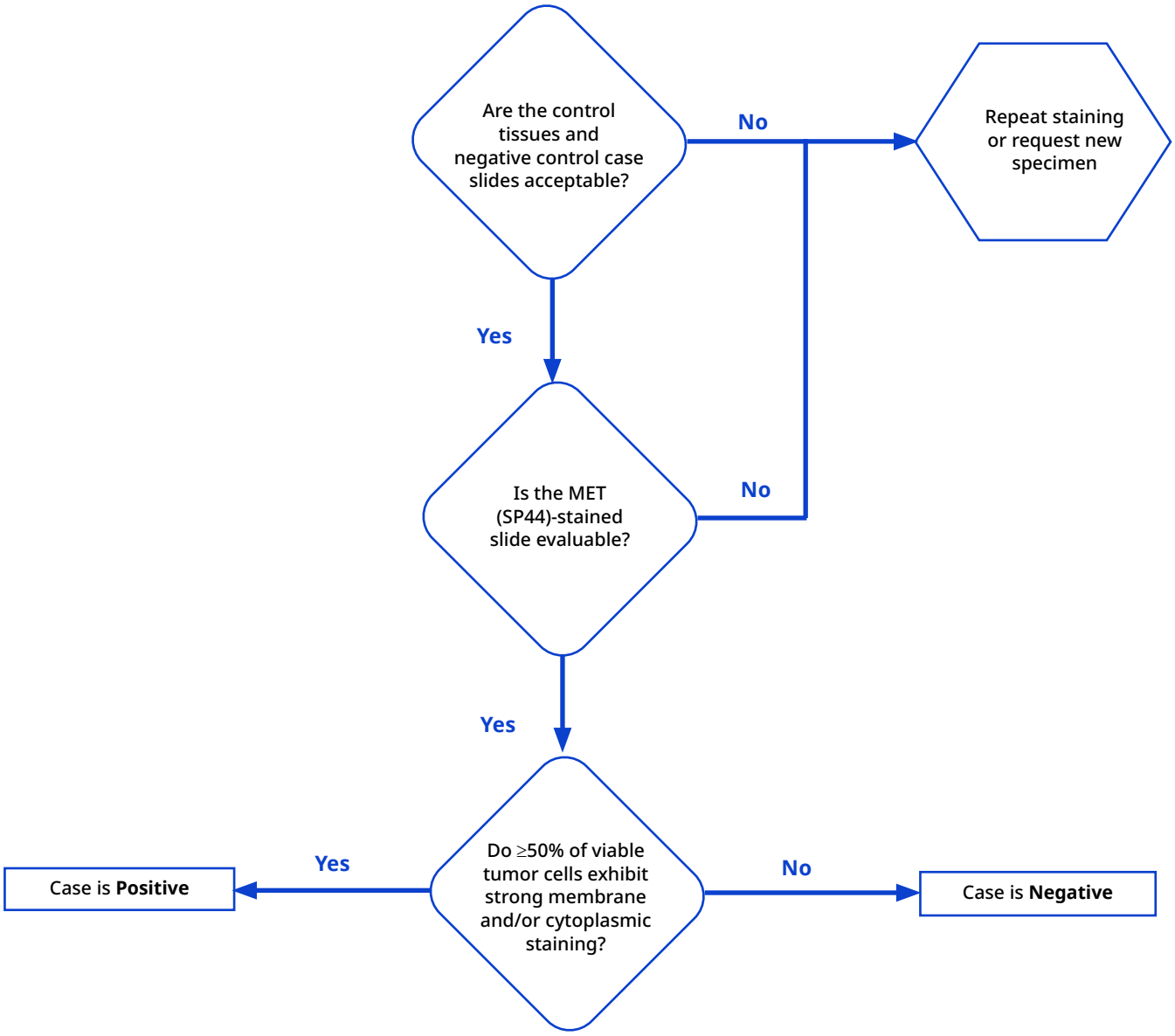
Specimen Flow



*Repeat can be on the same tissue or another patient tissue, as applicable

Decision Tree

Slides stained with the VENTANA MET (SP44) RxDx Assay should be evaluated using the approach noted in the figure below.

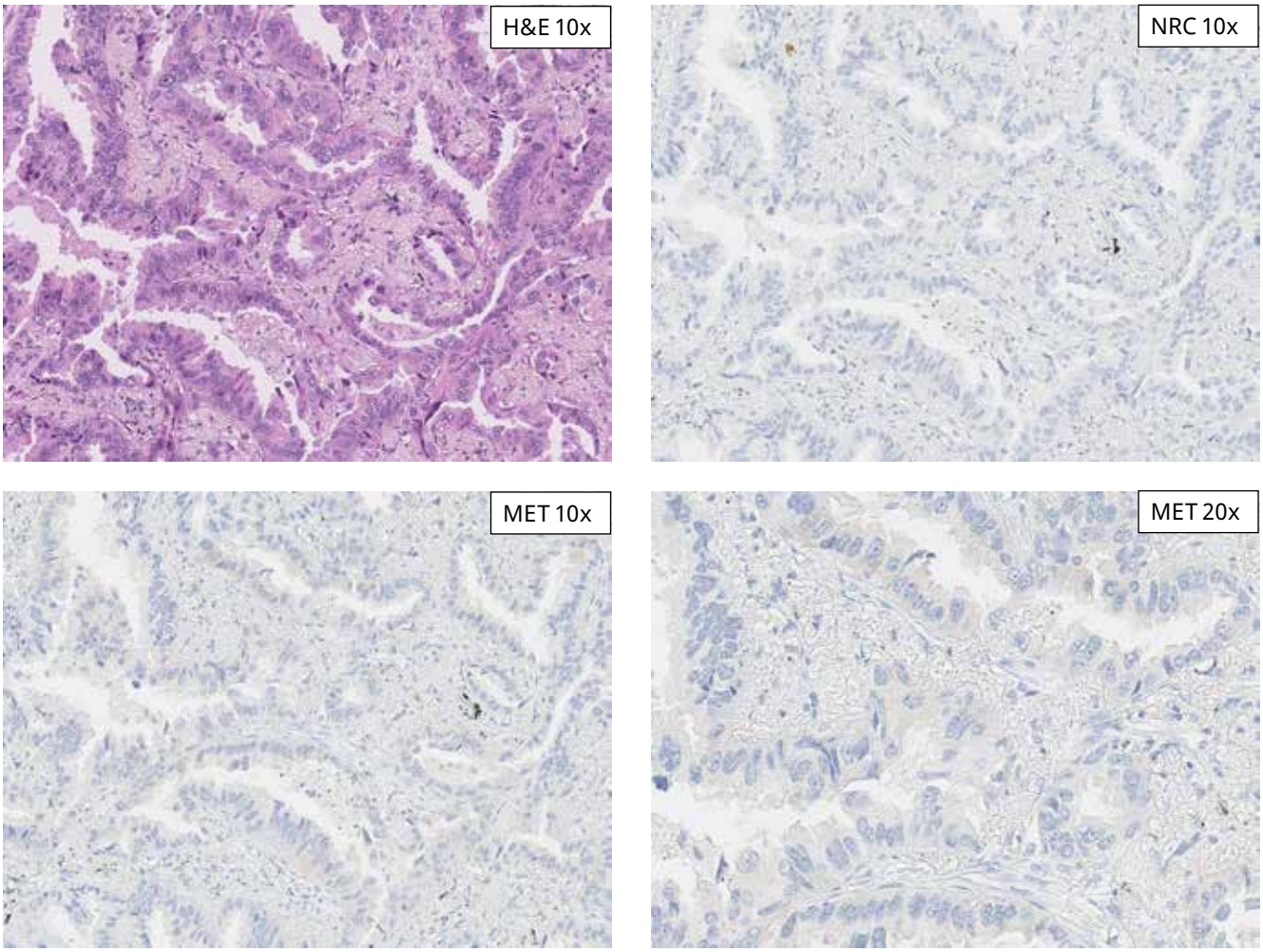


Reference Images

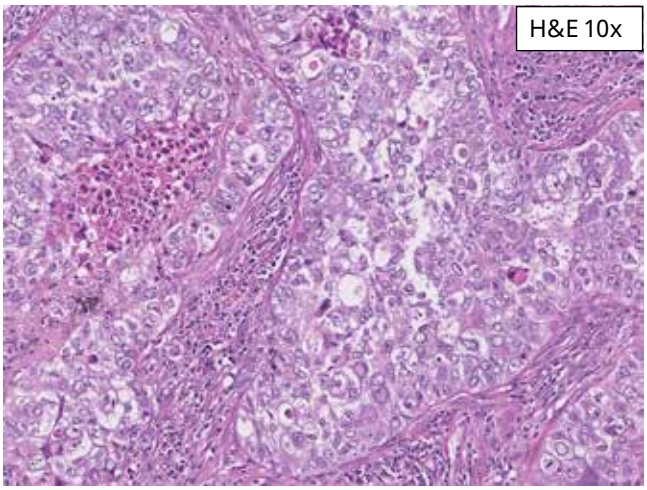
Representative Images for Assessing Percentage of strong Tumor Cell Staining

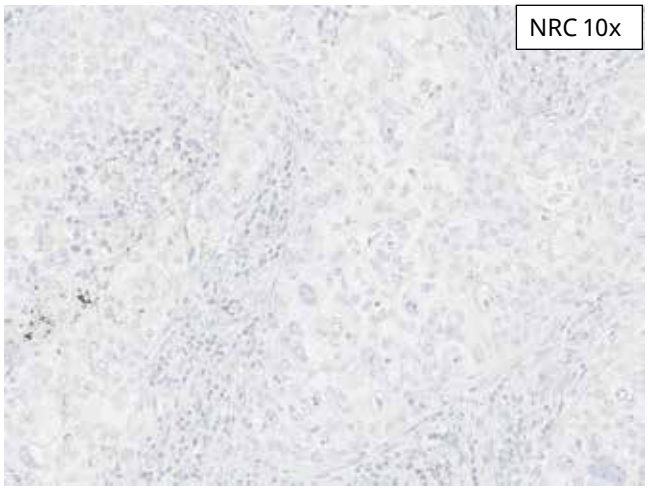
Examples given on the following pages illustrate the range of staining typically exhibited by the VENTANA MET (SP44) RxDx Assay. Cases are assessed for the percentage of tumor cells exhibiting strong membranous and/or cytoplasmic MET staining.

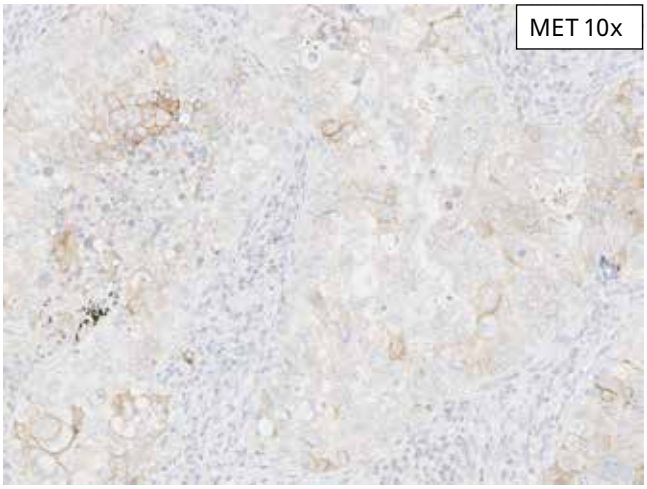
Negative Cases

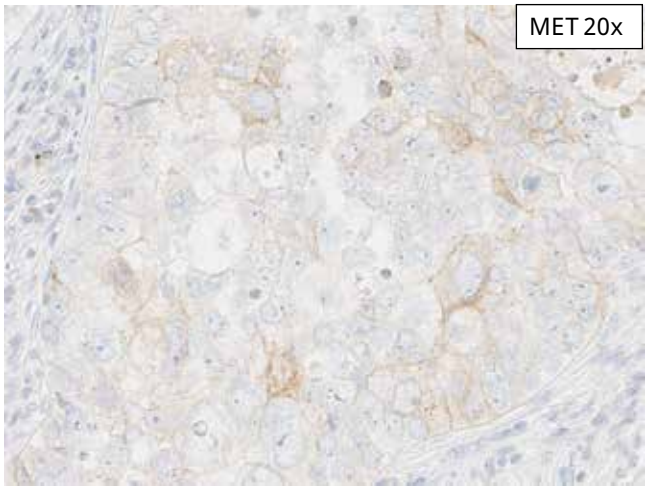


Negative Case Photoset 1: This case exhibits an absence of MET staining in tumor cells. This sample would be given a score of 0% staining at strong intensity (for the 10x field).

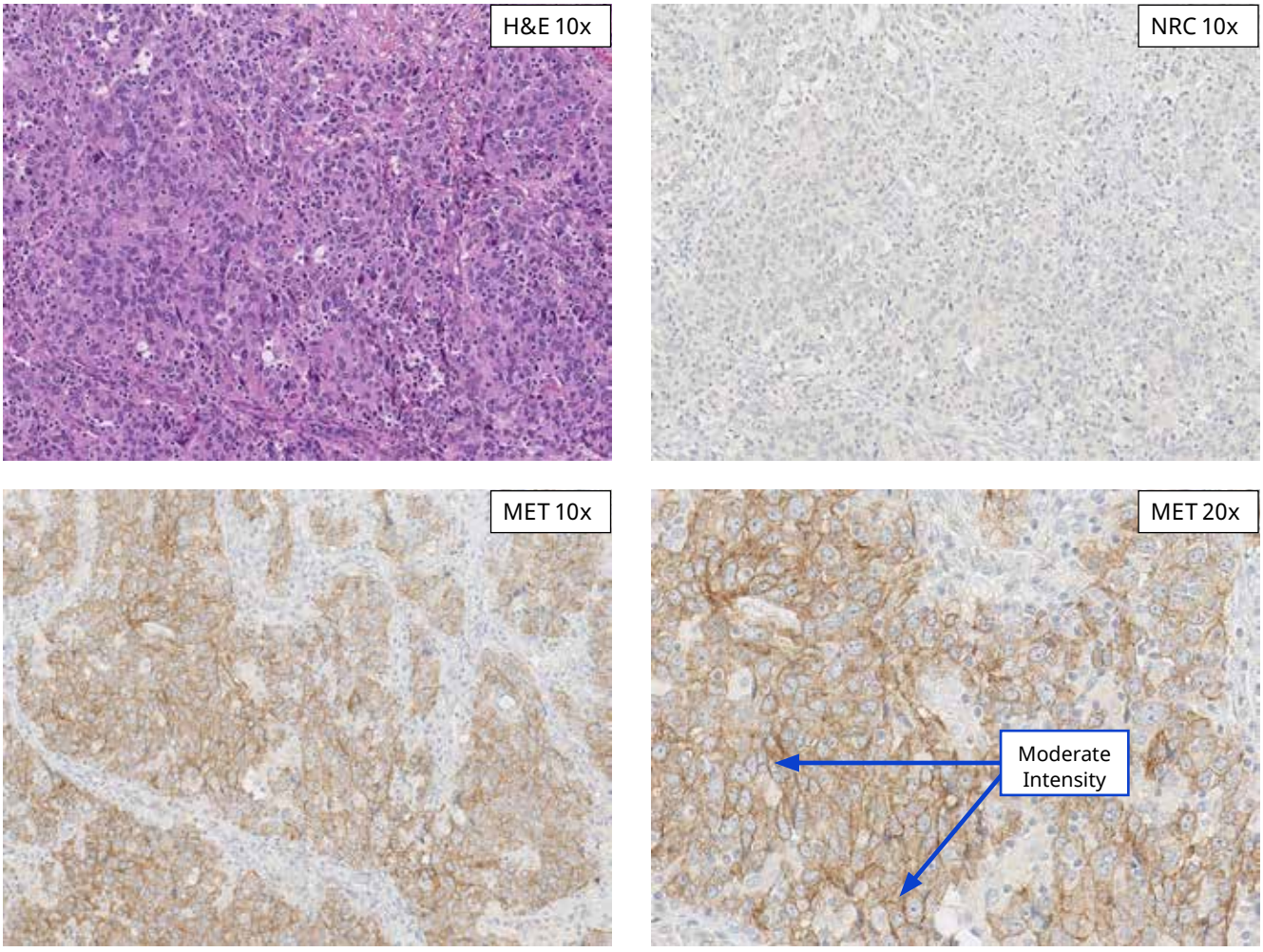




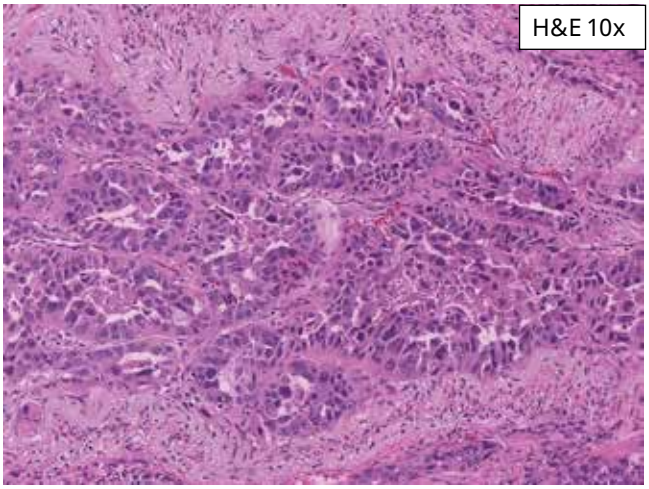


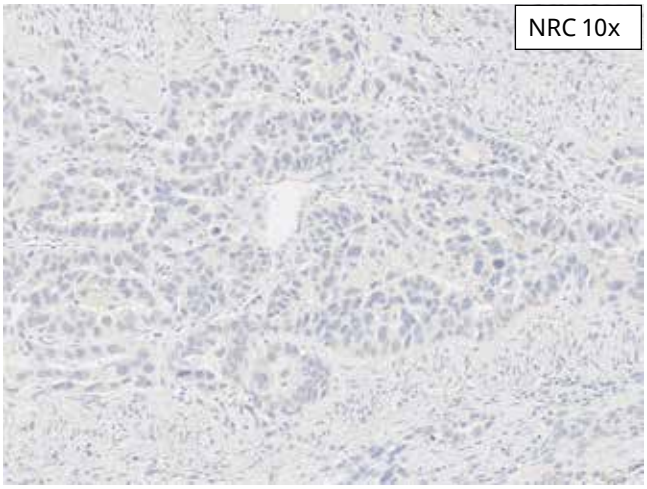


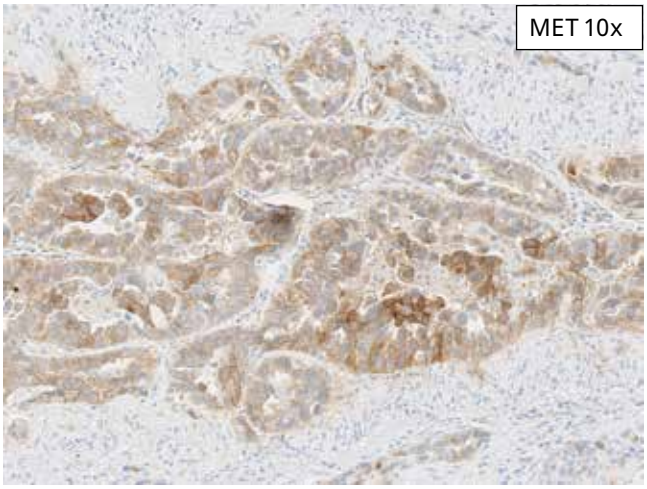
Negative Case Photoset 2: This case exhibits weak membranous and cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 0% staining at strong intensity (for the 10x field).

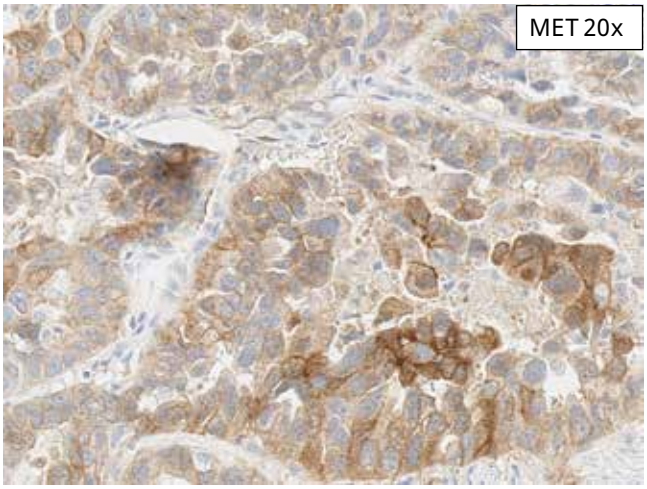


Negative Case Photoset 3: This case exhibits predominately moderate membranous intensity MET staining and weak cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 0% staining at strong intensity (for the 10x field).

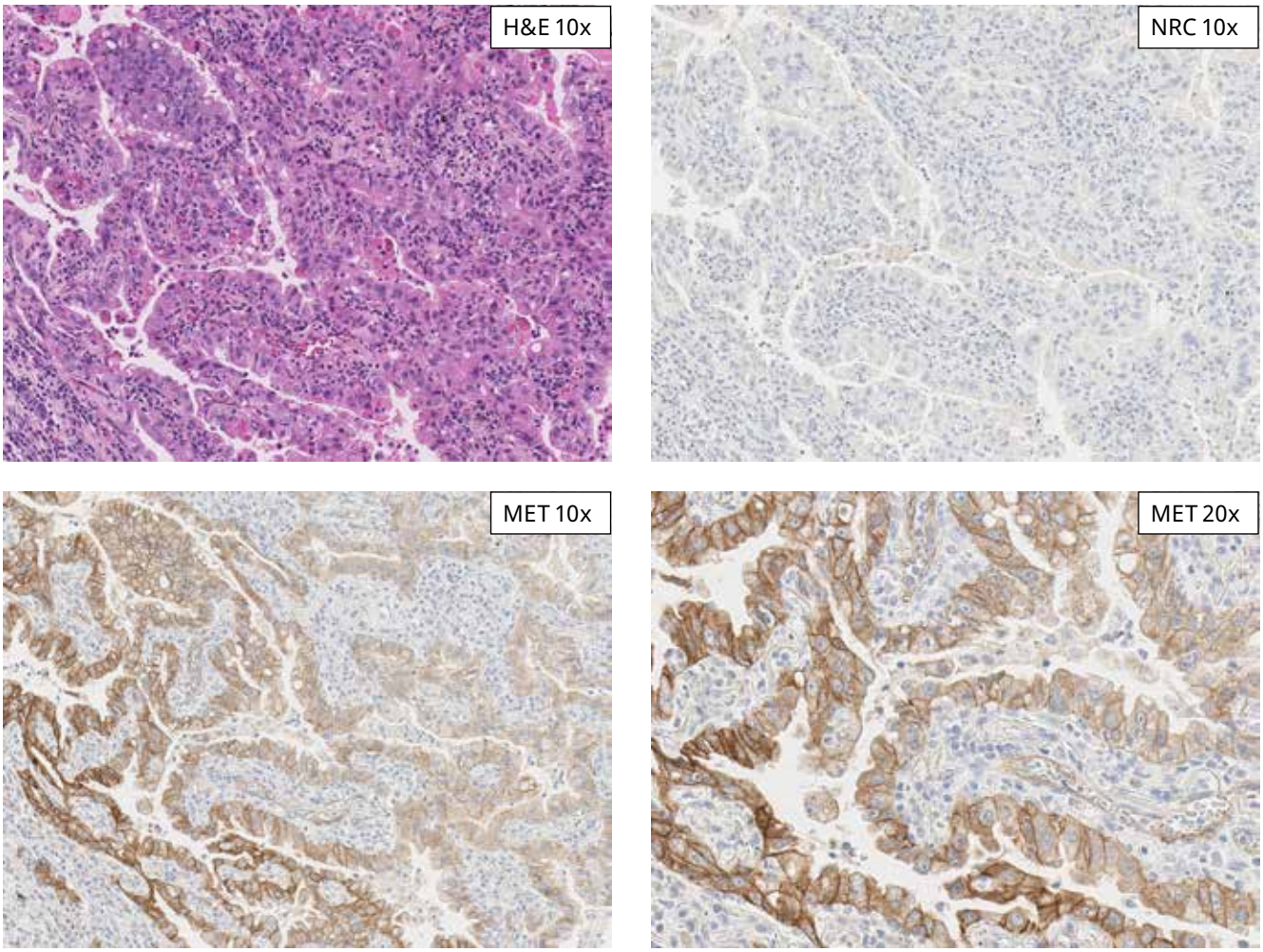






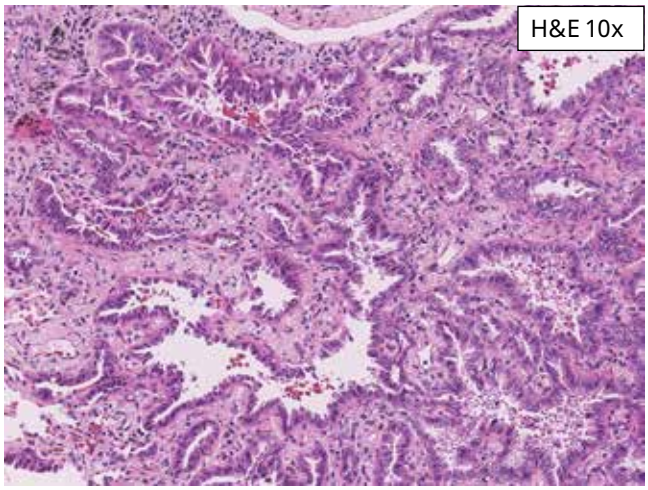


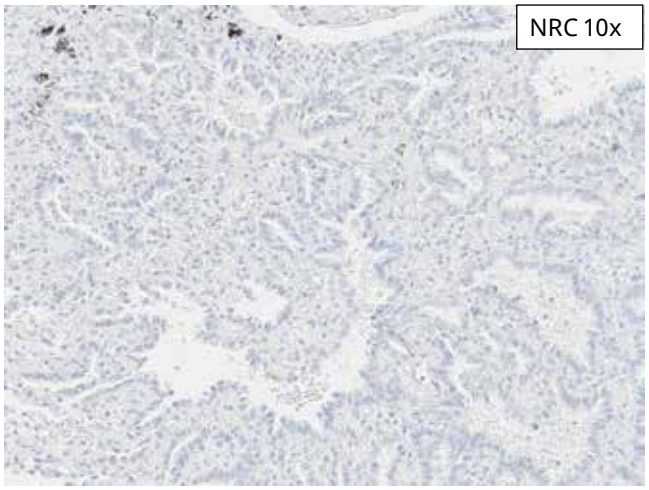
Negative Case Photoset 4: This case exhibits heterogeneous intensity MET staining in tumor cells. The majority of cells demonstrate weak cytoplasmic staining; there is focal strong membranous staining. This sample would be given a score of 5% staining at strong intensity (for the 10x field).

