

VENTANA MMR IHC Panel

Interpretation Guide for Staining of Colorectal Tissue

VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody

VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody

VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody

VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody

VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody

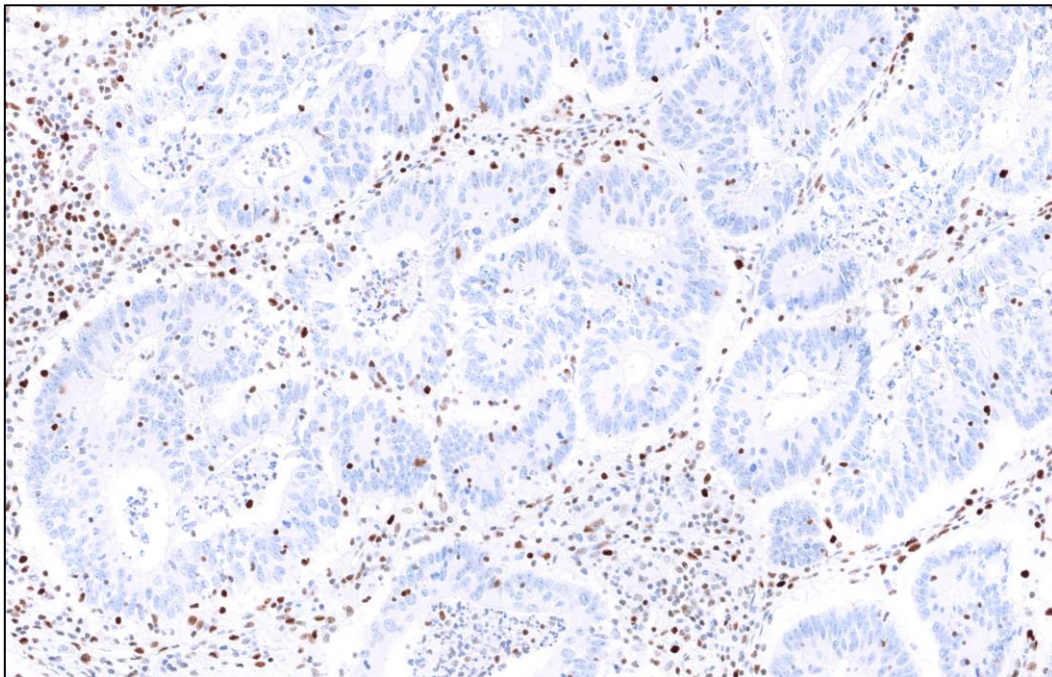


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Introduction

Colorectal Cancer and Lynch Syndrome

Colorectal cancer (CRC) is the third most common cancer in men, and the second in women, with an estimated 1.4 million new cases and 694,000 deaths occurring worldwide in 2012. Incidence rates vary widely by geographic location, with the highest estimated rates in Australia/New Zealand, Europe, and North America.¹ In the United States alone CRC represents 8.0% of all new cancer cases and an estimated 1.2 million people were living with colon and rectum cancer as of 2012.²

The risk of developing CRC is influenced by both environmental factors (e.g., dietary factors, obesity, smoking and alcohol use) and genetic factors. While the majority of CRC cases are sporadic in nature, 5-10% of cases are due to inherited autosomal dominant mutations.³ The most common subtype of hereditary CRC is Lynch syndrome, which accounts for 3% of all CRC diagnoses.⁴

Lynch syndrome (Hereditary Non-Polyposis Colon Cancer, HNPCC), was described in the 1960s and identified a link between the loss of DNA mismatch repair (MMR) function and cancer.⁵ Loss of any MMR protein (MLH1, PMS2, MSH2 or MSH6) may lead to microsatellite instability and a higher risk of not only colorectal cancer, but also cancer of stomach, brain, skin and, in women, endometrium and ovaries. Patients with Lynch syndrome have a 40-60% lifetime risk for colorectal cancer.⁶

Stratification for Lynch Syndrome in CRC

Using IHC assays for MLH1, PMS2, MSH2, and MSH6, the MMR status of the tumor may be determined. Detection of all four proteins in the tumor indicates normal or proficient mismatch repair status (pMMR). Loss of MLH1 expression is almost invariably accompanied with the loss of its heterodimer partner, PMS2. In sporadic occurrences of CRC, expression of the MLH1 gene may be suppressed by methylation of its promoter.