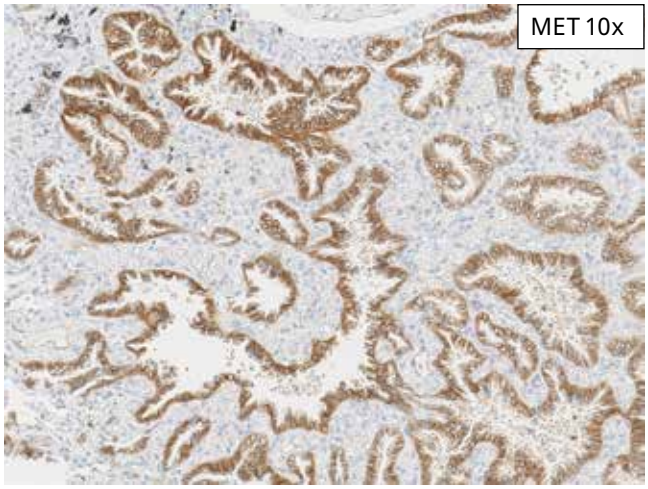


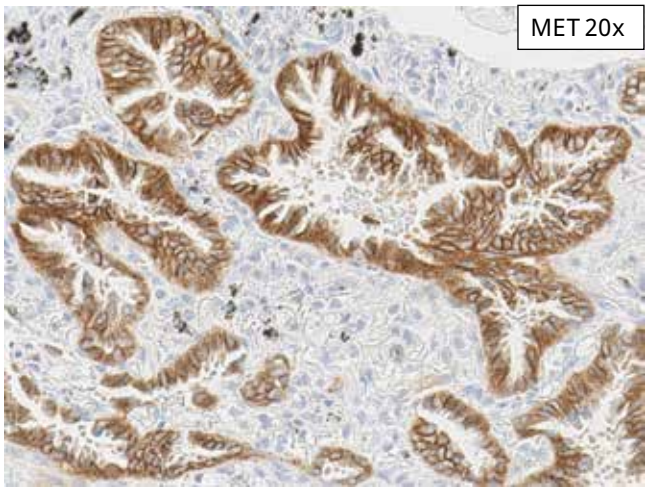
Negative Case Photoset 5: This case exhibits heterogeneous intensity MET staining in tumor cells. The majority of tumor cells demonstrate weak membranous and cytoplasmic staining, with focal strong membranous staining primarily localized in the lower left portion of the image. This sample would be given a score of 15% strong staining (for the 10x field).

Postive Cases

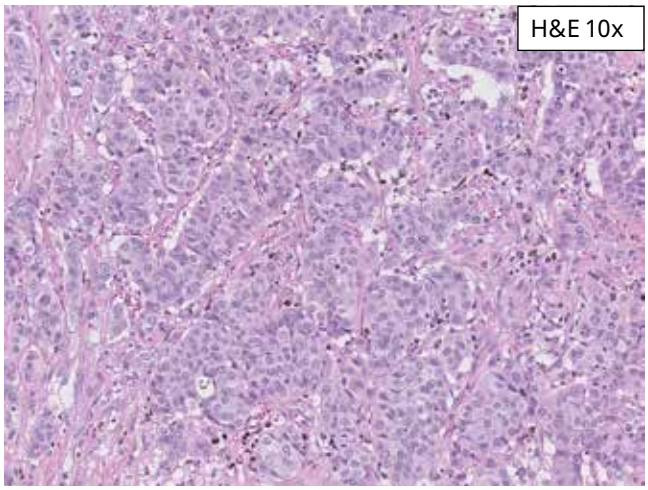


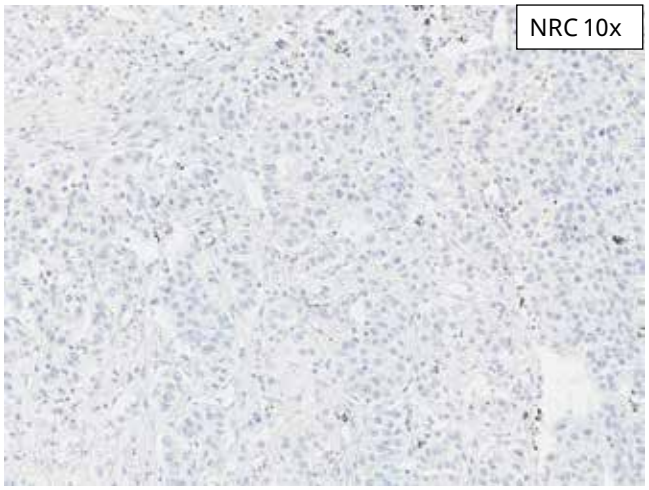


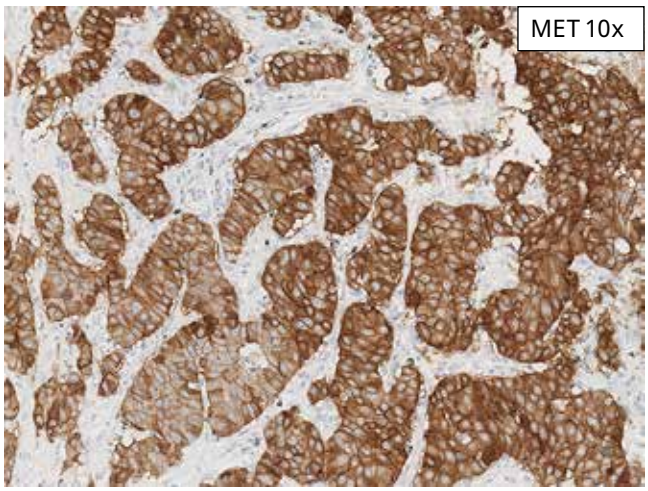


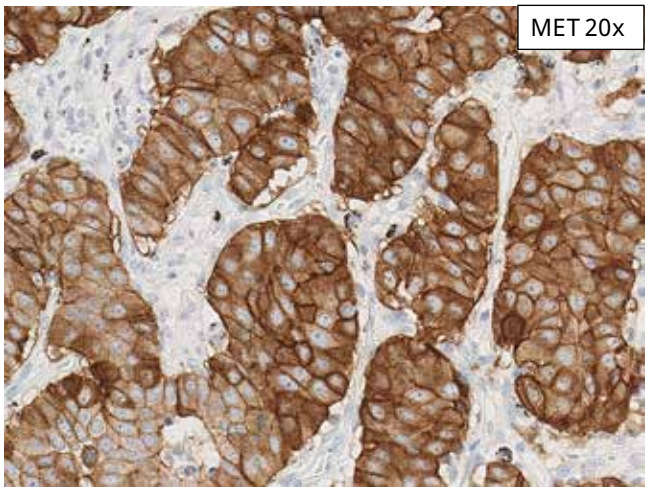


Positive Case Photoset 1: This case exhibits primarily strong membranous and/or cytoplasmic MET staining intensity in tumor cells. In this example, 70% of the tumor cells exhibit strong intensity staining (for the 10x field).

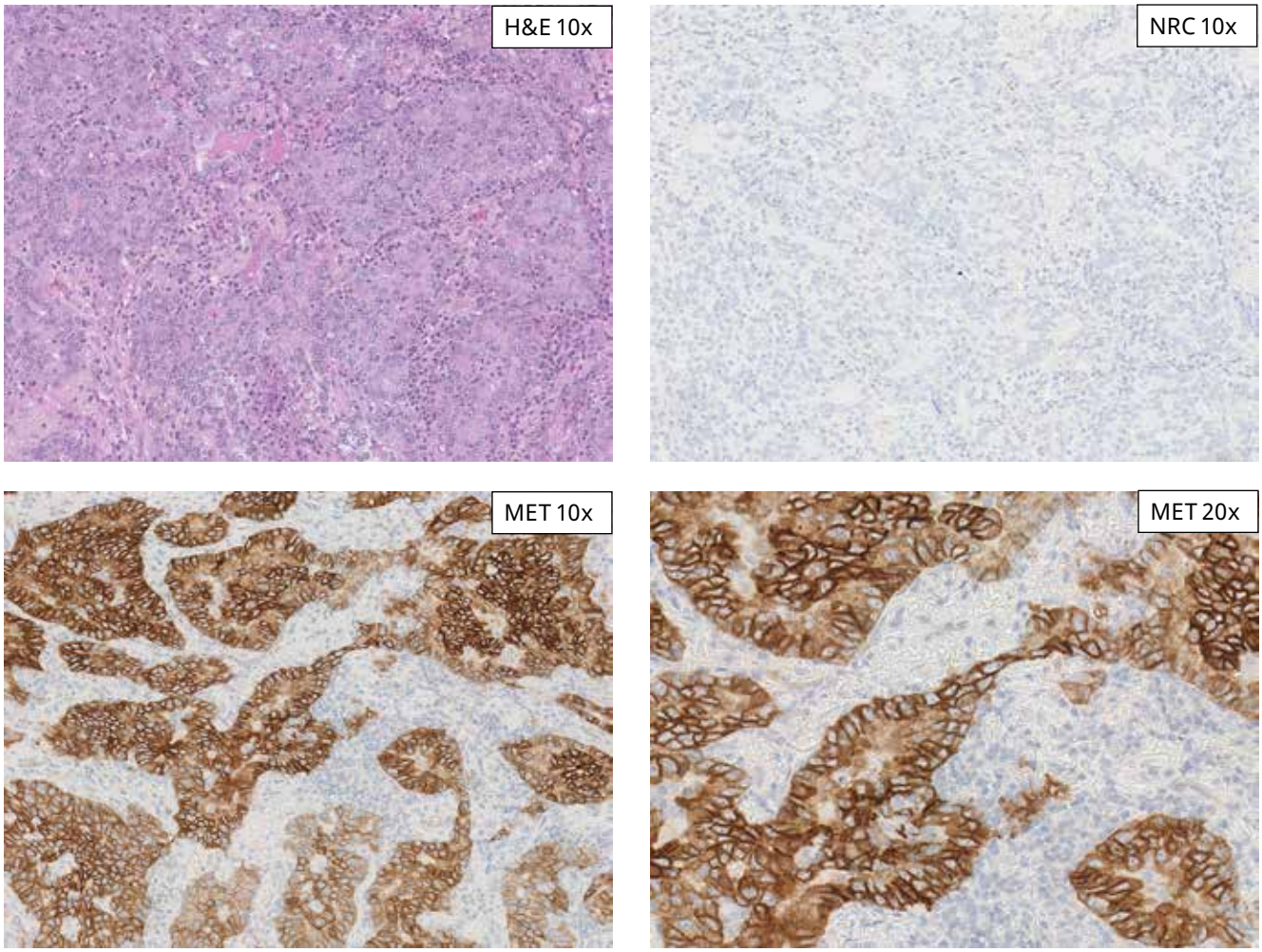




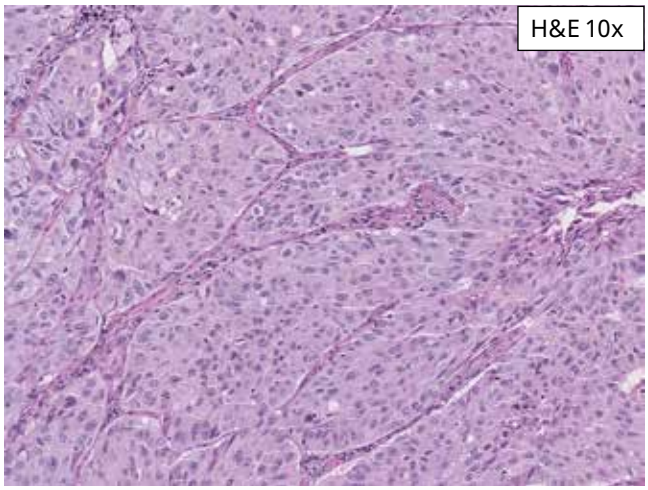


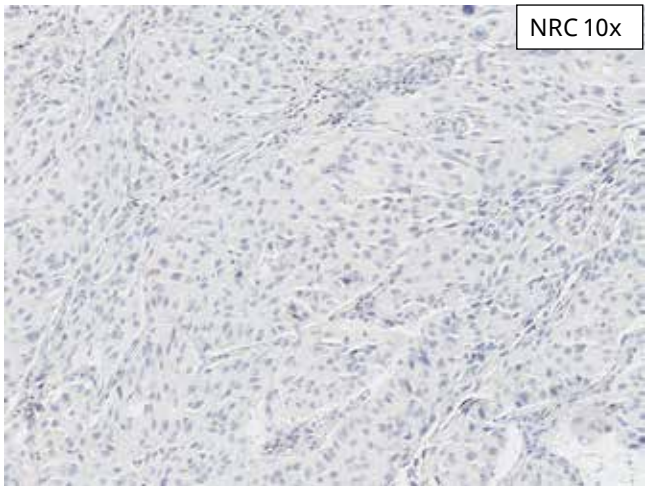


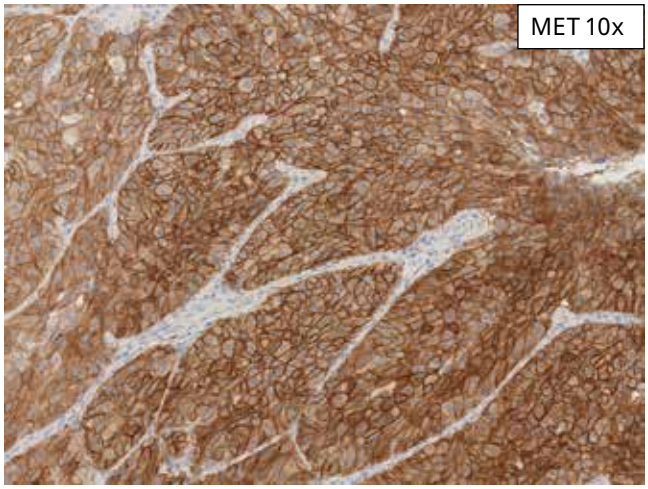
Positive Case Photoset 2: This case exhibits predominately strong membranous and/or cytoplasmic MET staining in tumor cells. This sample would be given a score of 80% staining at strong intensity (for the 10x field).

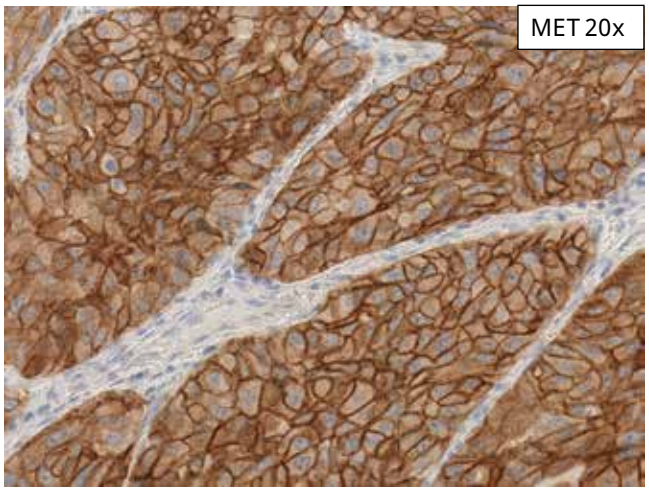


Positive Case Photoset 3: This case exhibits predominately strong membranous and/or cytoplasmic MET staining intensity in tumor cells. In this example, 80% of the tumor cells exhibit strong intensity staining (for the 10x field).

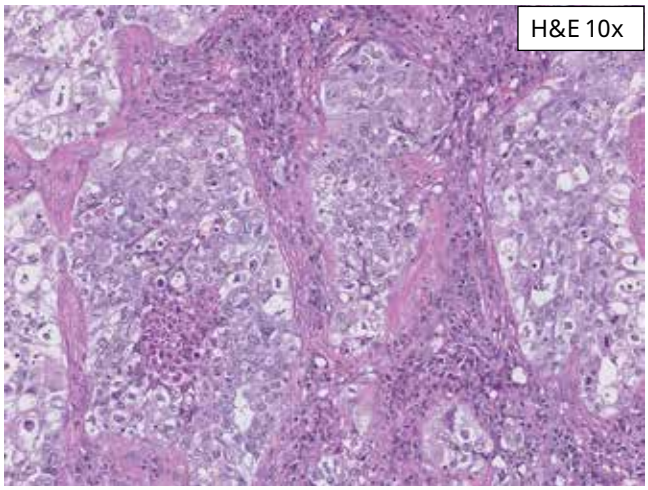


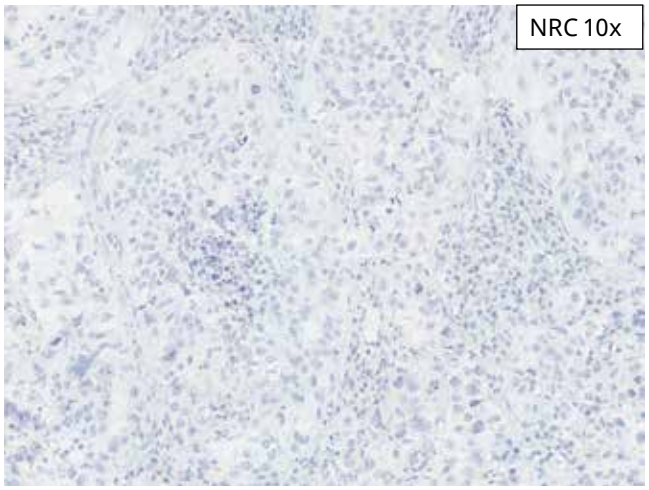


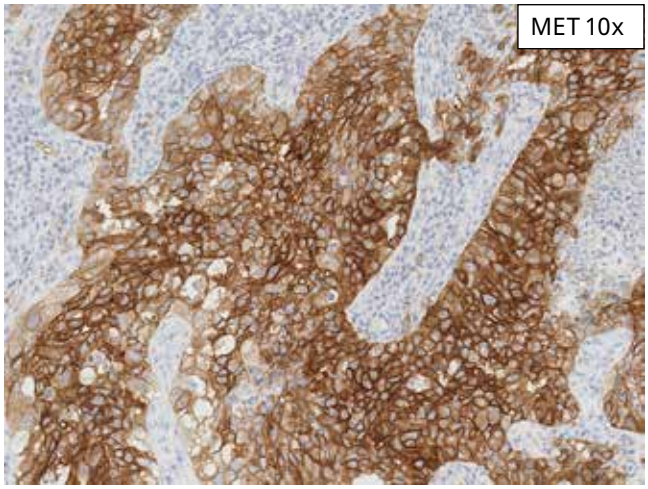


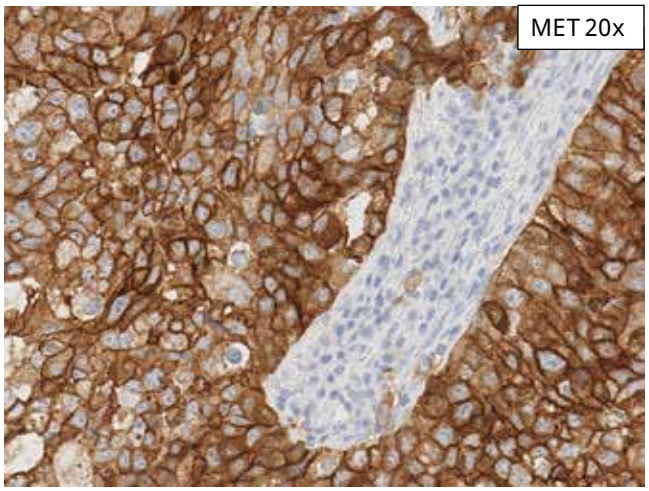


Positive Case Photoset 4: This case exhibits predominately strong membranous MET staining in tumor cells. Only the tumor on the left portion of the 10x field is staining at a moderate membranous intensity. The tumor cells exhibit predominately weak to moderate cytoplasmic staining. This sample would be given a score of 85% staining at strong intensity (for the 10x field).

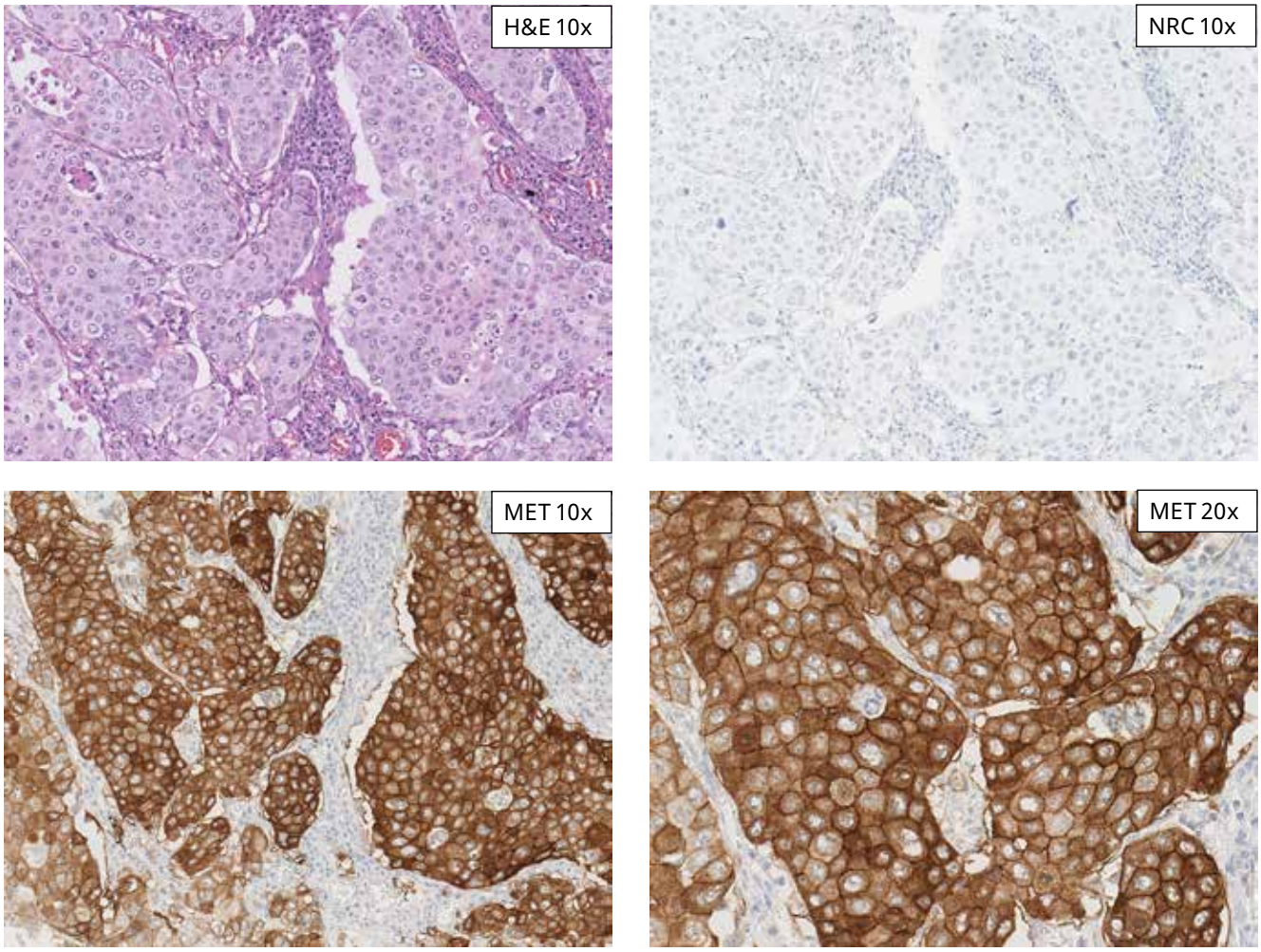




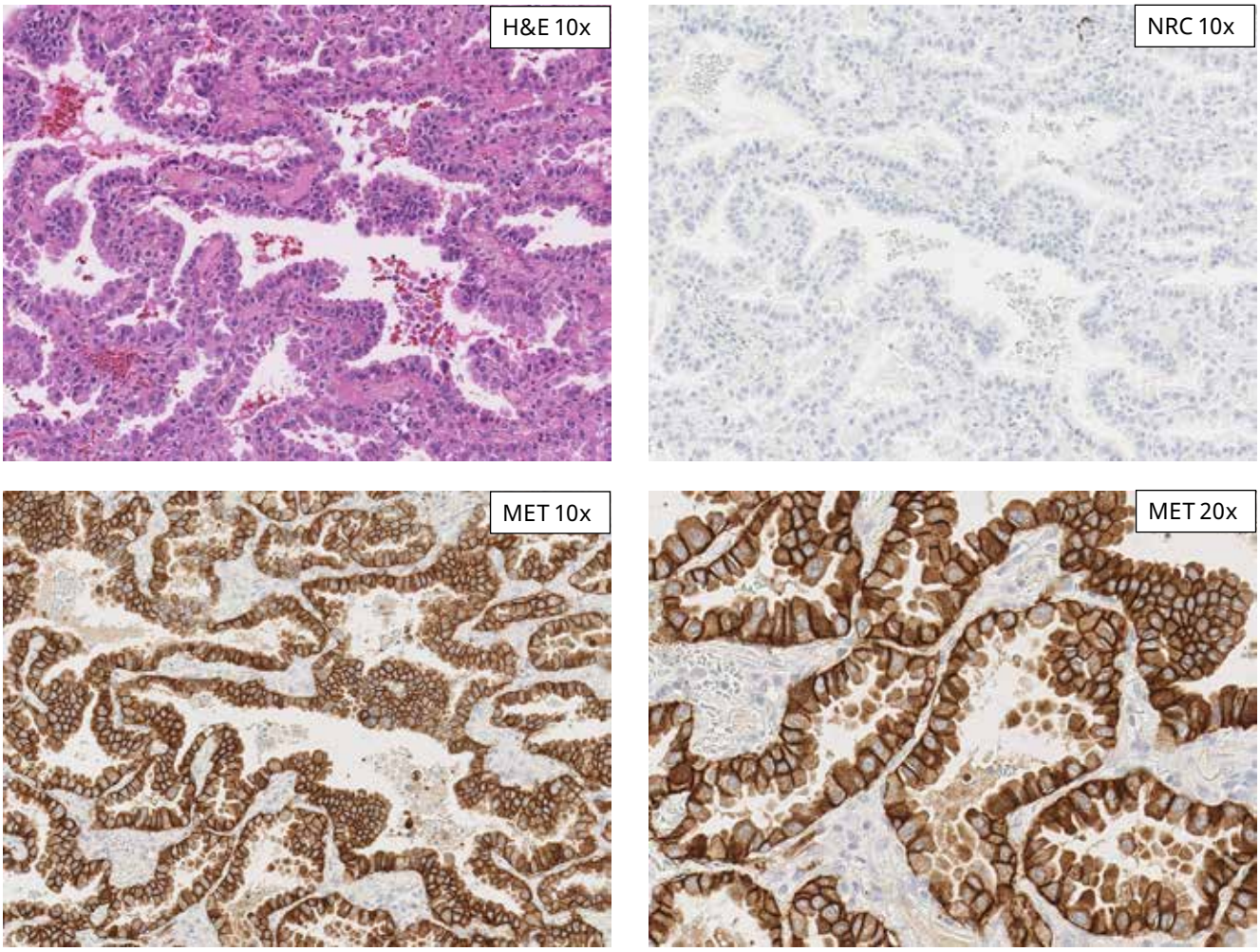




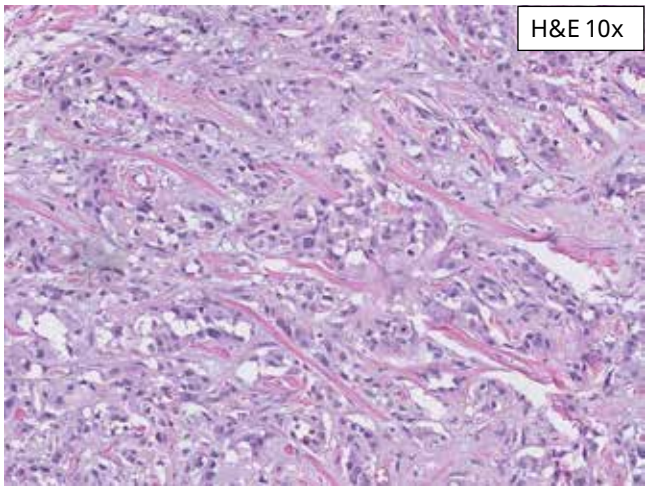
Positive Case Photoset 5: This case exhibits predominately strong membranous and/or cytoplasmic MET staining in tumor cells. This case would be given a score of 90% staining at strong intensity (for the 10x field).

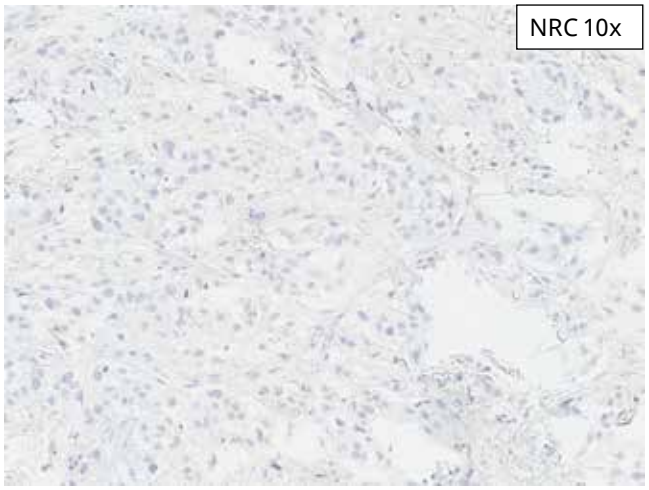


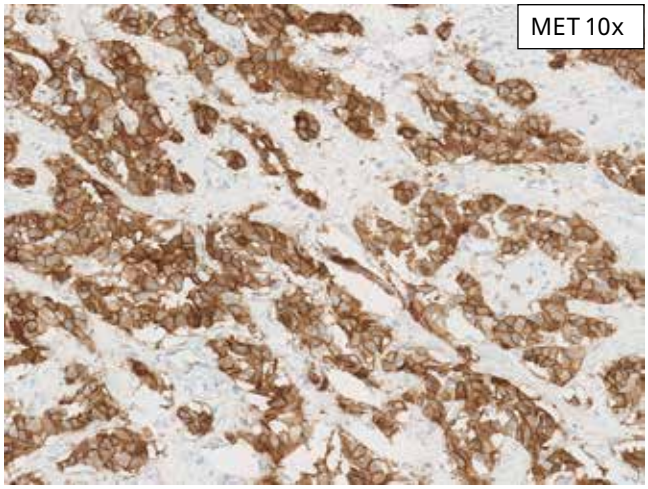
Positive Case Photoset 6: This case exhibits predominately strong membranous and/or cytoplasmic MET staining in tumor cells. This case would be given a score of 90% staining at strong intensity (for the 10x field).

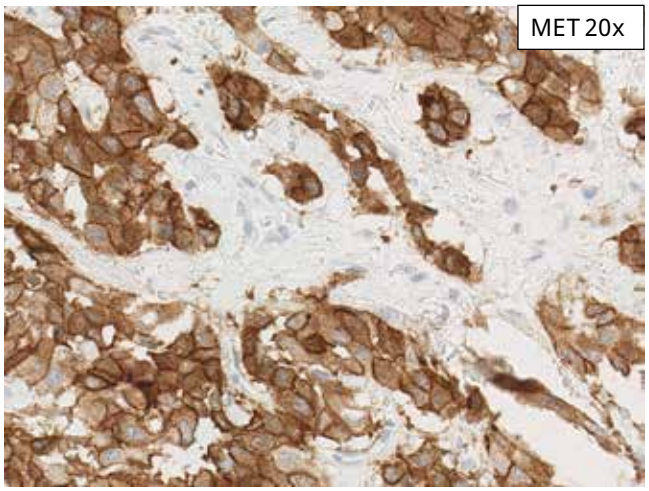


Positive Case Photoset 7: This case exhibits primarily strong membranous and/or cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 95% staining at strong intensity (for the 10x field).

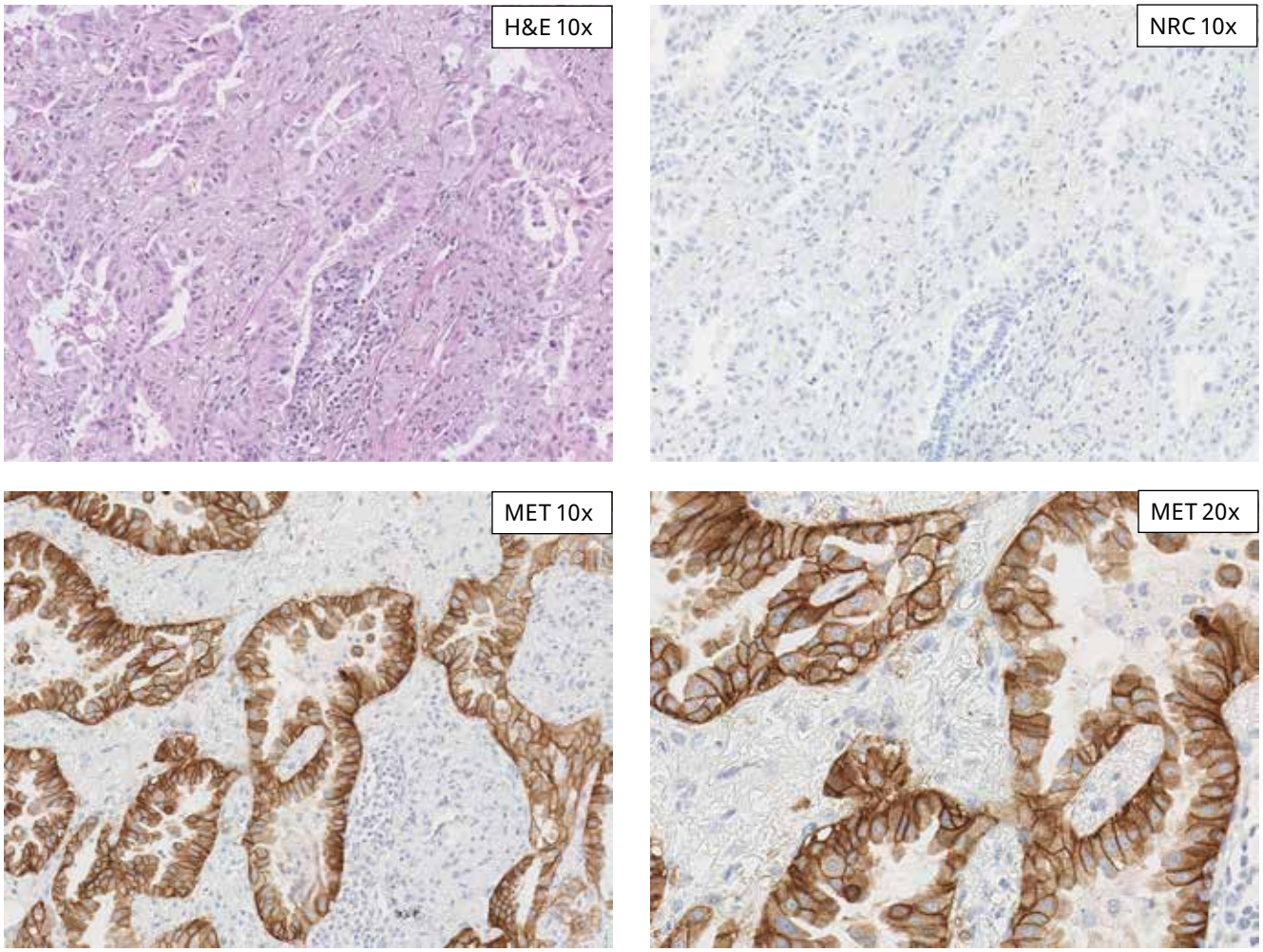




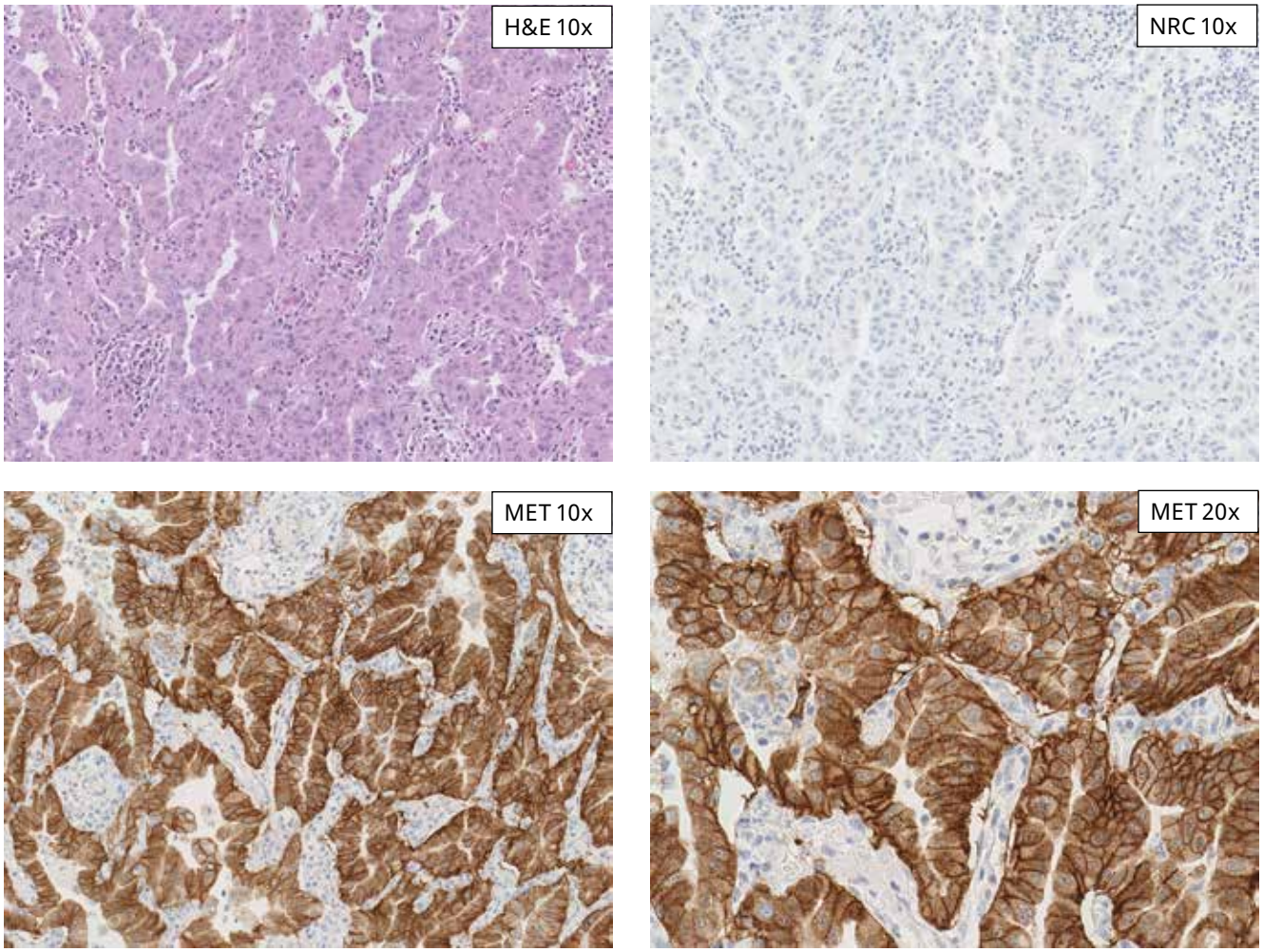




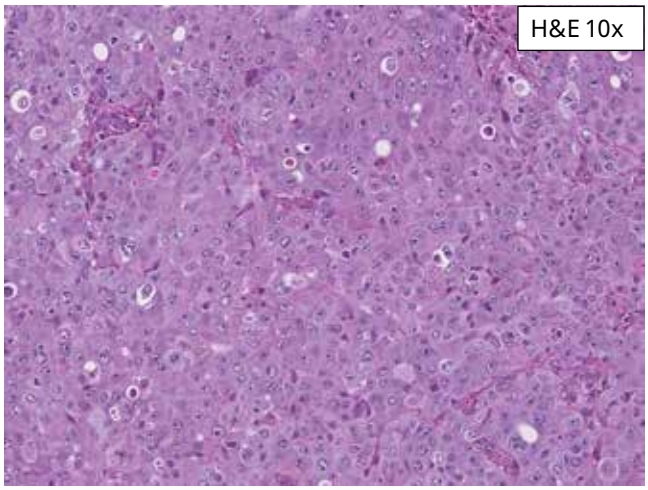
Positive Case Photoset 8: This case exhibits primarily strong membranous and/or cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 95% staining at strong intensity (for the 10x field).

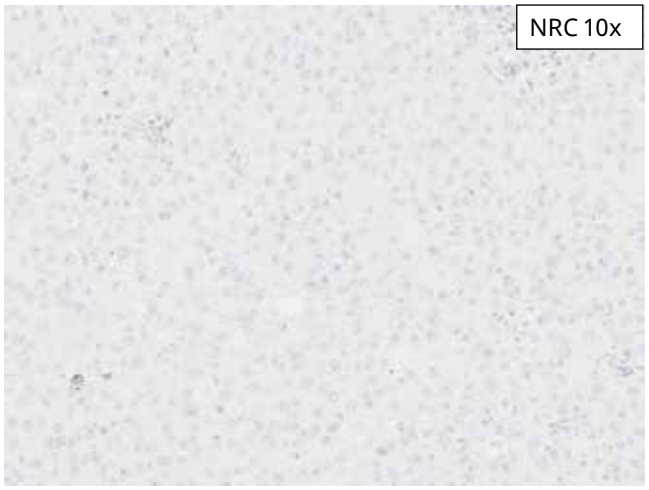


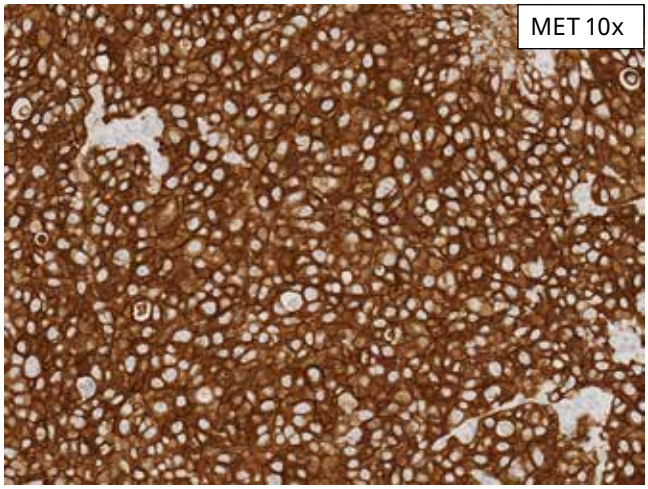
Positive Case Photoset 9: This case exhibits primarily strong membranous intensity MET staining and weak to moderate cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 95% staining at strong intensity (for the 10x field).

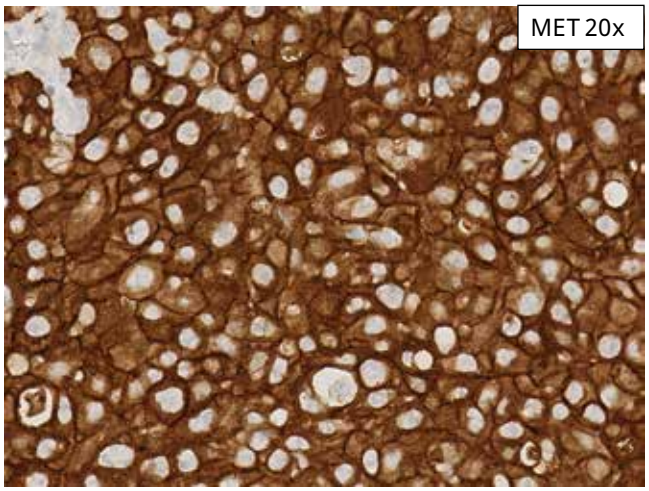


Positive Case Photoset 10: This case exhibits primarily strong membranous intensity MET staining and moderate to strong cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 95% staining at strong intensity (for the 10x field).