If the result indicates a loss of MLH1 protein, testing to see if the BRAF V600E mutation is present will stratify the tumor as sporadic or possible Lynch syndrome and indicate the need for additional testing of the MLH1 promoter methylation state. The presence of the BRAF V600E mutation with loss of MLH1 protein indicates the tumor is the result of a sporadic occurrence and makes Lynch syndrome as the underlying cause of malignancy highly unlikely.⁵ If BRAF V600E mutation and MLH1 promoter methylation is negative, the deficient DNA mismatch repair (dMMR) status is consistent with Lynch syndrome and warrants genetic testing for a confirmatory diagnosis.⁵

The use of IHC for the detection of PMS2, MSH2 and MSH6 proteins is a more direct indicator of germline mutational status. The loss of PMS2, in the presence of MLH1 expression, or loss of MSH2 or MSH6 expression designates the

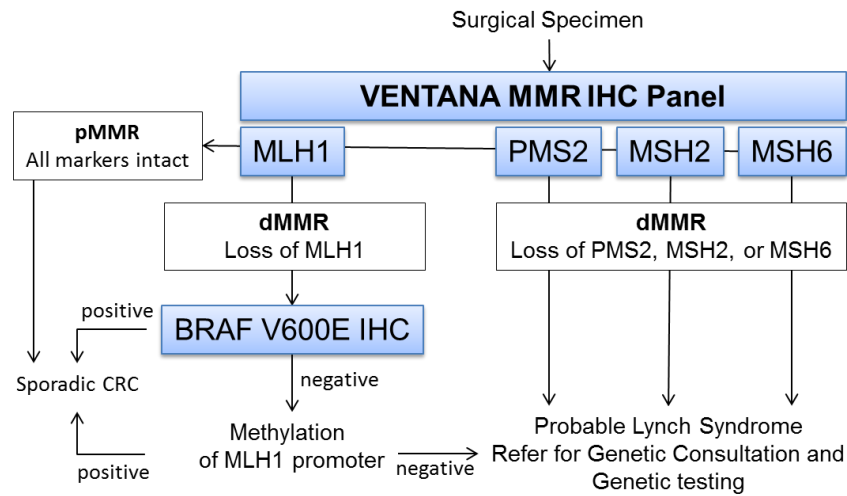
tumor as dMMR and is consistent with Lynch syndrome. All individuals with suspected Lynch syndrome would be referred for genetic counseling and further genetic testing to confirm the presence of the suspected mutation.

VENTANA MMR IHC Panel

Use of the VENTANA MMR IHC Panel to identify MMR Status and Probable Lynch Syndrome

The VENTANA MMR IHC Panel (VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody and VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody) aids in the identification of patients with a deficiency in MMR and the stratification of colorectal cancer (CRC) as sporadic or probable Lynch syndrome. This is summarized in Figure 1.

Figure 1: Use of VENTANA MMR IHC Panel



VENTANA MMR IHC Panel

Intended Use of Product

The VENTANA MMR IHC Panel is a qualitative immunohistochemistry (IHC) test intended for use in the light microscopic assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6) and BRAF V600E proteins in formalin-fixed, paraffin-embedded colorectal cancer (CRC) tissue sections. The OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6 and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 detection. The VENTANA MMR IHC Panel is for use on the VENTANA BenchMark ULTRA instrument. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody, and VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody.

The VENTANA MMR IHC Panel is indicated in patients diagnosed with colorectal cancer (CRC) to detect mismatch repair (MMR) proteins deficiency as an aid in the identification of probable Lynch syndrome and to detect BRAF V600E protein as an aid to differentiate between sporadic CRC and probable Lynch syndrome.

Results from the VENTANA MMR IHC Panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

The clinical performance of this device to guide treatment of MMR deficient patients has not been established.