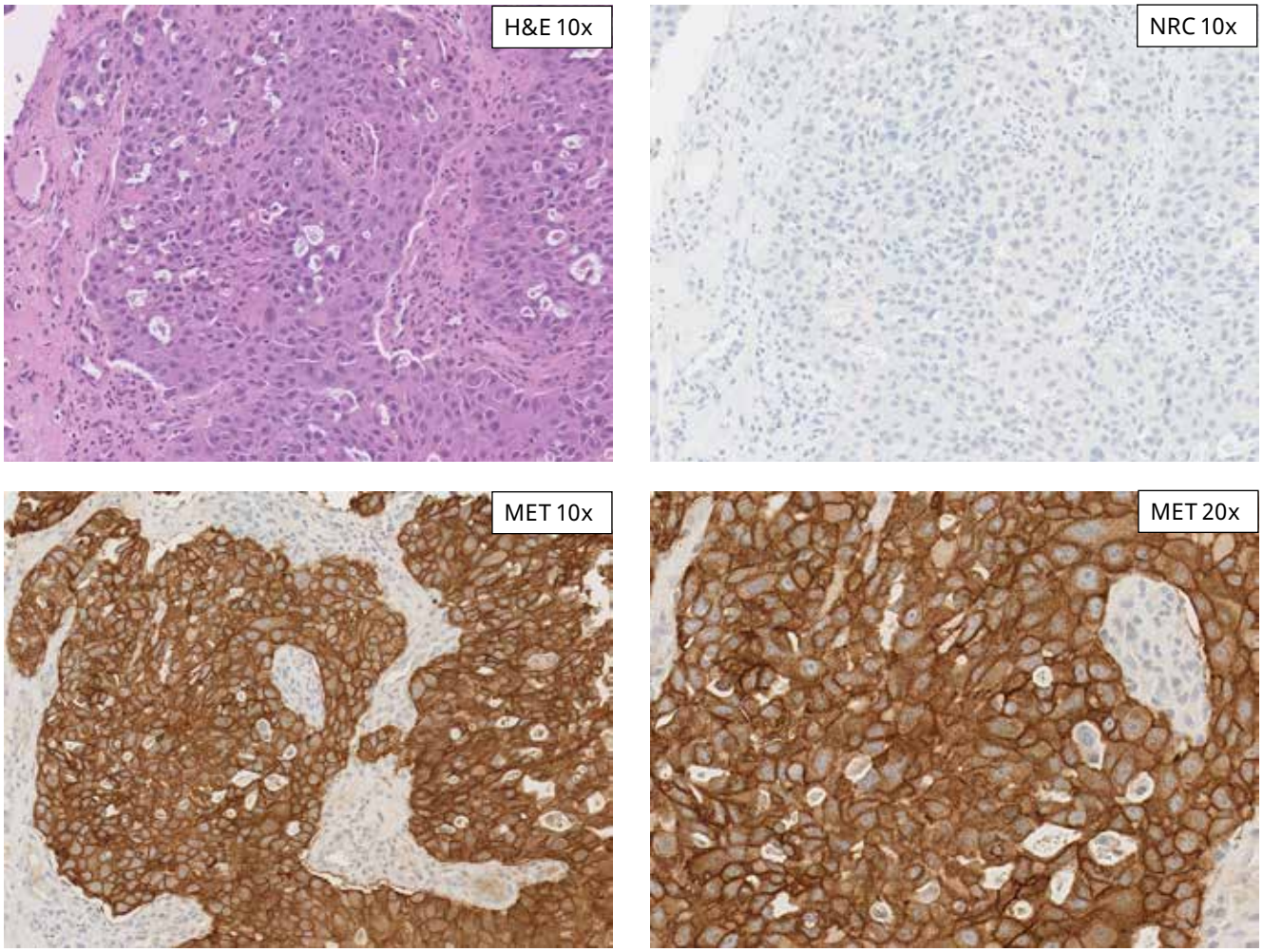




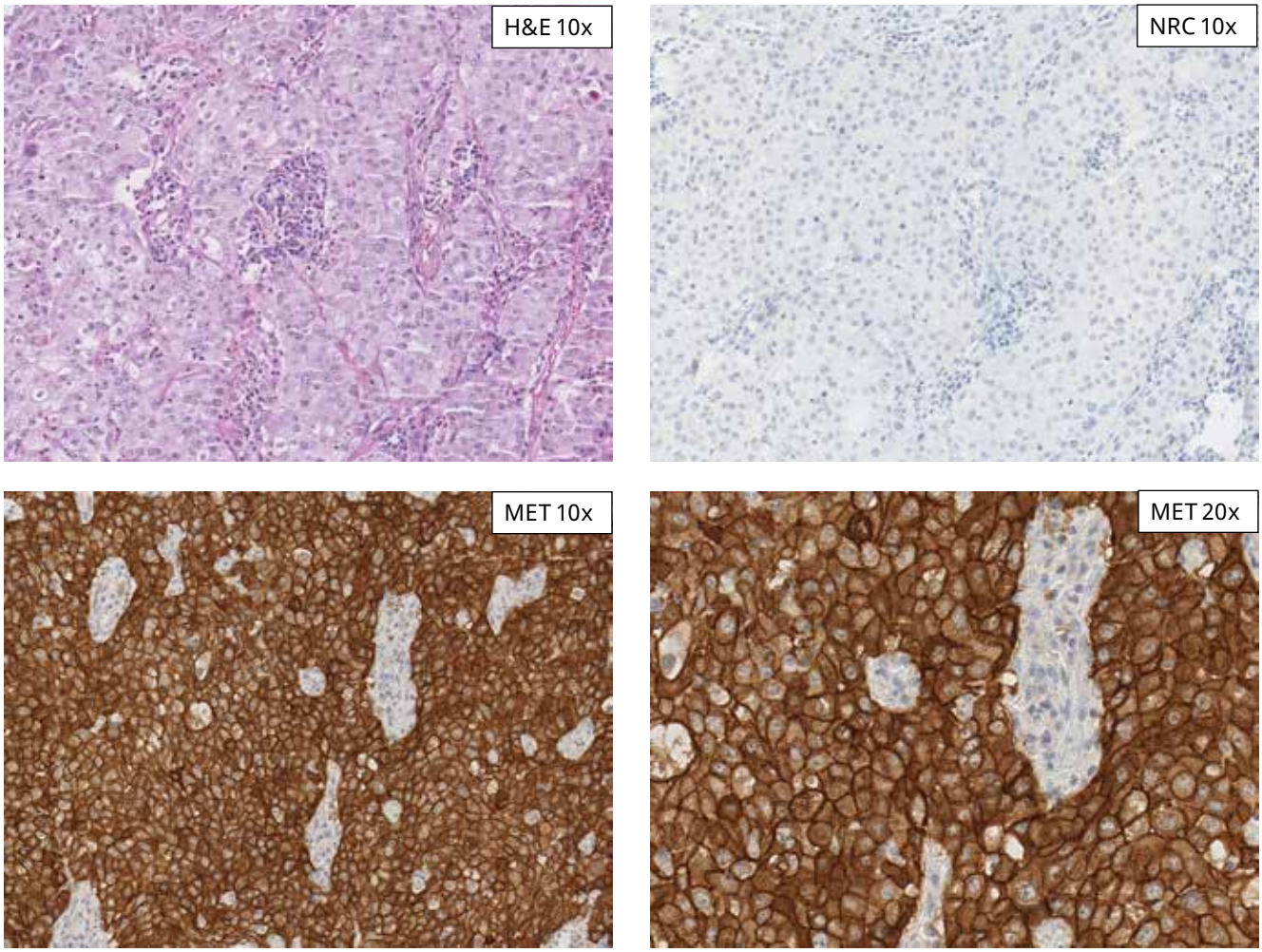




Positive Case Photoset 11: This case exhibits strong membranous and/or cytoplasmic MET staining in tumor cells. This case would be given a score of 100% staining at strong intensity (for the 10x field).



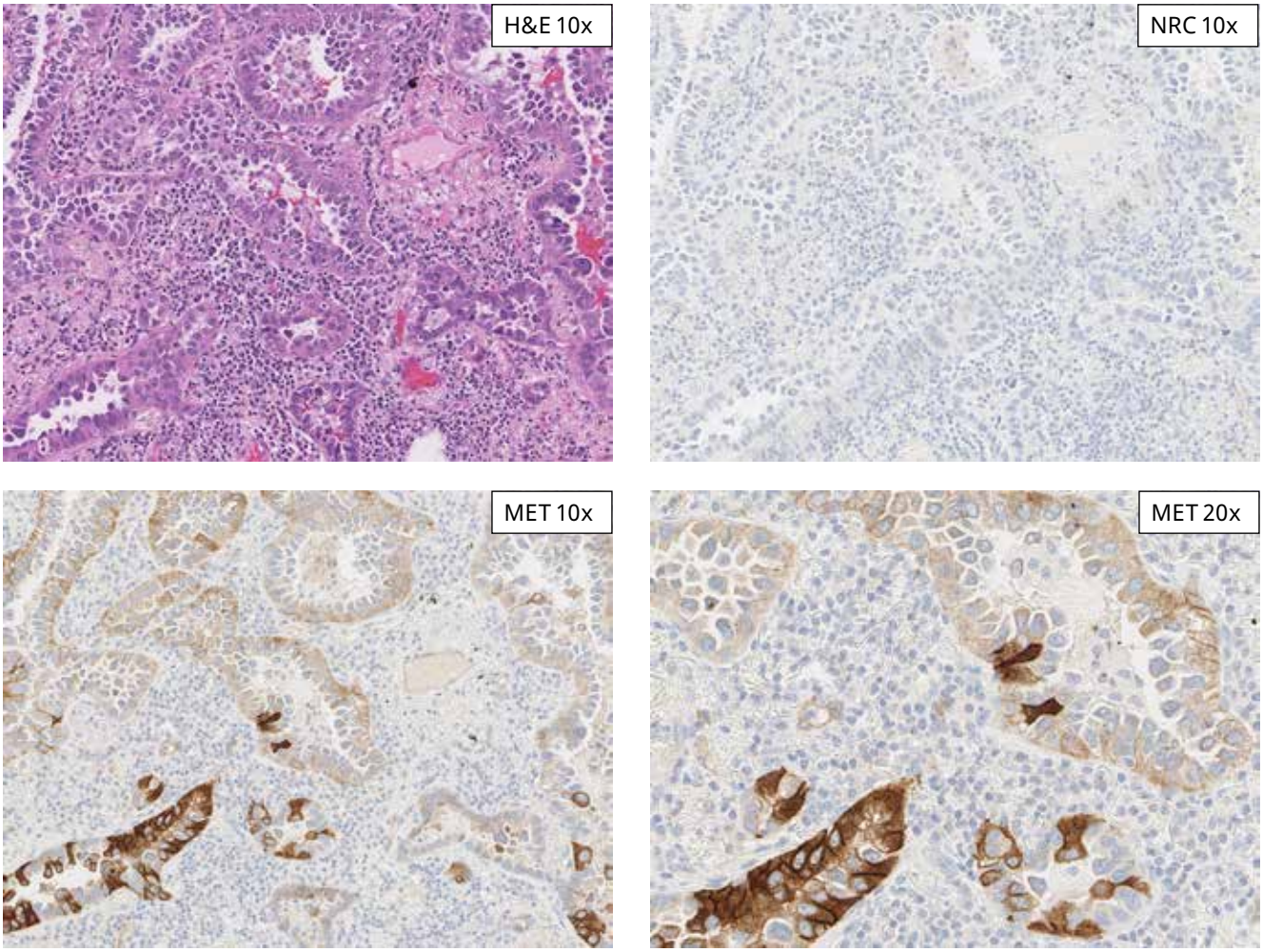
Positive Case Photoset 12: This case exhibits primarily strong membranous intensity MET staining and moderate to strong cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 100% staining at strong intensity (for the 10x field).



Positive Case Photoset 13: This case exhibits primarily strong membranous and/or cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 100% staining at strong intensity (for the 10x field).

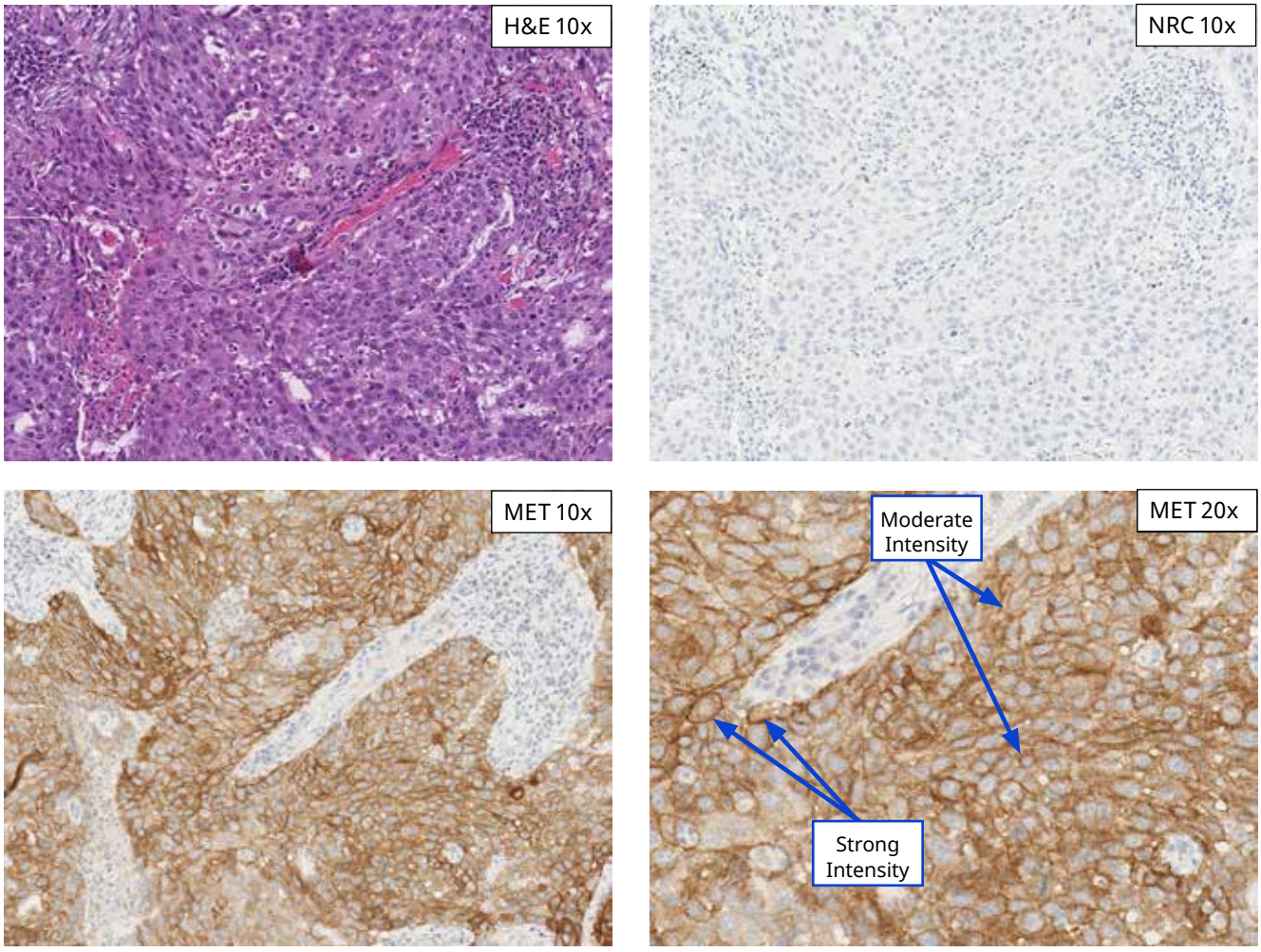
Challenging Cases

Heterogeneity

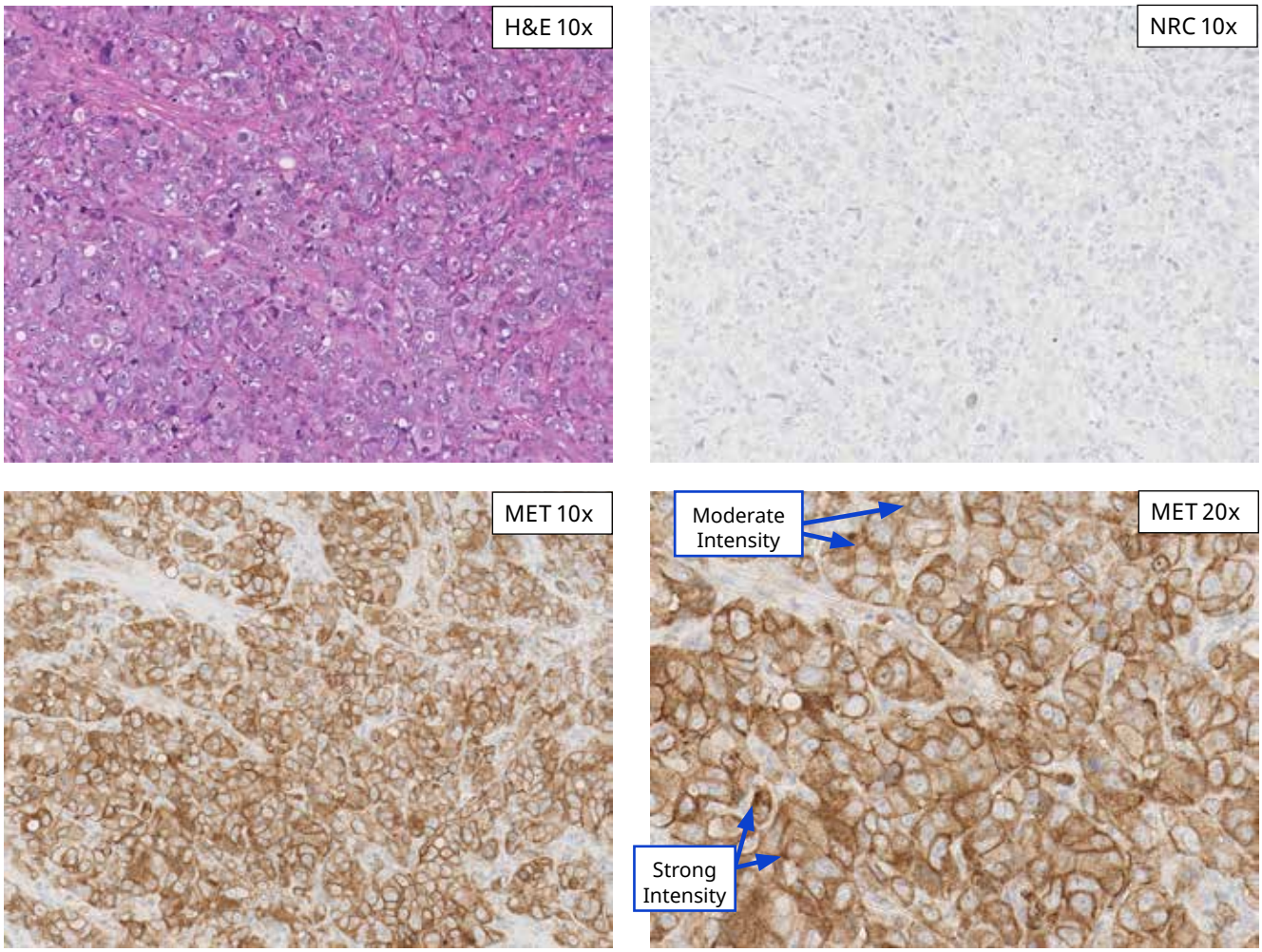


Challenging Case Photoset 1: This case exhibits heterogeneous intensity MET staining in tumor cells. A portion of the tumor cells in this sample exhibit strong membranous and cytoplasmic MET staining (20%) (for the 10x field).

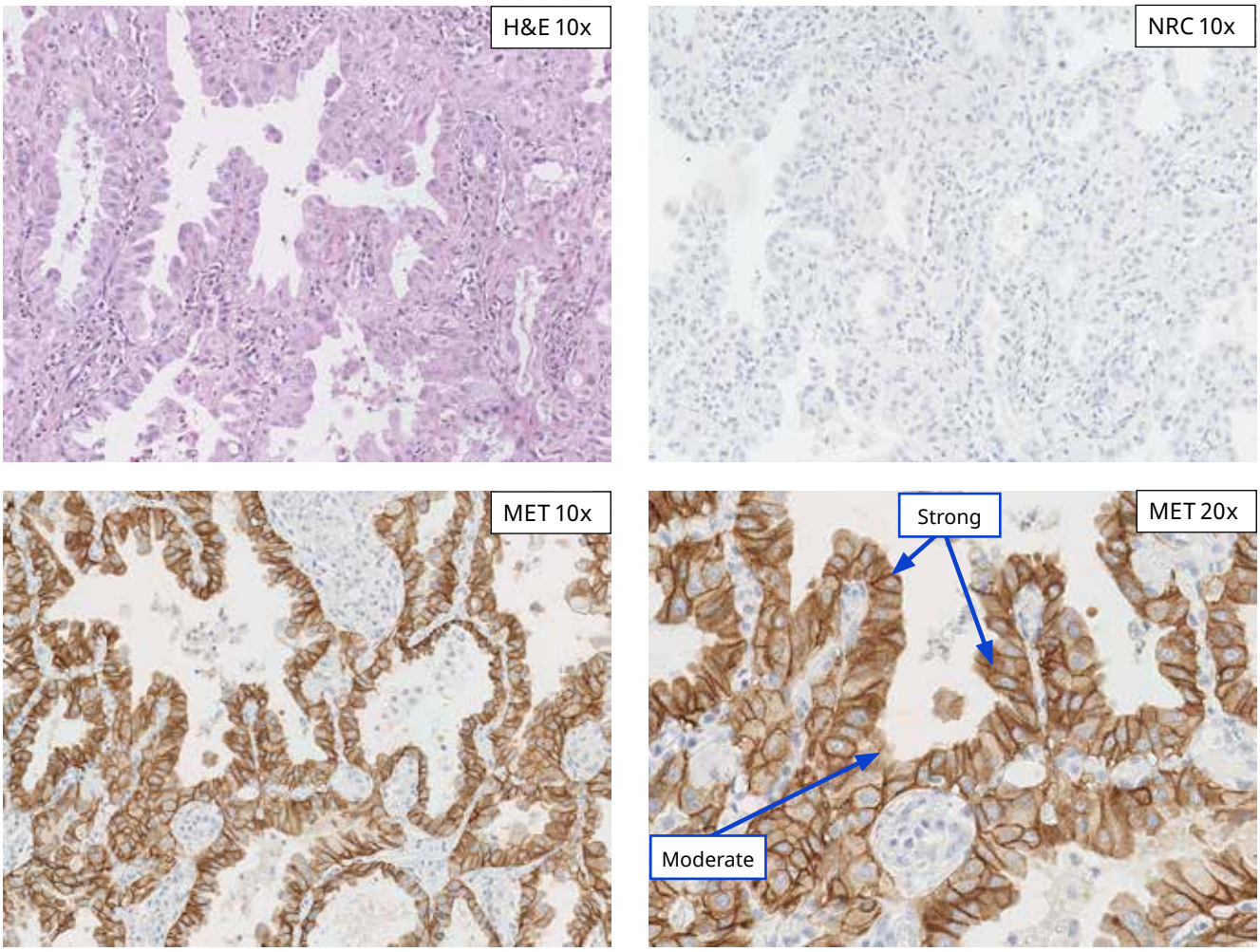
Borderline Intensity



Challenging Case Photoset 2: This case exhibits both moderate and strong membranous MET staining in tumor cells. The tumor cells on the left side of the image highlighted by the arrows demonstrate strong membranous staining that is at the lower end of strong intensity. The tumor cells predominately exhibit weak cytoplasmic staining. This sample would be given a score of 30% staining at strong intensity (for the 10x field).