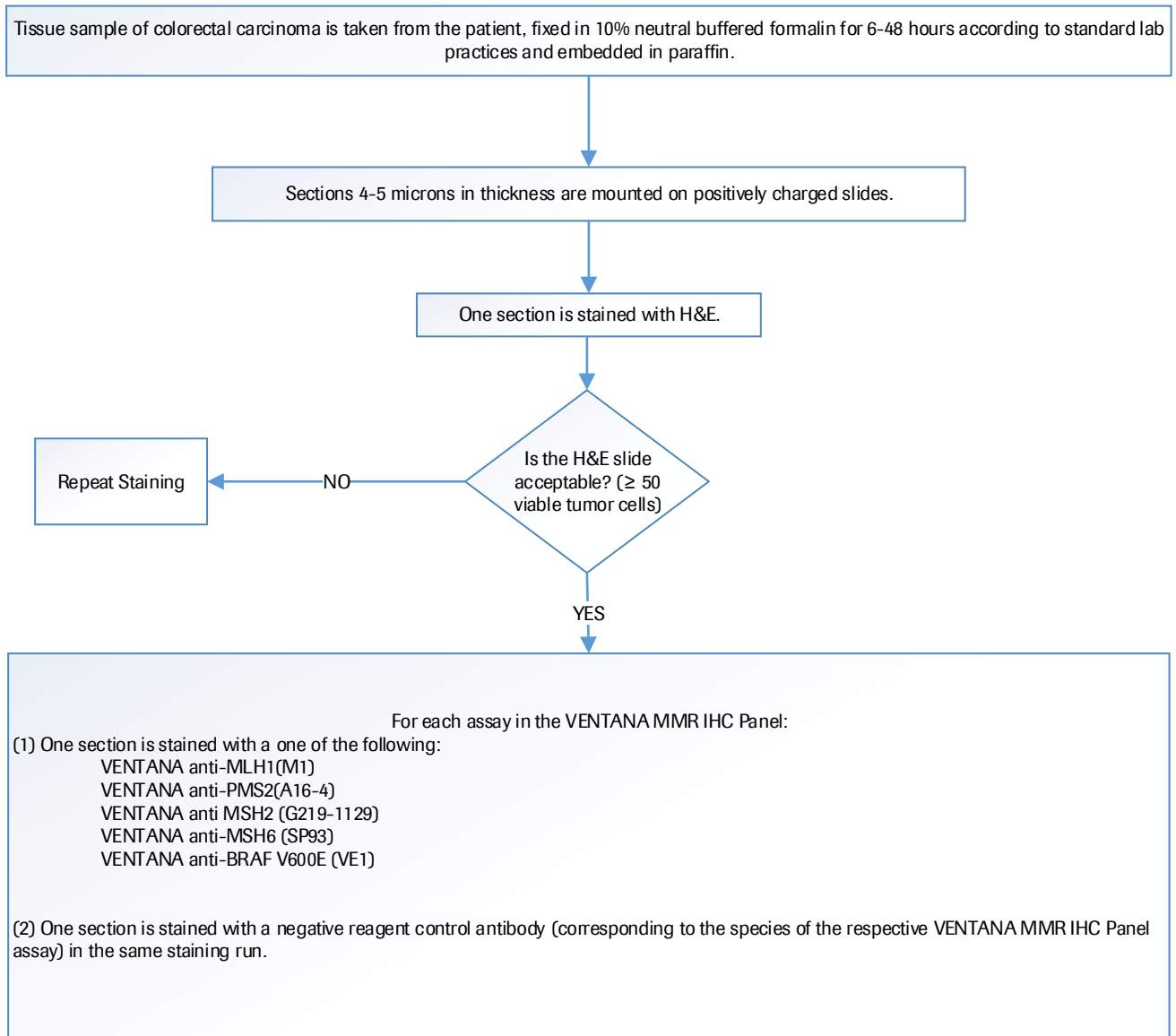
Intended for *in vitro* diagnostic (IVD) use. Prescription Use Only.

Purpose of Interpretation Guide

This guide is intended to aid pathologists in the clinical evaluation of formalin-fixed, paraffin-embedded (FFPE) colorectal carcinoma sections stained with the assays that comprise the VENTANA MMR IHC Panel in accordance with the proposed product labeling. Specifically this guide:

- Provides photographic images that illustrate the patterns and intensities of staining that may result from staining of colorectal carcinoma tissues with the assays that comprise the VENTANA MMR IHC Panel.
- Provides a reference for relating staining patterns and intensities to specific MMR biomarker clinical scores.
- Provides examples of challenging cases.
- Discusses other controls that may be used with the assay but are not provided by Ventana

Specimen Flow for Staining with the VENTANA MMR IHC Panel



Clinical Evaluation of MMR IHC Assays

Evaluating Staining Patterns and Intensities

Cells labeled with the IHC assays for the four MMR proteins (MLH1, PMS2, MSH2 and MSH6) are evaluated for presence or loss of the diaminobenzidine (DAB) signals. In colorectal carcinoma, the immunohistochemical staining of the four MMR proteins follows a nuclear staining pattern. In CRC cases with Lynch syndrome or somatic mutations, loss of any one of the four MMR proteins detected by the MMR IHC Assays is observed in the nuclei of tumor cells. The signal is classified as Intact or Loss based on nuclear localization only.