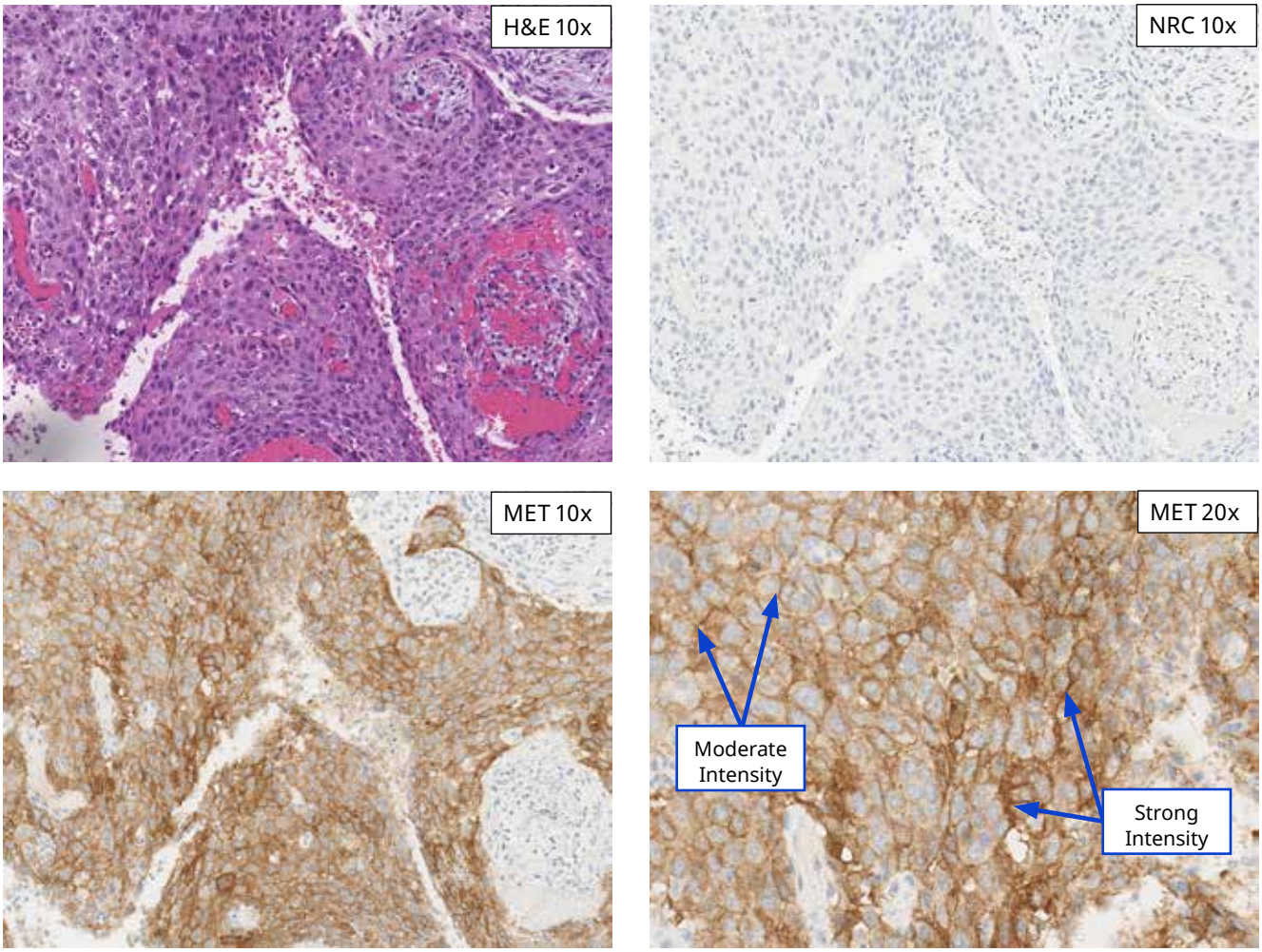


Challenging Case Photoset 3: This case exhibits both moderate and strong MET membranous staining, with many of the tumor cells exhibiting DAB signal near the threshold of strong intensity. The tumor cells exhibit predominately weak to moderate cytoplasmic staining. In this example, 60% of the tumor cells exhibit strong intensity staining (for the 10x field).

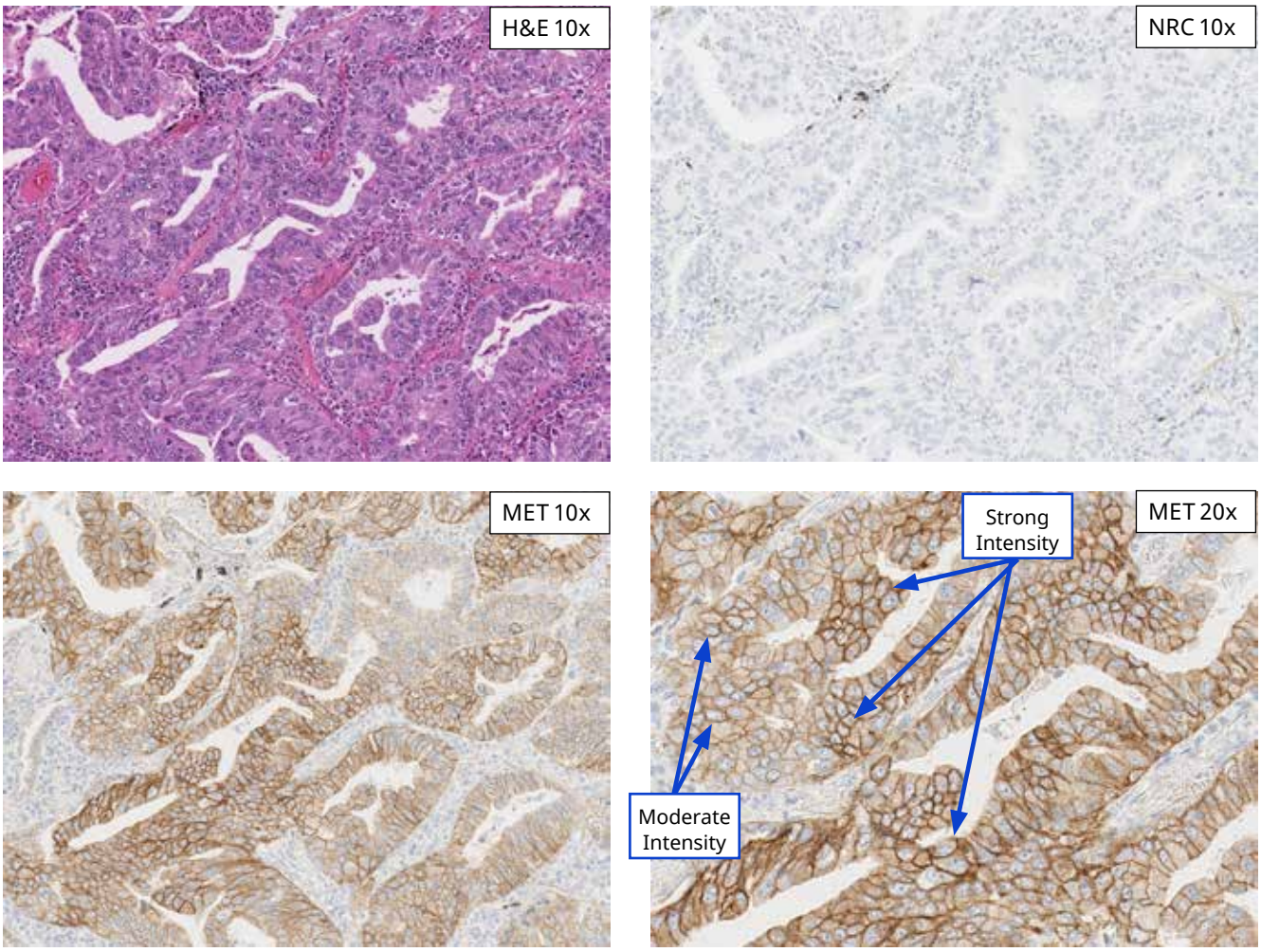


Challenging Case Photoset 4: This case exhibits predominately strong membranous MET staining and weak cytoplasmic MET staining in tumor cells. Some of the tumor cells exhibit membranous staining near the lower threshold for strong intensity. Tumor cells must exhibit dark brown to black hue to be interpreted as strong. This case would be given a score of 90% staining at strong intensity (for the 10x field).

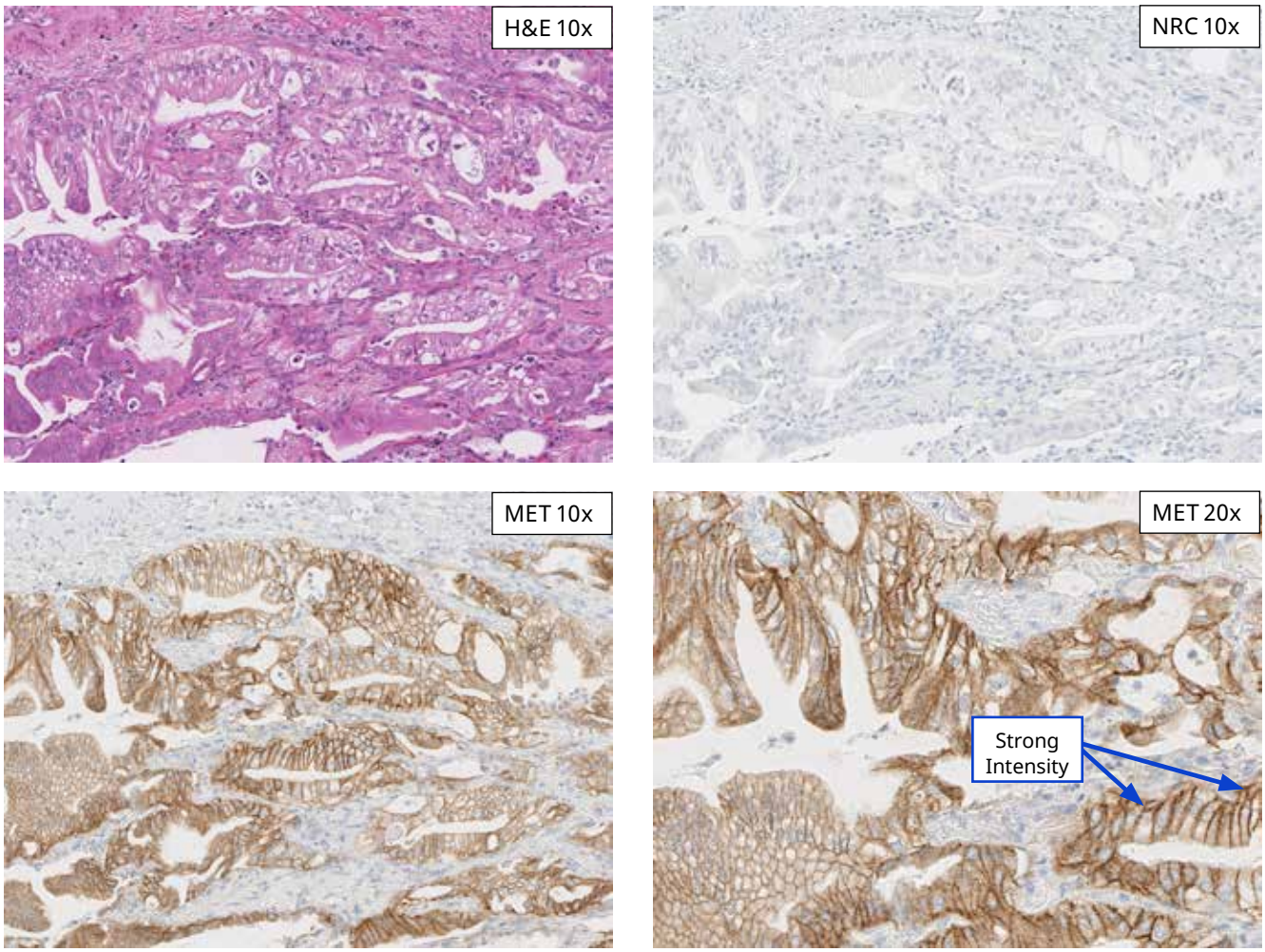
Borderline % Strong Tumor Cell Staining



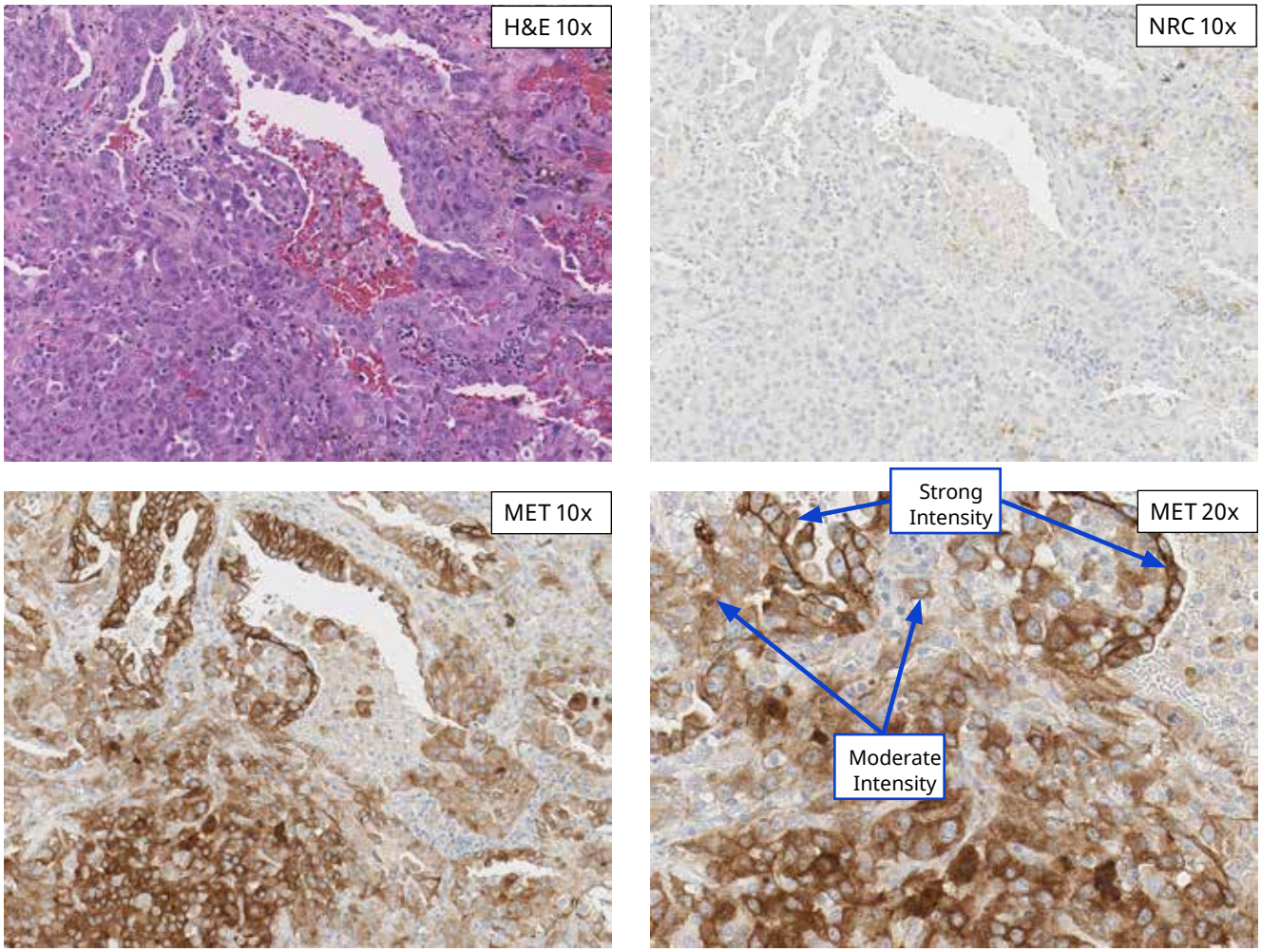
Challenging Case Photoset 5: This case exhibits both moderate and strong MET membranous staining. Most tumor cells show weak cytoplasmic staining, with focal areas of moderate to strong cytoplasmic staining. In this example, 40% of tumor cells exhibit strong intensity staining (for the 10x field).



Challenging Case Photoset 6: This case exhibits heterogeneous intensity MET staining in tumor cells. The arrows highlight tumor cells with moderate and strong membranous staining. The tumor cells demonstrate weak cytoplasmic staining. A portion of the tumor cells in this sample would be classified as strong intensity staining (40%) (for the 10x field).

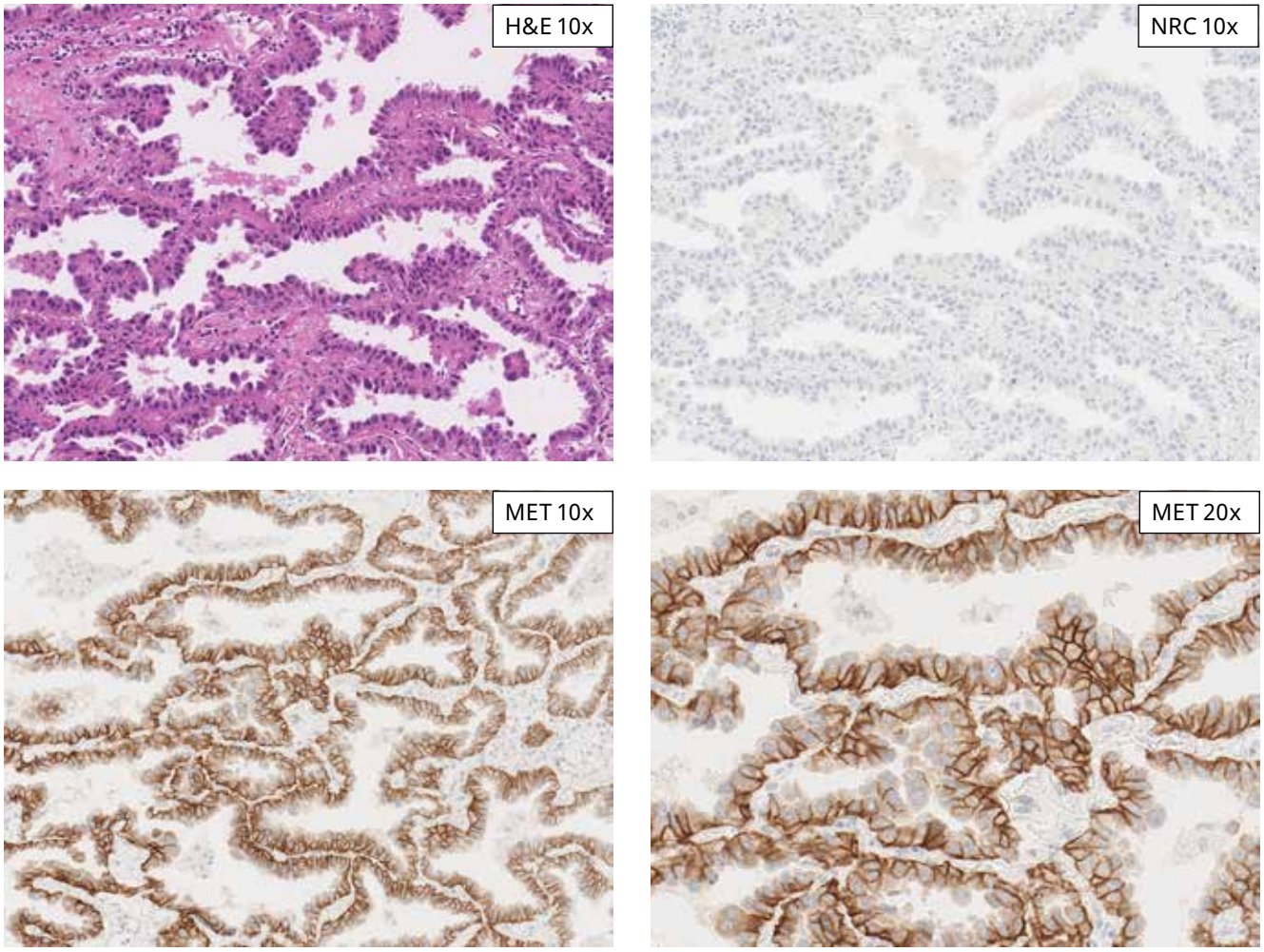


Challenging Case Photoset 7: This case exhibits heterogeneous intensity MET staining in tumor cells, with weak to moderate cytoplasmic staining. Greater than 50% of the tumor cells in the sample demonstrate strong membranous staining. This sample would be given a score of 60% staining at strong intensity (for the 10x field).

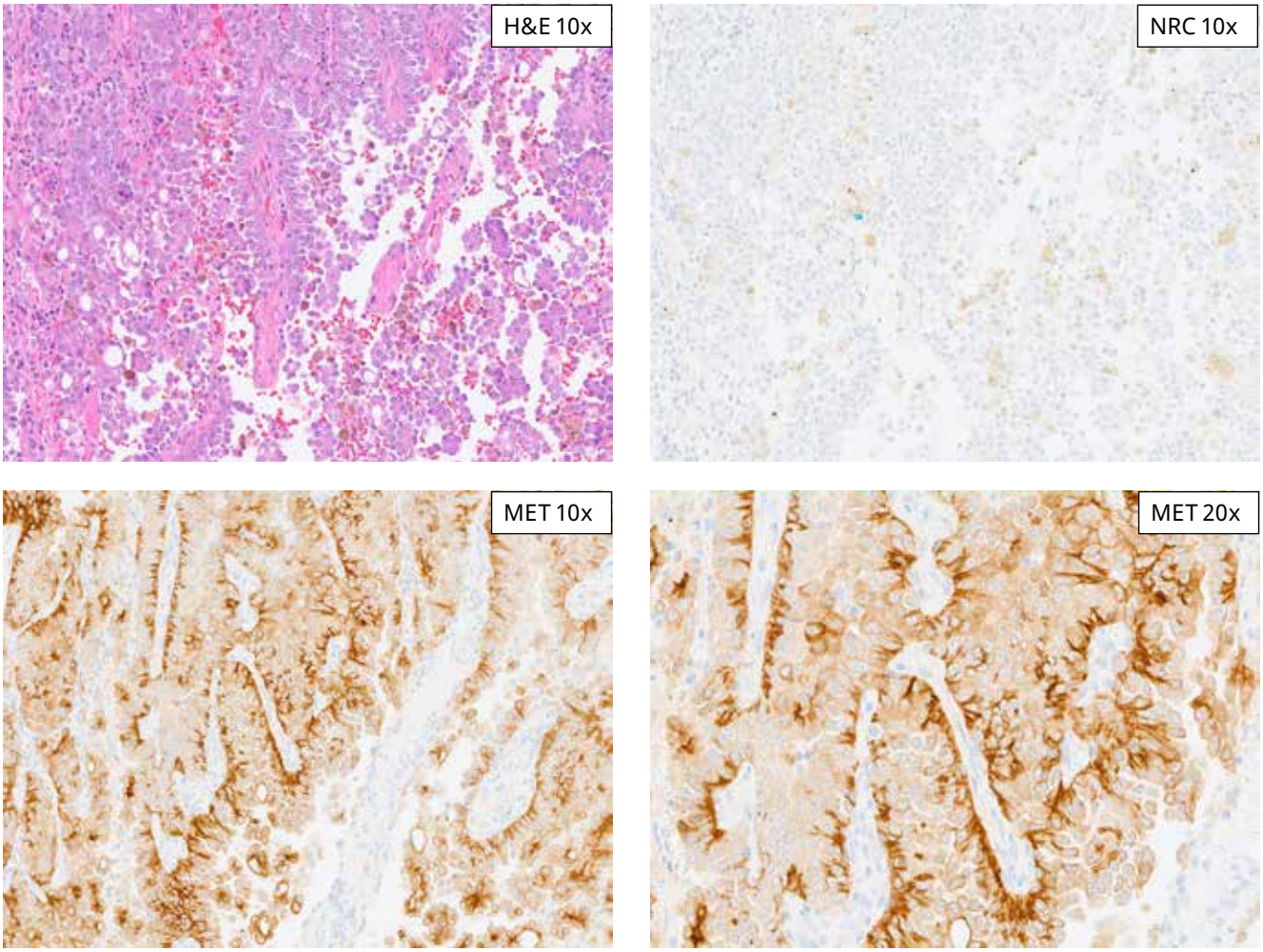


Challenging Case Photoset 8: This case exhibits heterogeneous intensity MET staining in tumor cells. Greater than 50% of the tumor cells demonstrate strong membranous and/or cytoplasmic staining. The remainder of the tumor cells exhibit weak to moderate membranous and/or cytoplasmic staining. A portion of the tumor cells in this sample would be classified as strong intensity staining (60%) (for the 10x field).

Basolateral Staining Pattern

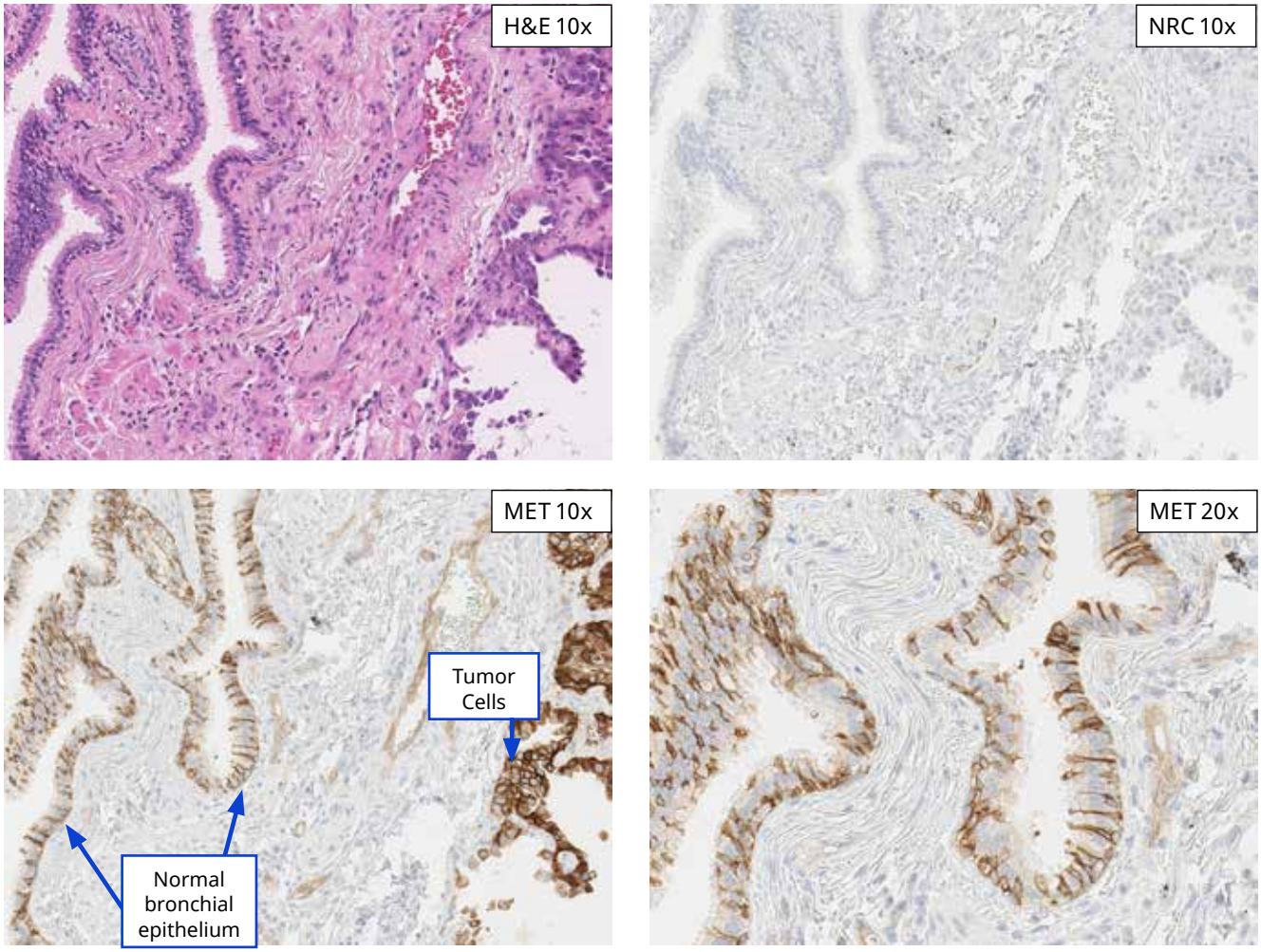


Challenging Case Photoset 9: This case exhibits a strong basolateral membranous staining pattern. Any type of MET-specific strong membrane counts towards the % strong staining estimation. The tumor cells exhibit predominately weak cytoplasmic staining. This sample would be given a score of 95% staining at strong intensity (for the 10x field).

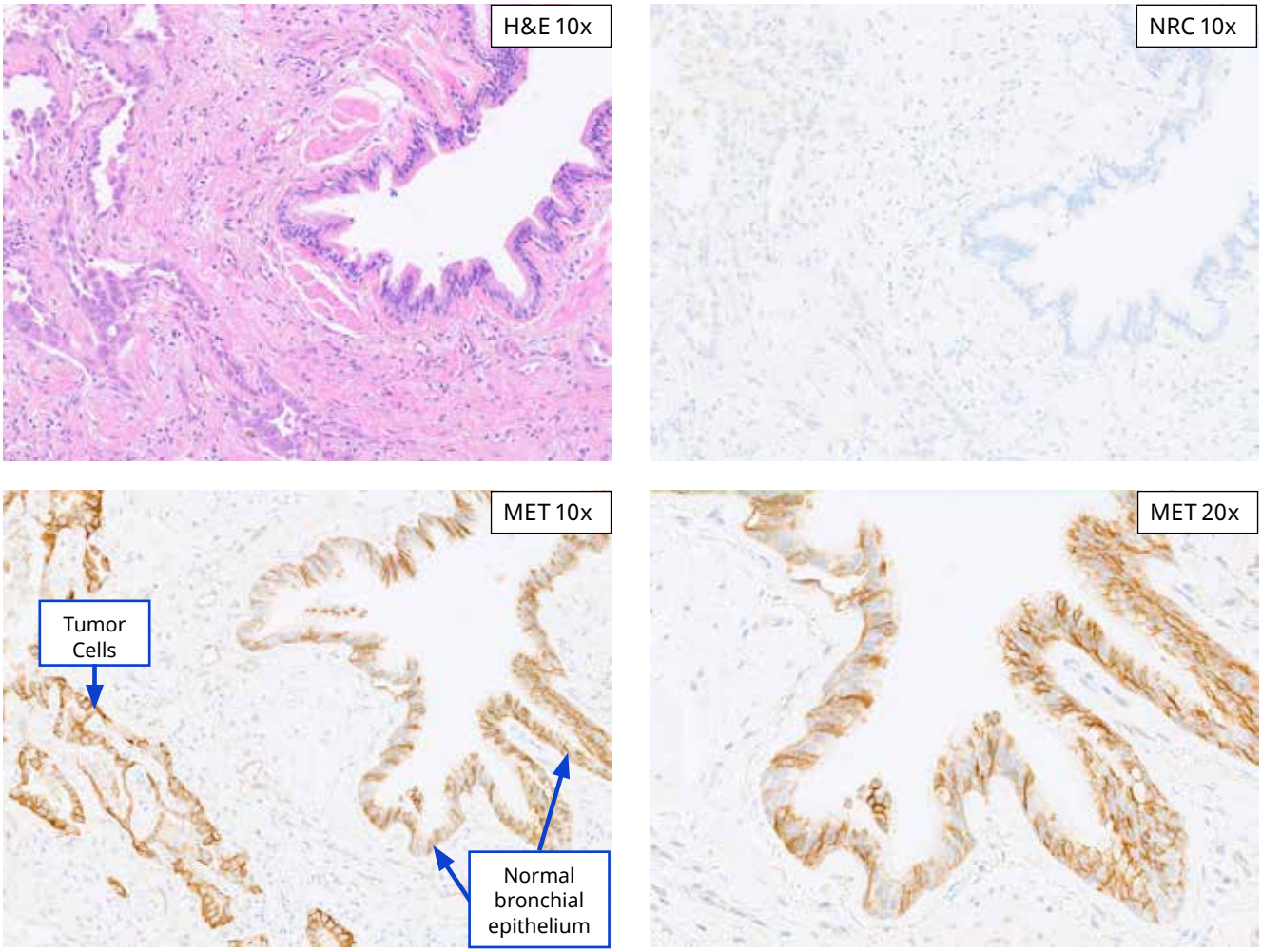


Challenging Case Photoset 10: This case exhibits a strong basolateral membranous staining pattern. Examination at higher magnification for this type of staining pattern is important as the strong staining may not be as apparent at lower magnification. Any type of MET-specific strong membrane counts towards the % strong staining estimation. The tumor cells exhibit predominately weak cytoplasmic staining. This sample would be given a score of 70% of the tumor cells staining at strong intensity (for the 10x field).

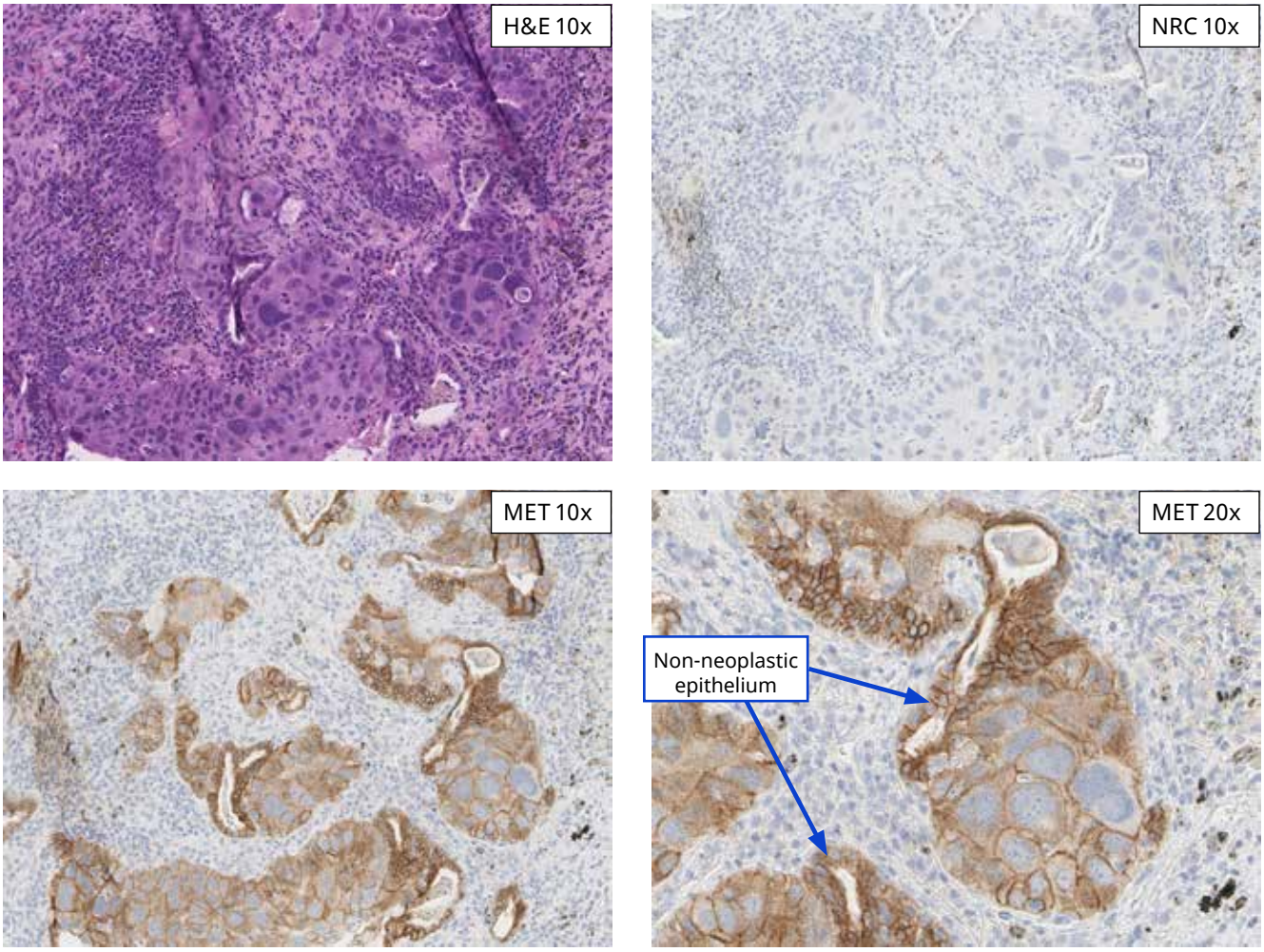
Non-Neoplastic Cell Staining



Challenging Case Photoset 11: This case exhibits moderate to strong staining of non-neoplastic epithelial cells of the lung adjacent to specifically-stained tumor cells. Non-neoplastic epithelial cells should not be counted towards % strong staining estimation (10x and 20x).

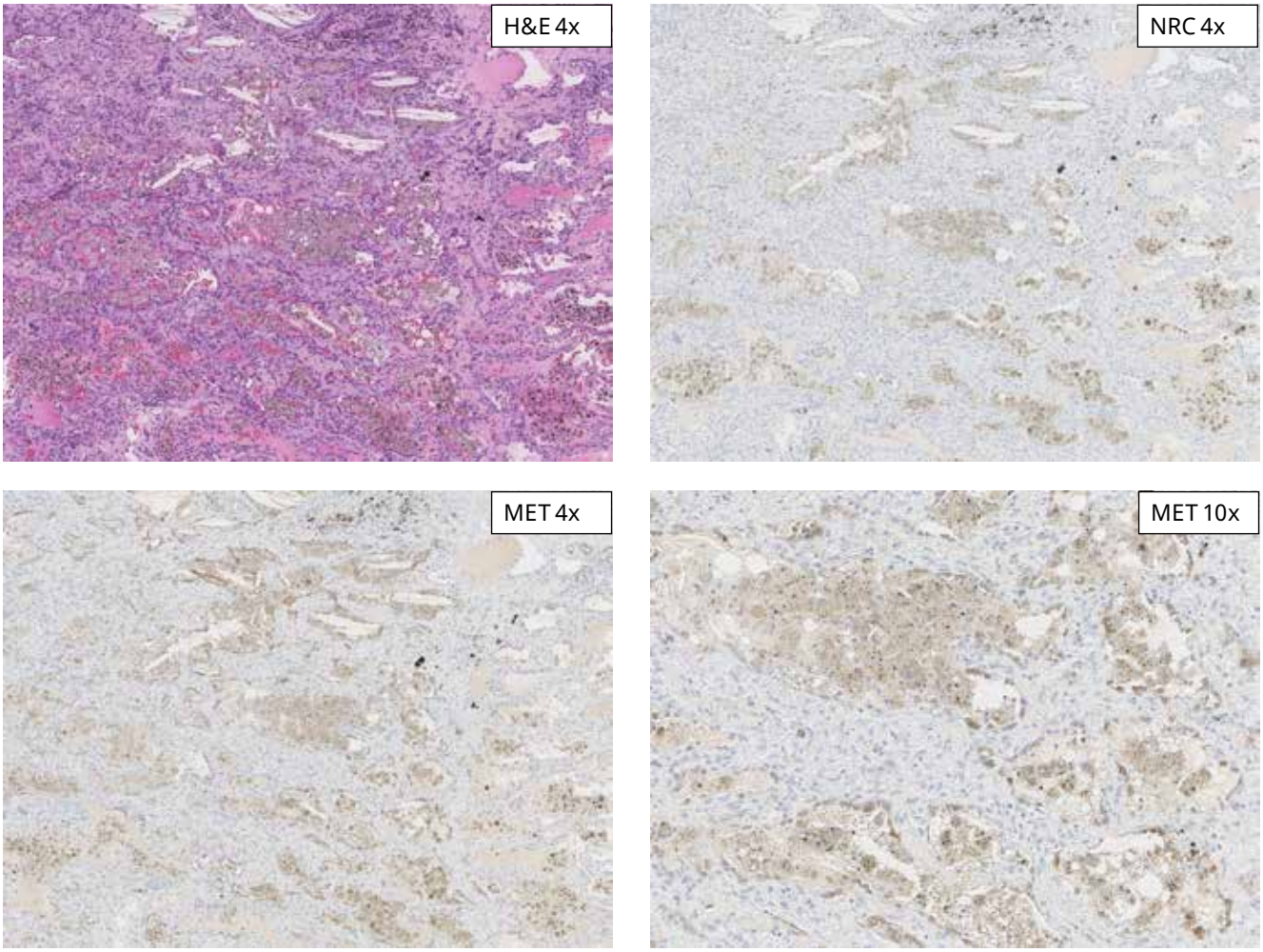


Challenging Case Photoset 12: This case exhibits moderate to strong staining of non-neoplastic epithelial cells of the lung adjacent to specifically-stained tumor cells. Normal bronchial epithelium can stain similarly to tumor cells and should not be counted towards % strong staining estimation.

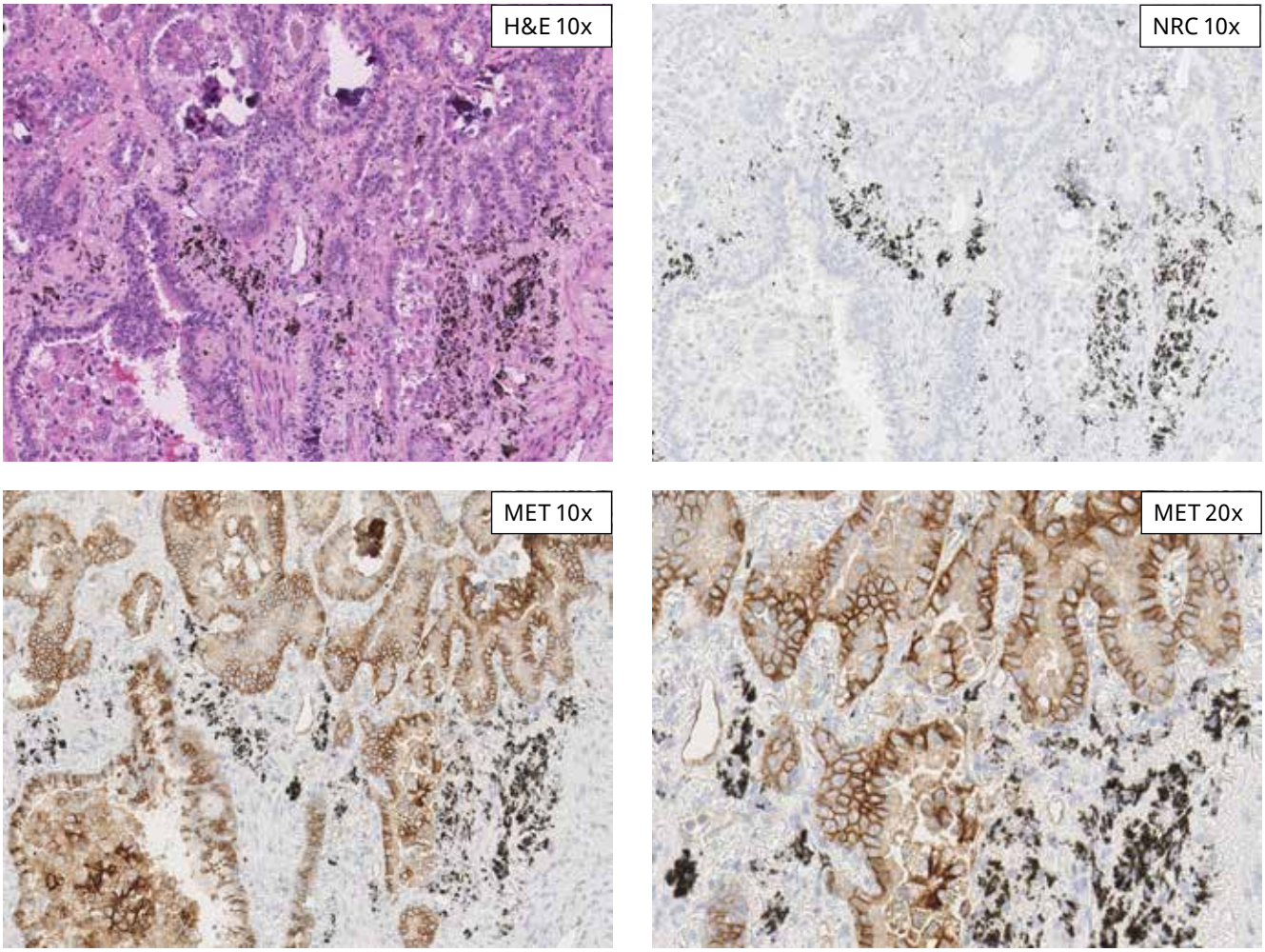


Challenging Case Photoset 13: This case exhibits staining of non-neoplastic glands adjacent to tumor. Ensure that non-neoplastic cell staining is not counted towards % strong staining estimation.

Endogenous Pigment

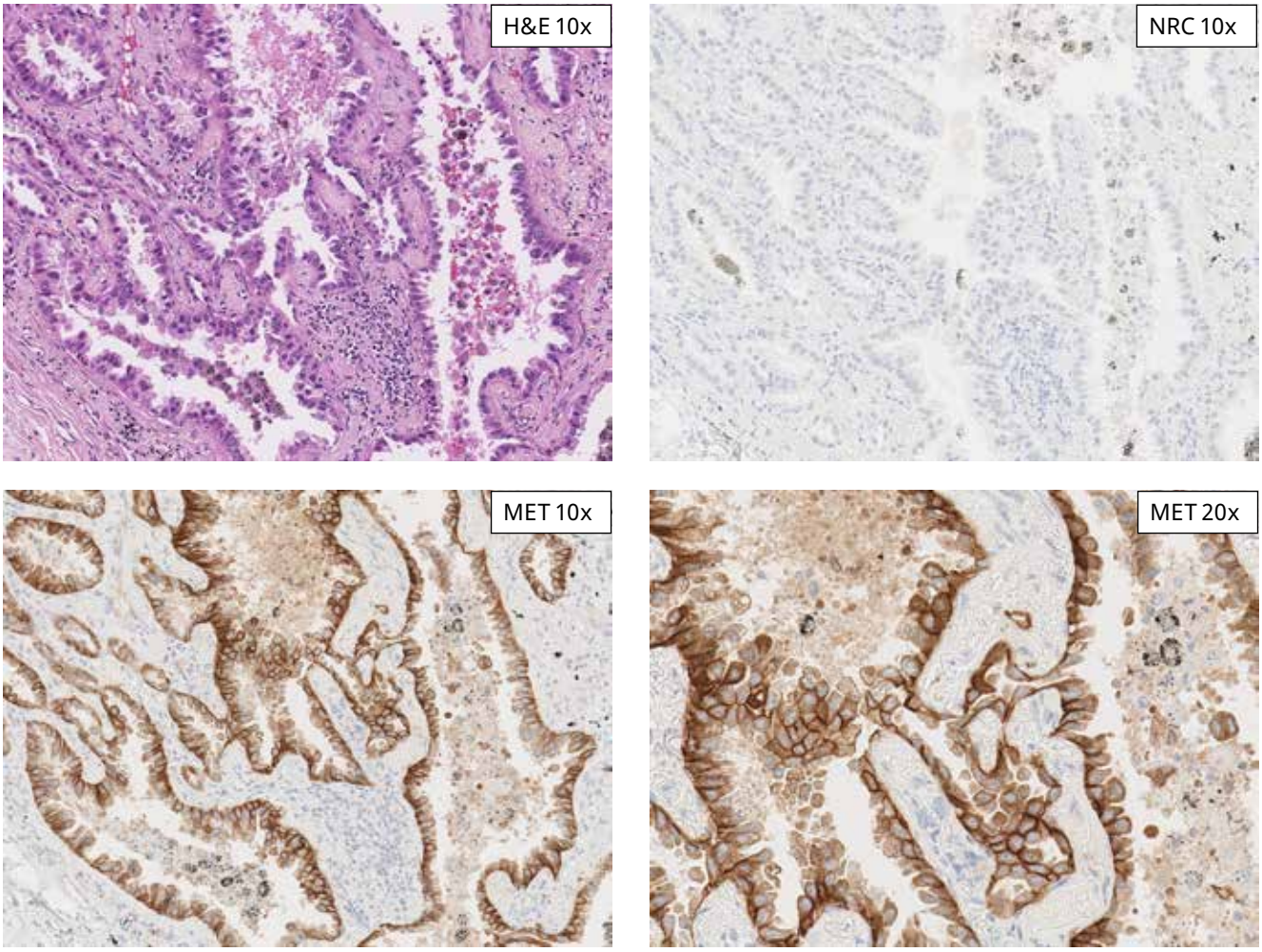


Challenging Case Photoset 14: This case exhibits some pigments that may distract the reader. Anthracosis and nonspecific serum staining can be seen in this example. These features are also seen in the NRC, which should be used to aid the reader in determining what must be excluded during % strong staining estimation.