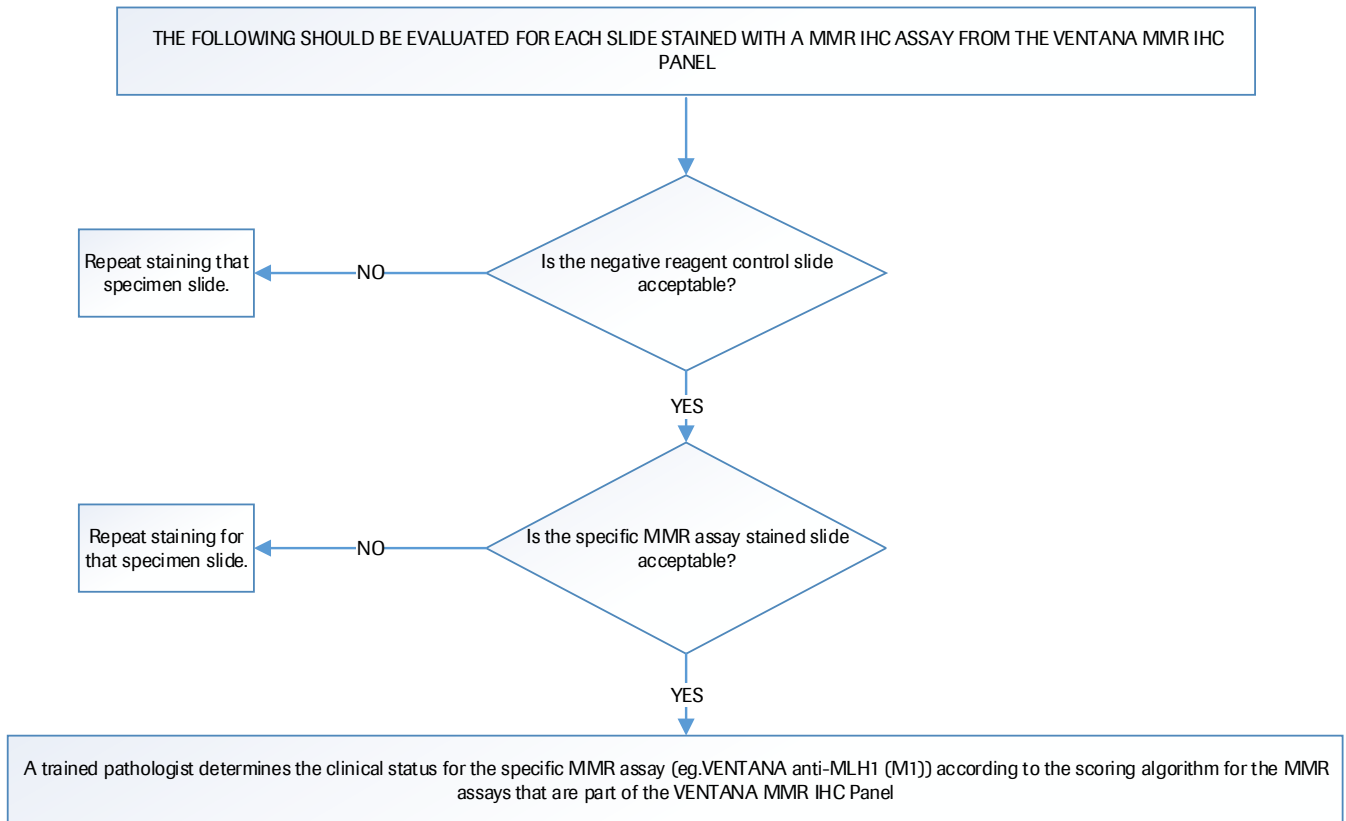
- **Positive (Intact)** signal is characterized by tumor cells that exhibit unequivocal nuclear staining of any intensity above background.
- **Negative (Loss)** signal intensity is characterized by an absence of any detectable signal or pale grey or tan nuclear discoloration in tumor cells.

The DAB signal may be distributed homogeneously, having a uniform level of intensity throughout the neoplastic portions of the tumor or distributed heterogeneously having more than one intensity level. A species-matched negative control antibody is used to evaluate the presence of background in test samples and establish a staining intensity baseline.

Each assay requires three serial tissue sections from each tissue specimen, one section for hematoxylin and eosin (H&E) staining, a second section for negative reagent control antibody staining, and a third section for staining with one of the MMR antibodies. A pre-qualified CRC tissue with an MMR status of intact may be used as a positive system-level control.

If the H&E evaluation indicates that the patient specimen is inadequate (for example, if fewer than 50 viable tumor cells are present), then a new specimen should be obtained. Repeat staining of a specimen for a particular MMR IHC assay within the panel (e.g., VENTANA anti-MLH1 (M1) antibody) should be carried out on unstained slides if (1) the system-level positive control slide stained with that assay does not exhibit acceptable staining; (2) the case slide stained with the appropriate negative reagent control for that assay does not exhibit acceptable staining, or (3) if the case slide stained with that particular MMR IHC assay (e.g., VENTANA anti-MLH1 (M1) antibody) is not evaluable. If the last of these slides is not interpretable due to no staining in the internal positive control cells, artifacts, edge effects, necrosis, lack of tissue, or any other reason, then the slide cannot be used for clinical evaluation. If controls are acceptable and the VENTANA MMR assay-stained slide is evaluable, the slide can be evaluated by a trained pathologist as described in the Scoring Criteria.