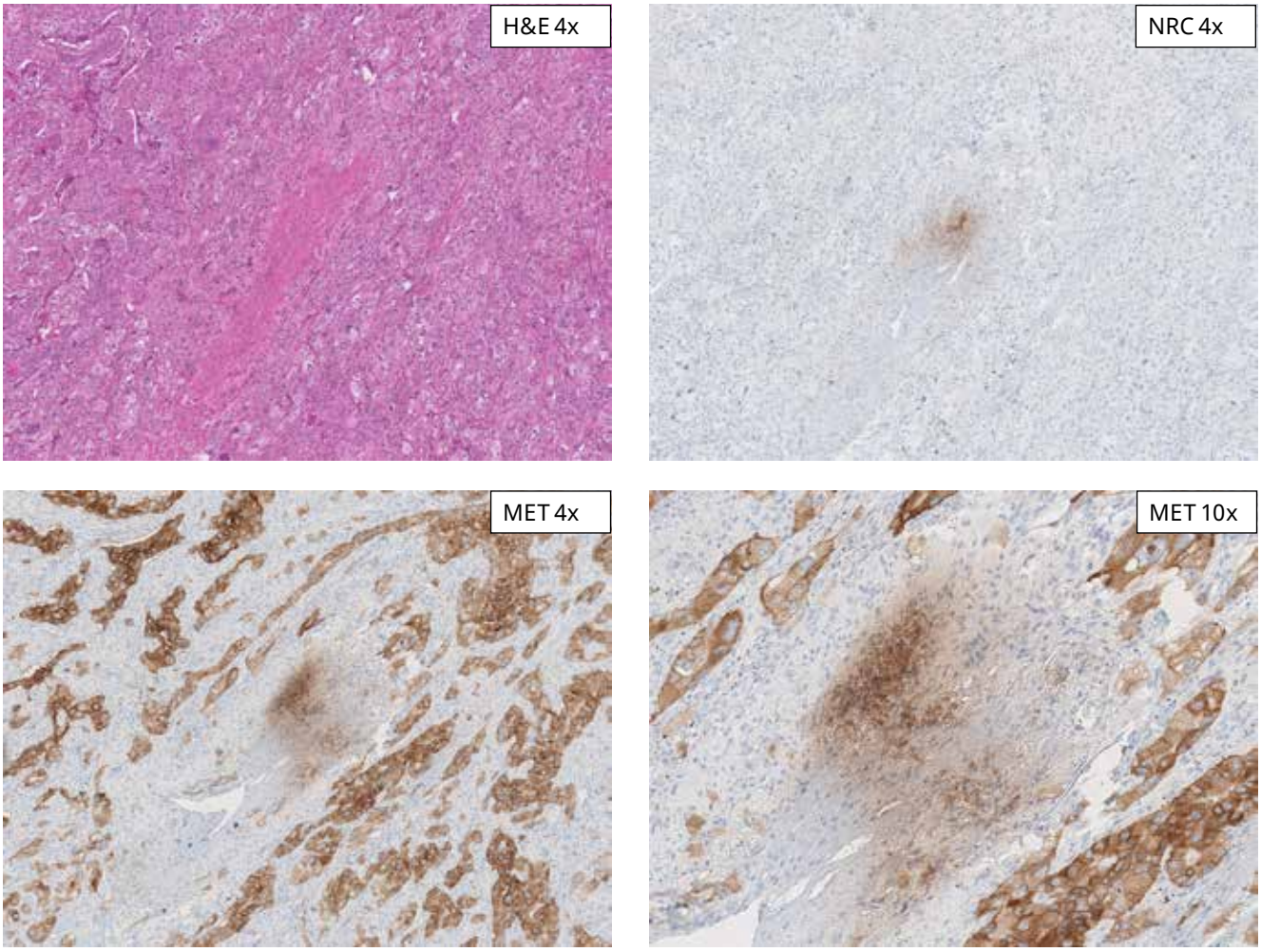
Challenging Case Photoset 15: This case exhibits anthracosis. In some circumstances, anthracosis can resemble saturated DAB signal. Using the NRC will assist the reader in excluding anthracosis from inclusion in % strong staining estimation.

Non-Specific Staining



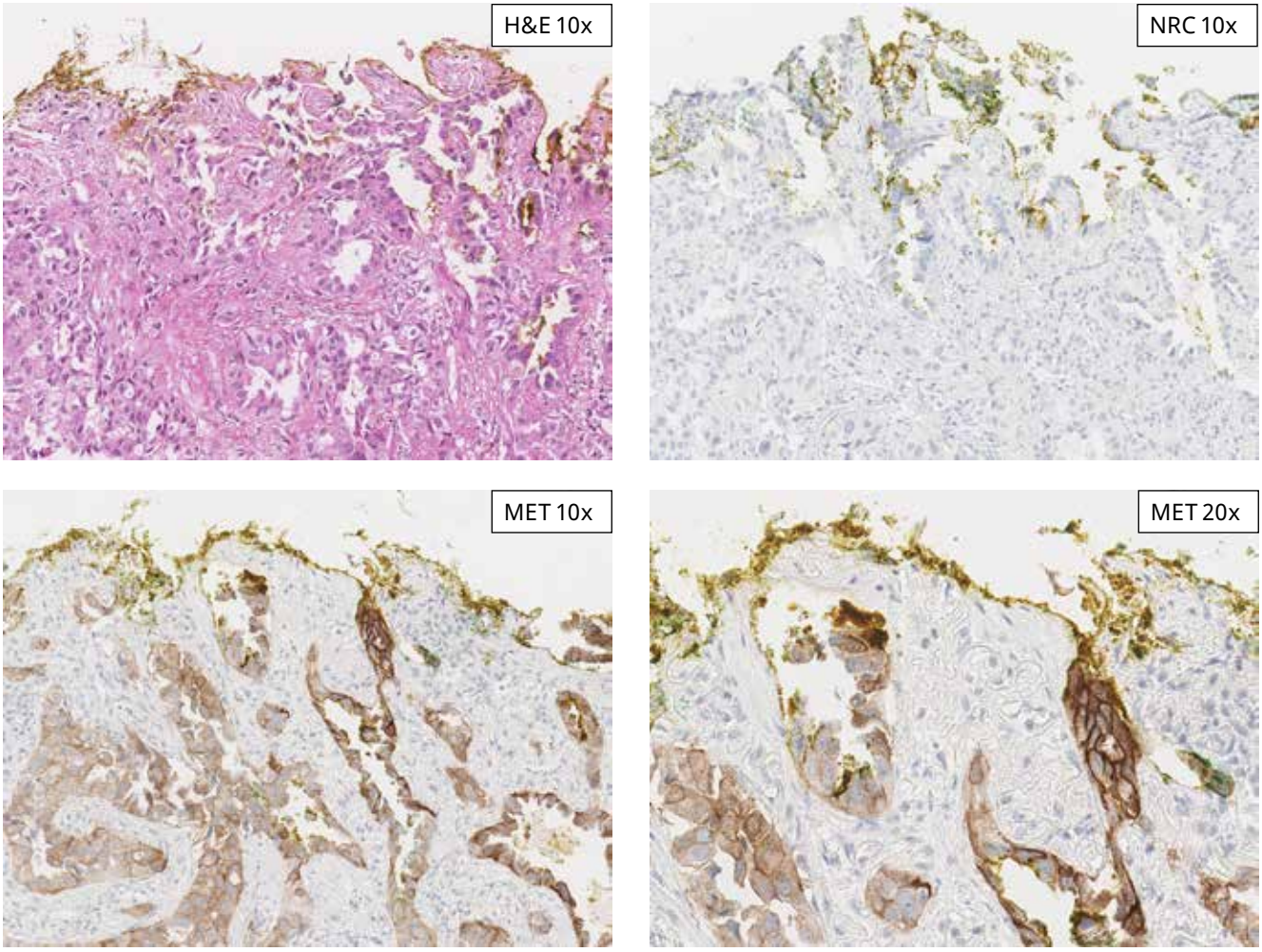
Challenging Case Photoset 16: This case exhibits strong staining of tumor cells with weak staining of luminal debris. Non-viable tumor cells must be excluded from estimation of % strong staining. Always review the corresponding NRC-stained slide to ensure that non-specific background staining is within acceptable limits.

DAB Trapping



Challenging Case Photoset 17: This case exhibits DAB trapping. DAB trapping could be mistaken for specific signal, so the reader must take care to exclude any such artifact from inclusion in % strong staining estimation. DAB trapping may be observed on NRC and VENTANA MET (SP44) RxDx Assay-stained slides. The presence of these artifacts may require repeat staining if they interfere with interpretation of VENTANA MET (SP44) RxDx Assay staining.

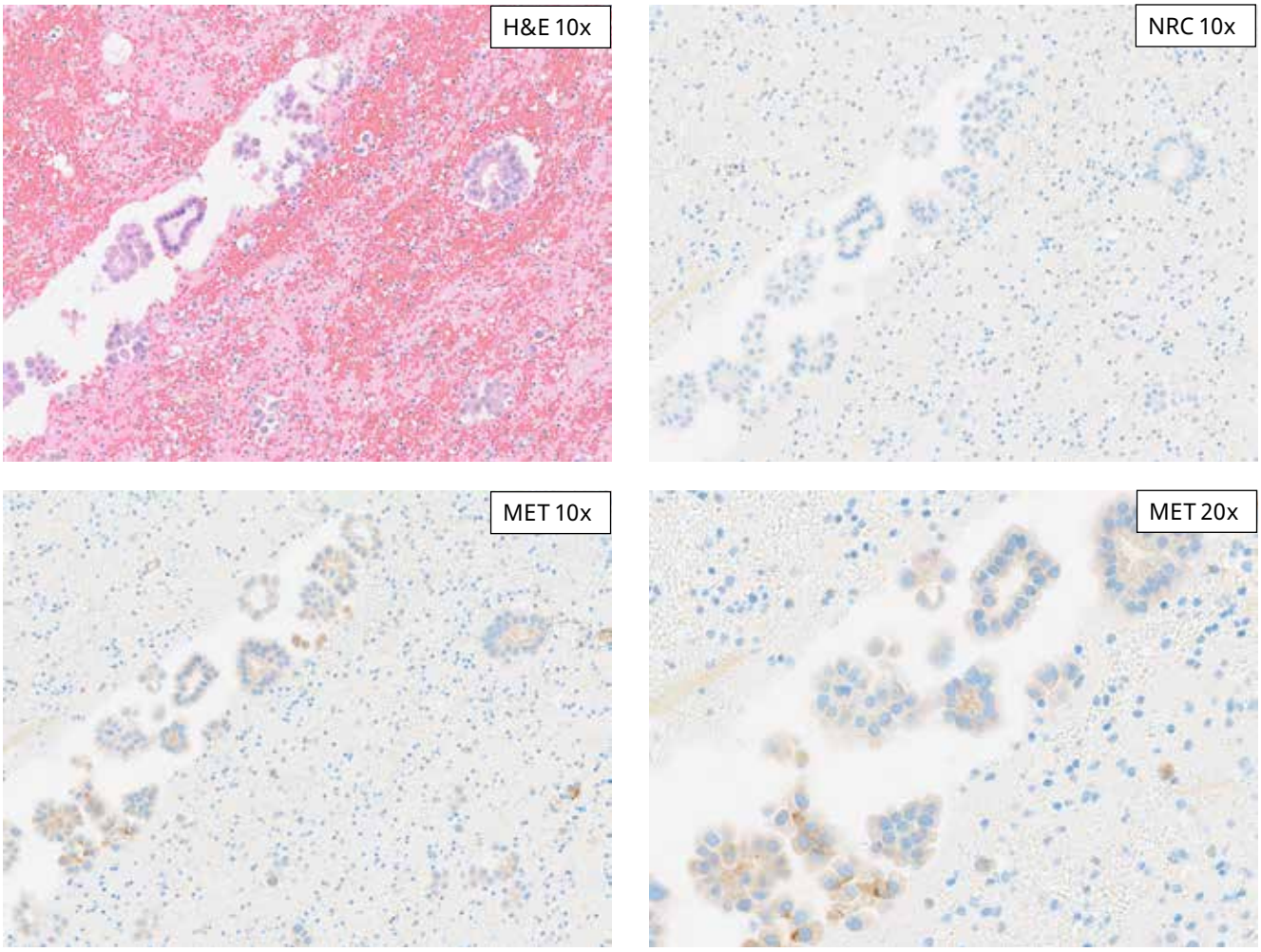
Ink on Specimen



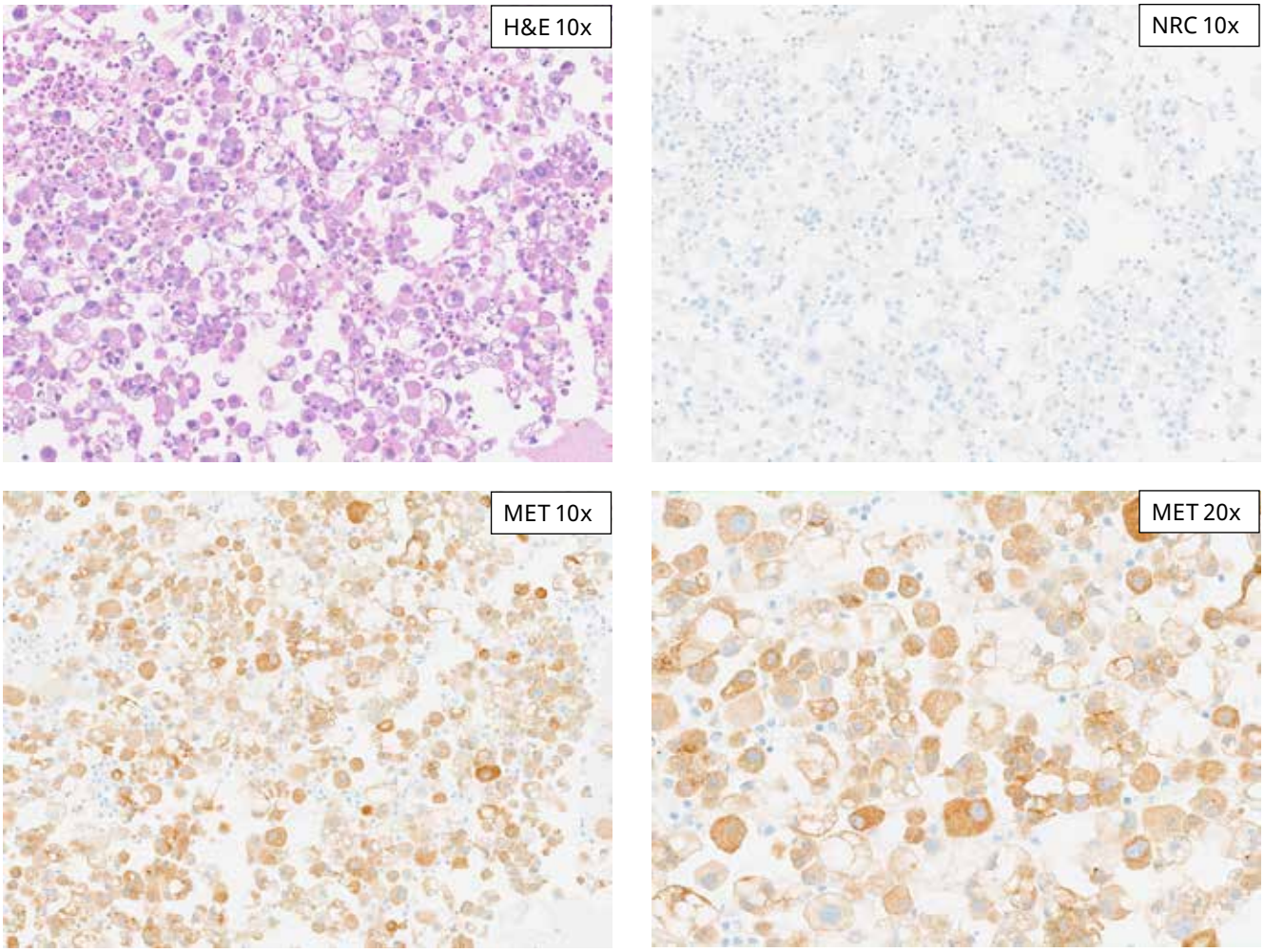
Challenging Case Photoset 18: This case exhibits ink within the sample. In some circumstances, ink may be of a hue that resembles DAB signal. Using the NRC will assist the reader in excluding surgical dye from inclusion in % strong staining estimation.

Cell Block Cases

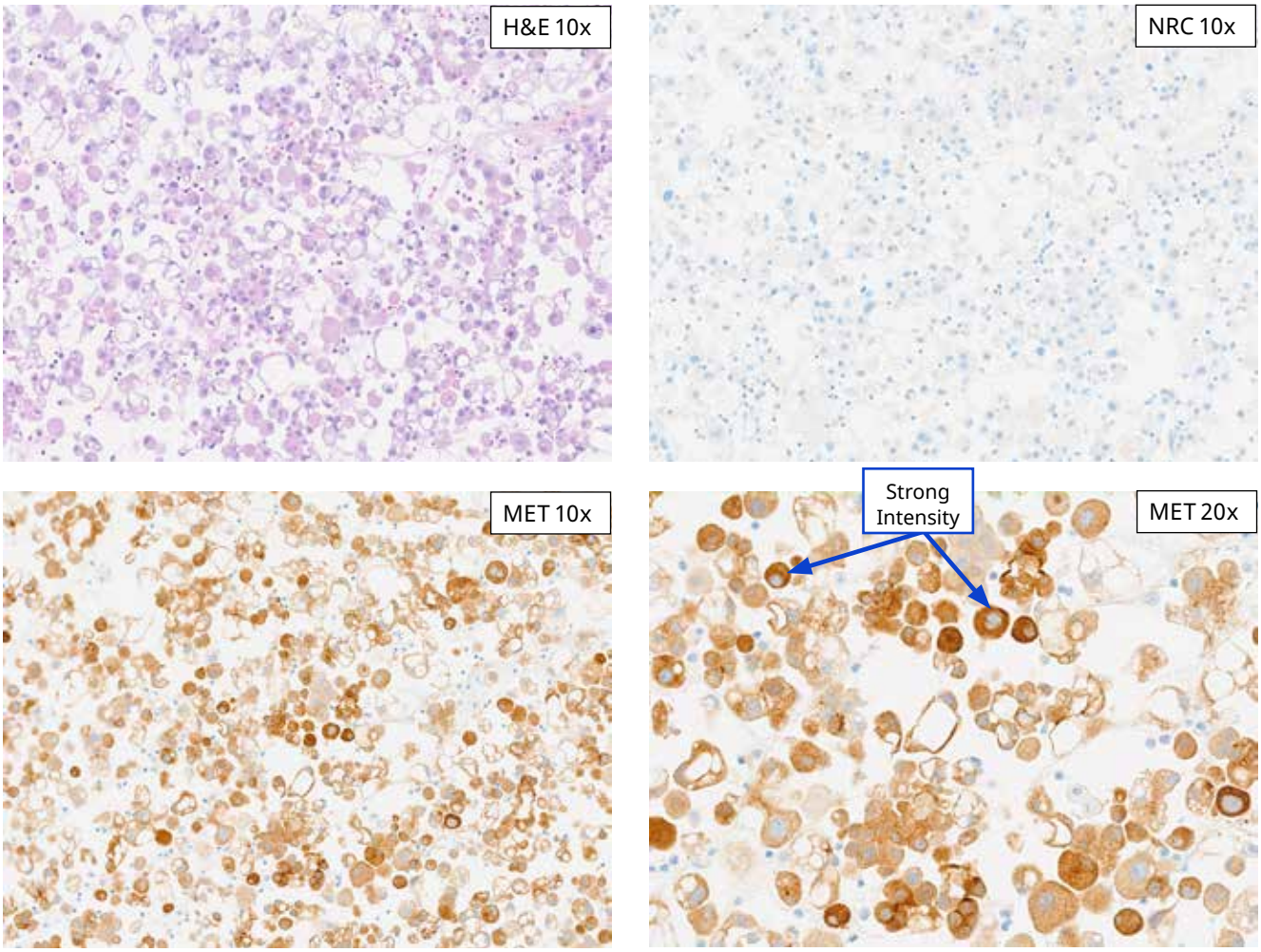
Cell block cases encompass the entire dynamic range of staining, with cytoplasmic staining frequently being more prominent. A cell block case is assigned a MET status based on the percent of tumor cells exhibiting strong membranous and/or cytoplasmic staining.



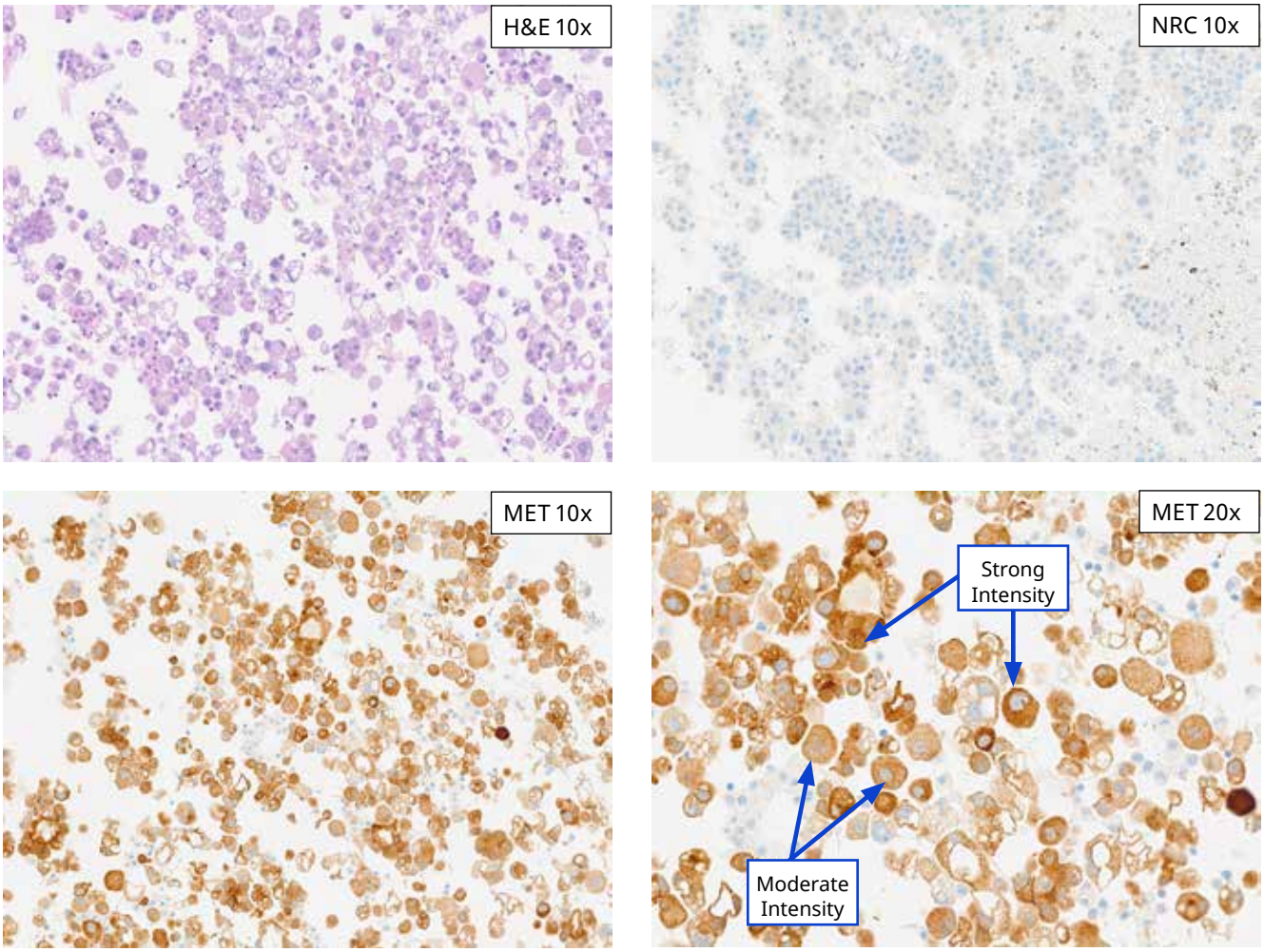
Cell Block Cases Photoset 1: This case exhibits primarily weak cytoplasmic intensity MET staining in tumor cells. This sample would be given a score of 0% staining at strong intensity (for the 10x field).



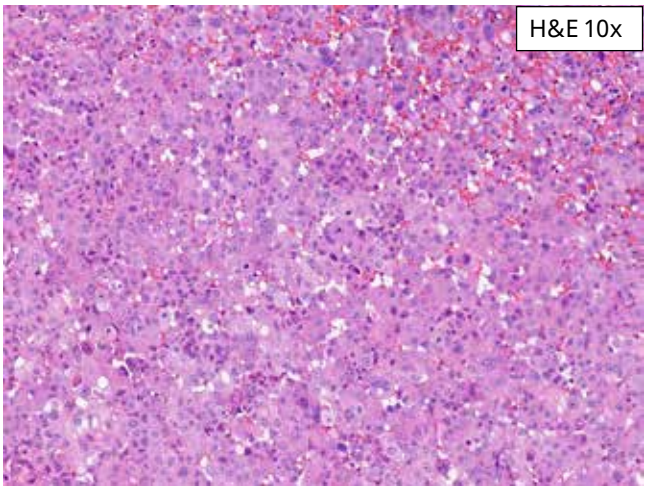
Cell Block Cases Photoset 2: This case exhibits primarily weak cytoplasmic MET staining in tumor cells. This sample would be given a score of <1% staining at strong intensity (for the 10x field).