Specimen Flow for MMR IHC Assays



Morphology and Background Acceptability Criteria

For each case slide stained with an MMR IHC assay, tissue morphology and background acceptability are assessed using the criteria described below in Table 1.

Table 1: Morphology and Background Acceptability Criteria

	Acceptable	Unacceptable
Morphology	Cellular elements of interest are visualized, allowing clinical interpretation of the stain	Cellular elements of interest are not visualized, compromising clinical interpretation of the stain
Background	Background does not interfere with clinical interpretation of the stain	Background interferes with ability to interpret the stain

System-Level Control

A known positive control tissue fixed and processed in the same manner as the patient specimens should be run for each set of test conditions and with every MMR IHC antibody staining protocol performed, to serve as a system-level control. The control tissue should be a fresh biopsy/surgical specimen prepared and fixed as soon as possible in a manner identical to patient specimens. This tissue is 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. A positive tissue with moderate staining is more suitable for quality control than one that stains strongly; it can be used to detect minor levels of reagent degradation or out-of-specification issues that might be instrument-related. Positive control tissue may be on the same slide as the patient case or on a separate slide that is stained on the same run as the patient case.

Pre-qualified CRC tissue with an IHC Clinical MMR status of Intact may be used as a positive system-level control. Alternatively pre-qualified normal colon tissue fixed and processed in the same manner as the patient tissue can also be used as a positive system-level control. Normal colon will stain positive for all MMR IHC antibodies. Acceptable staining with a system-level control will confirm that all the reagents for that particular MMR IHC assay (e.g., VENTANA anti-MLH1 (M1)) were applied and the instrument functioned properly. The positive tissue control should be used only to monitor the correct performance of processed tissues, test reagents and instruments and not as an aid in formulating a specific diagnosis of patient samples.

Pre-qualified CRC tissue with an IHC Clinical MMR status of Loss may be used as a negative system-level control. Since the MLH1, PMS2, MSH2, and MSH6 proteins are expressed in all tissues, a normal negative tissue control does not exist for these biomarkers. The negative tissue control should be used only to monitor the correct performance of processed tissues, test reagents and instruments and not as an aid in formulating a specific diagnosis of patient samples.

Table 2: Staining Criteria for a Positive System-Level Control

	Acceptable	Unacceptable
Intact Staining Pattern	Unequivocal nuclear staining in viable tumor cells, in the presence of internal positive controls (nuclear staining in lymphocytes, fibroblasts or normal epithelium in the vicinity of the tumor)	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls

Internal Positive Controls

In colorectal carcinoma samples, unequivocal nuclear staining in lymphocytes, fibroblasts or normal epithelium in the vicinity of the tumor will serve as internal positive controls. Representative images of the internal control for the MMR IHC assays are shown below in Figure 2.