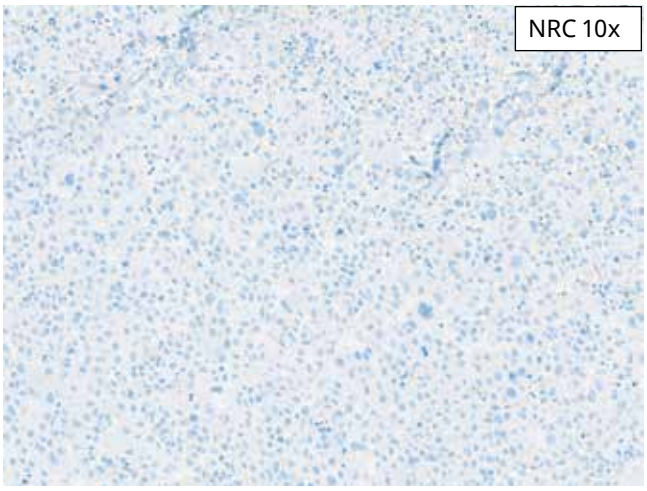


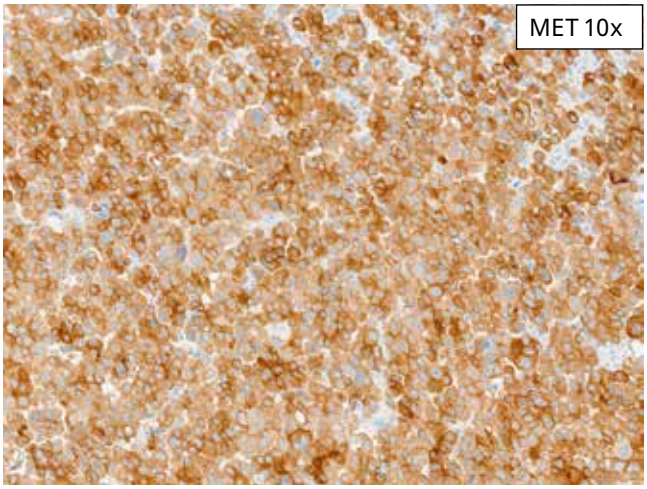
Cell Block Cases Photoset 3: This case exhibits primarily weak to moderate cytoplasmic MET staining in tumor cells with focal strong staining. This sample would be given a score of 10% staining at strong intensity (for the 10x field).

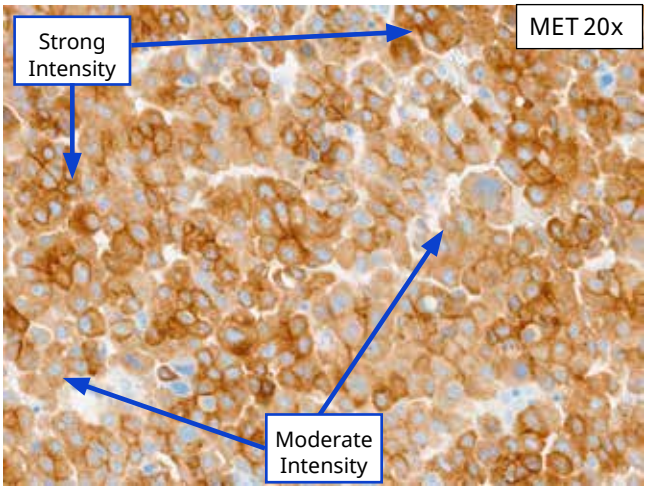


Cell Block Cases Photoset 4: This case exhibits primarily moderate cytoplasmic MET staining in tumor cells. The arrows highlight the slight differences that may be observed between moderate and strong staining. Tumor cells must exhibit dark brown to black hue to be interpreted as strong. This sample would be given a score of 30% staining at strong intensity (for the 10x field).

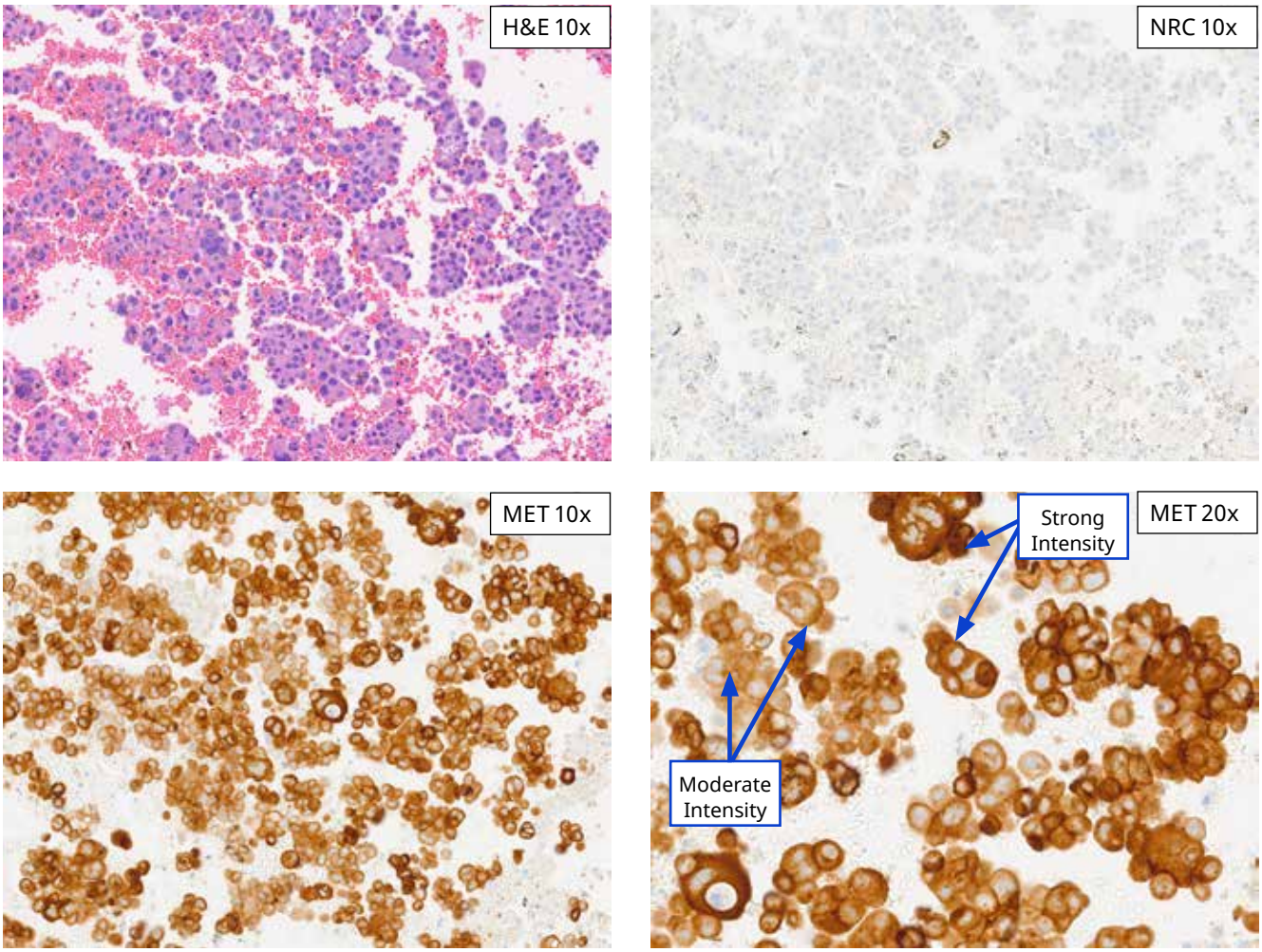




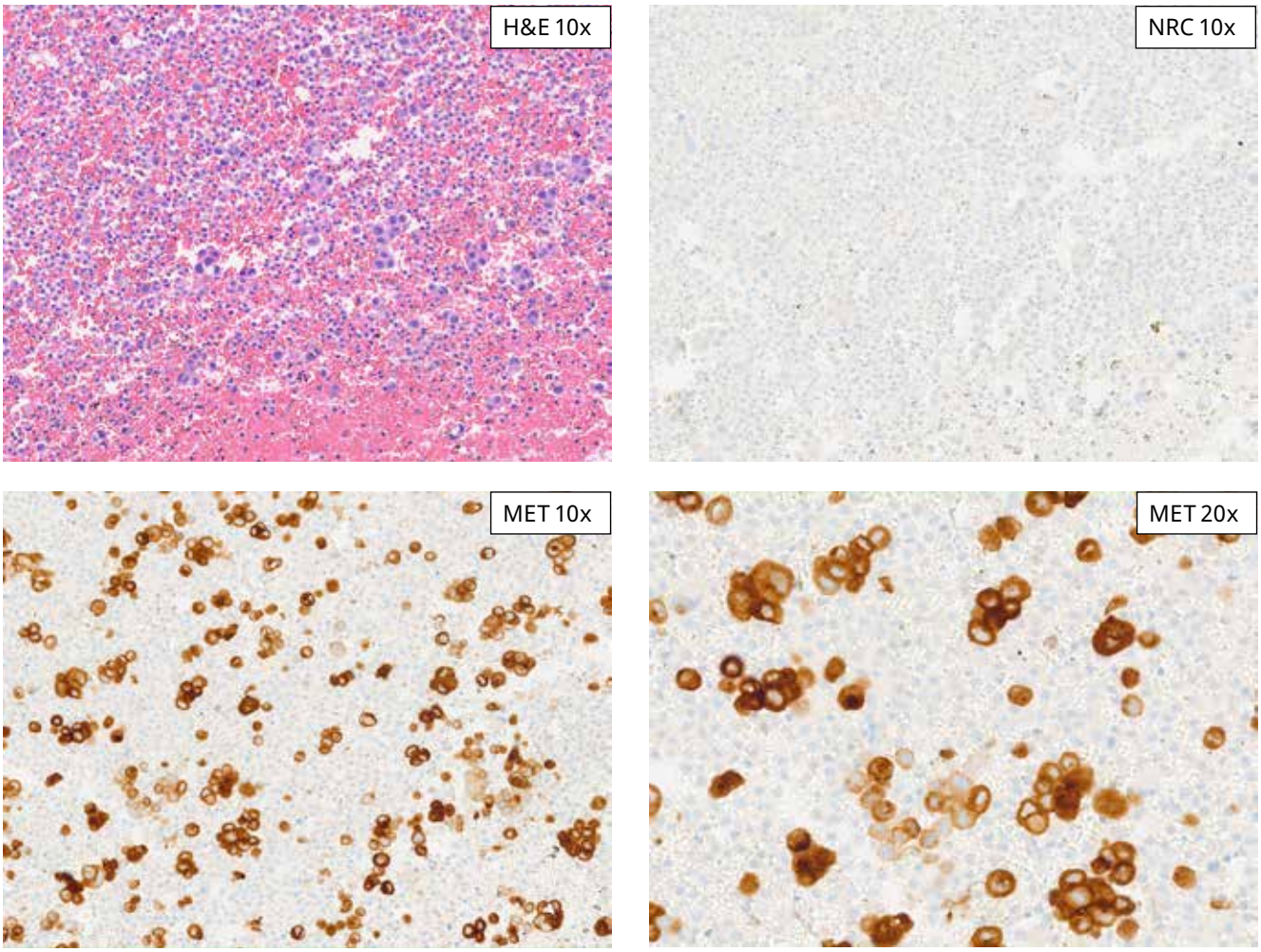




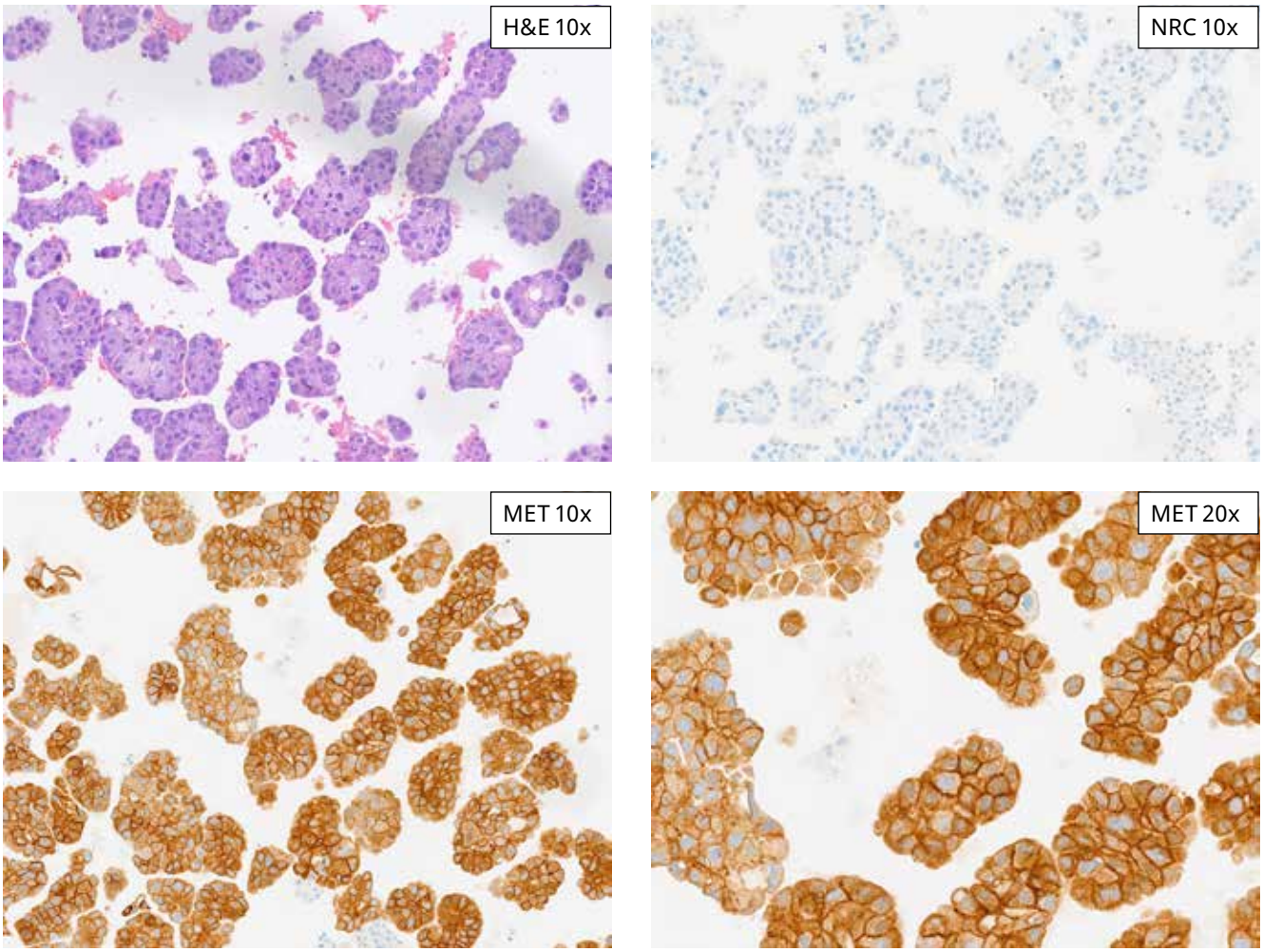
Cell Block Cases Photoset 5: This case exhibits moderate and strong membranous and cytoplasmic MET staining in tumor cells. The membrane staining present is predominately of strong intensity. This sample would be given a score of 50% staining at strong intensity (for the 10x field).



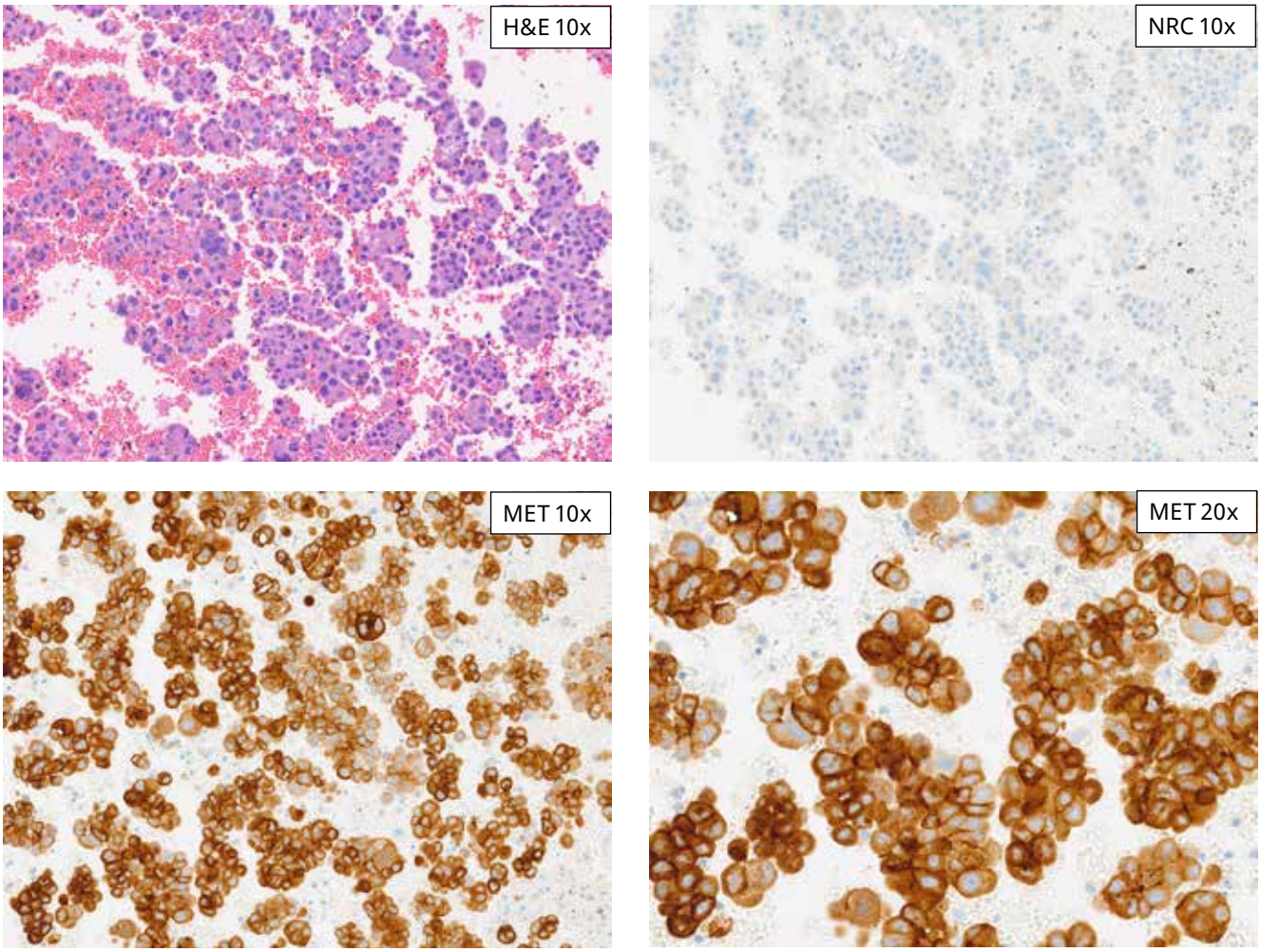
Cell Block Cases Photoset 6: This case exhibits moderate and strong cytoplasmic MET staining in tumor cells. This sample would be given a score of 60% staining at strong intensity (for the 10x field).



Cell Block Cases Photoset 7: This case exhibits predominately strong cytoplasmic MET staining in tumor cells. This sample would be given a score of 70% staining at strong intensity (for the 10x field).



Cell Block Cases Photoset 8: This case exhibits predominately strong membranous MET staining and moderate to strong cytoplasmic MET staining in tumor cells. This sample would be given a score of 80% staining at strong intensity (for the 10x field).



Cell Block Cases Photoset 9: This case exhibits predominately strong membranous and/or cytoplasmic MET staining in tumor cells. This sample would be given a score of 90% staining at strong intensity (for the 10x field).

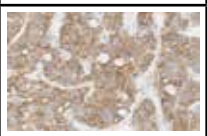
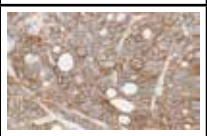
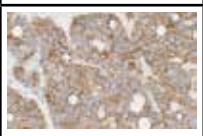
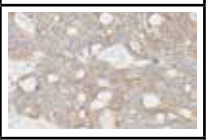
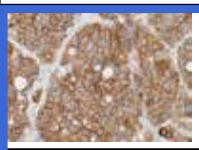
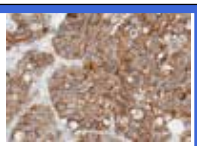
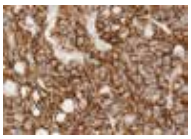
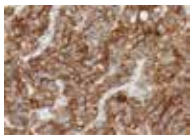
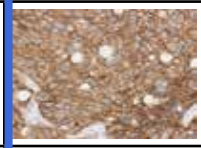
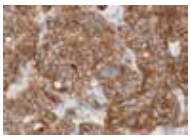
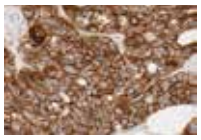
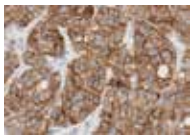
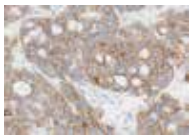
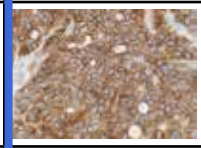
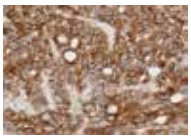
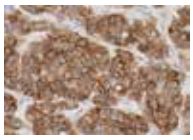
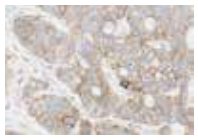
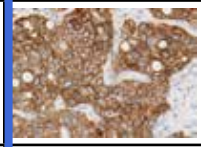
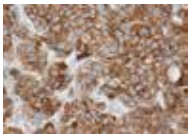
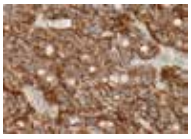
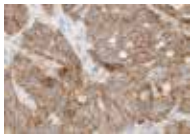
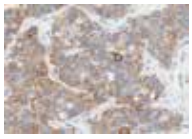
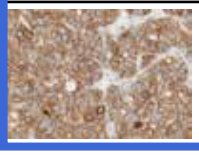
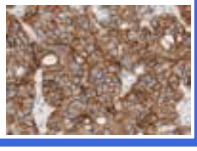
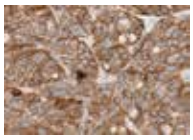
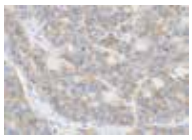
Impact of Pre-analytical Conditions on VENTANA MET (SP44) RxDx Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results

- Roche Diagnostics recommends fixation in 10% neutral buffered formalin (NBF) for 6-72 hours. See acceptable fixatives and fixation times in rectangular box below.

- Cytology specimen processing requirements for the use of cytology fixatives such as CytoLyt® and SurePath™ collection media have not been established. Cytology specimens may be collected into and rinsed in 10% NBF and cell blocks may be fixed in 10% NBF.

Table 4: Fixation Conditions

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

The following fixatives and fixation times are not recommended:

*Less than 6 hour fixation is not recommended

** Use of alcohol-formalin-acetic acid (AFA), 95% ethanol, Z-Fix and PREFER are not recommended due to variability in percent tumor cell staining and staining intensity, and, therefore, a potential for change in MET status

Impact of Tissue Thickness

The impact of tissue section thickness on the VENTANA MET (SP44) RxDx Assay was assessed using several NSCLC and gallbladder specimens. Each specimen was sectioned at 2, 3, 4, 5, 6, and 7 µm.

To reduce possible fluctuations in the percentage of strong staining tumor cells, cut sections at 4-5 µm and mount on positively charged slides for use with the VENTANA MET (SP44) RxDx Assay.

Cut Slide Stability

Roche Diagnostics has determined that the VENTANA MET (SP44) RxDx Assay should not be performed on cut slides that have been stored longer than 45 days. Store unstained cut slides at 2-8°C.

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Revision History

Rev	Updates
14-May-2025	Rev A, First Publication.

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