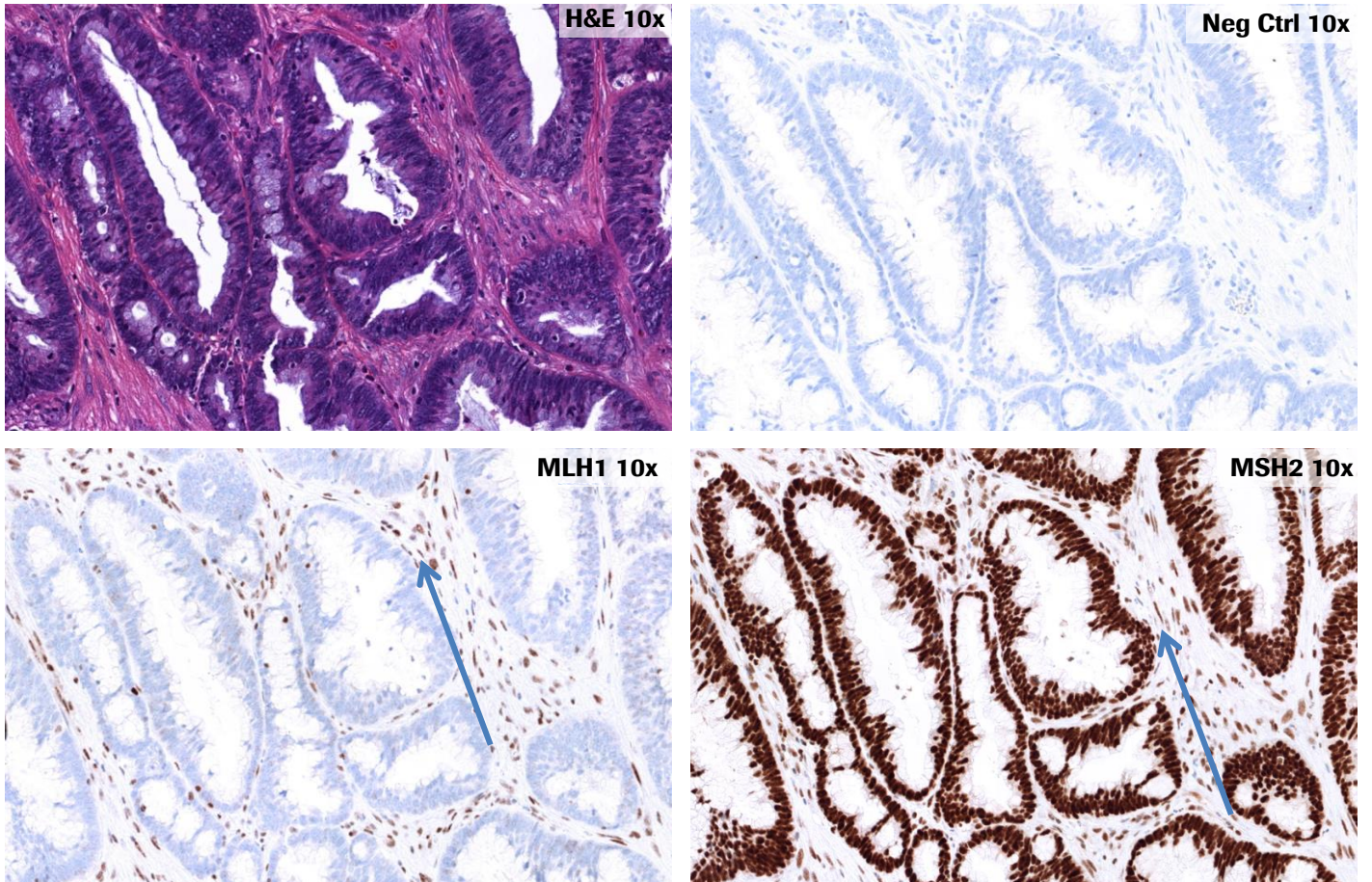


Figure 2: Internal Positive Controls Strong fibroblast staining (indicated by the blue arrows) is shown in a MLH1 loss status case and a MSH2 intact status case.

CRC Case Criteria for Clinical Evaluation

Table 3 summarizes the criteria for a CRC tissue slide to be evaluated for MMR status with any one of the MMR IHC assays.

Table 3: Evaluable and Non-evaluable Criteria

Clinical Interpretation	Staining Pattern
Evaluable (all must be true)	<ol style="list-style-type: none"> 1) H&E has \geq 50 viable tumor cells 2) Negative Reagent Control Slide is acceptable 3) Morphology is acceptable 4) Background is acceptable 5) System-level control is acceptable 6) Internal positive controls have unequivocal nuclear staining
Not Evaluable (if one criteria in this section is true the slide staining should be repeated)	<ol style="list-style-type: none"> 1) H&E has $<$ 50 viable tumor cells 2) Negative Reagent Control Slide is unacceptable 3) Morphology is unacceptable 4) Background is unacceptable 5) System-level control is unacceptable 6) Internal positive controls do not have unequivocal nuclear staining 7) Interpretation is not possible due to tissue loss, tumor absence, artifacts and/or edge artifacts.

Scoring Algorithm for MMR IHC Assays

Clinical Status is assigned by a trained pathologist based on their evaluation of the presence or absence of specific staining with the four MMR IHC assays in the VENTANA MMR IHC Panel. A Clinical Status of Intact is assigned to cases with presence of nuclear staining for all four markers and a Clinical Status of Loss is assigned to cases with absence of nuclear staining in any one of the markers.

Clinical interpretation of colorectal carcinoma cases stained with the MMR IHC assays should be based on the criteria noted in Table 4 below. Images of various Intact and Loss staining patterns are provided after the table.

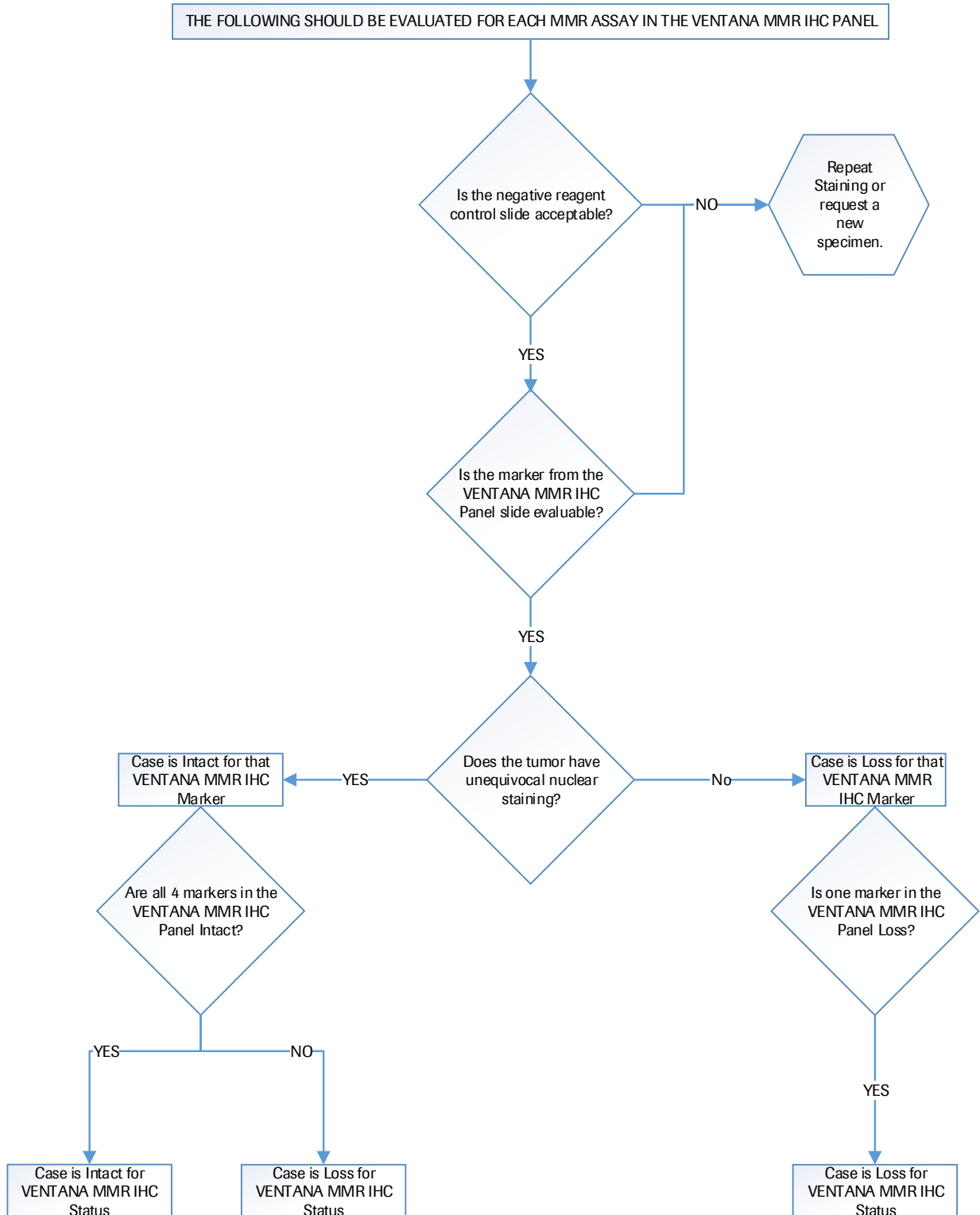
Table 4: Clinical Interpretation Criteria for the MMR IHC Assays

Intact Protein Expression	Loss of Protein Expression
Unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (nuclear staining in lymphocytes, fibroblasts or normal epithelium in the vicinity of the tumor)	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls

If unequivocal nuclear stain is absent in internal positive controls and/or background staining interferes with interpretation, the assay should be considered unacceptable and repeated. Punctate nuclear staining of tumor cells should be considered negative (loss). In cases with focal tumor cell staining, the intensity of the nuclear staining should be at least that of the internal positive controls along with the confluent /continuous staining of the nuclei in a few epithelial glands or nests for the case to be given a Clinical Status of Intact. In the absence of this, a Clinical Status of Loss is given to the case.

Decision Tree for MMR IHC Assays

Slides stained with VENTANA MMR IHC assays should be evaluated using the Clinical Interpretation Criteria (Table 4), the approach is summarized in the decision tree below.



Cases with Intact MMR Status

CRC Cases with Intact MMR protein expression

An intact status staining result is characterized by the presence of detectable nuclear signal in the presence of appropriately stained internal controls.

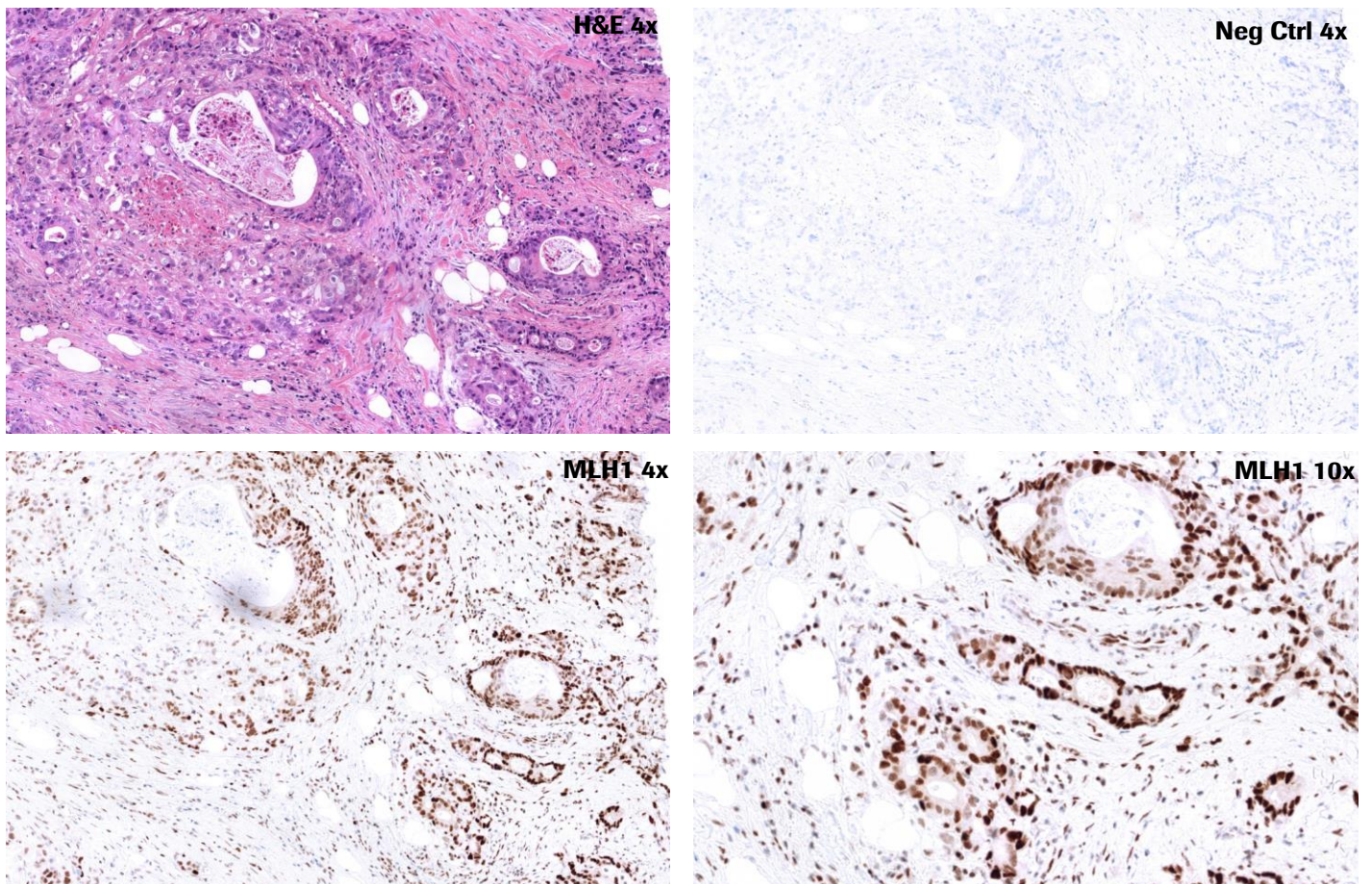


Figure 3: MLH1 Intact Status tissue exhibits strong nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

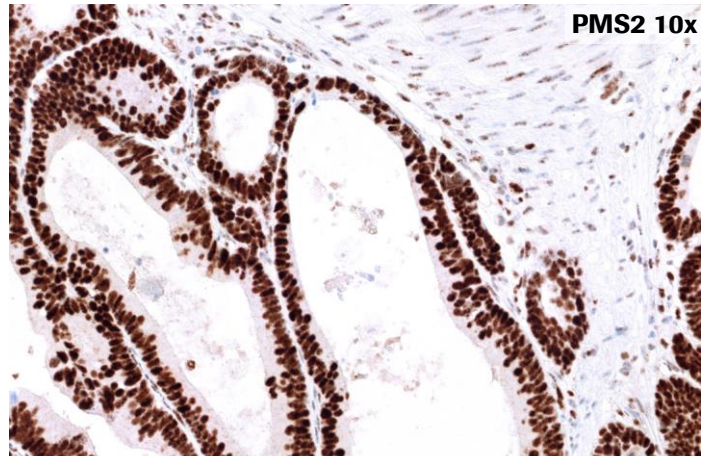
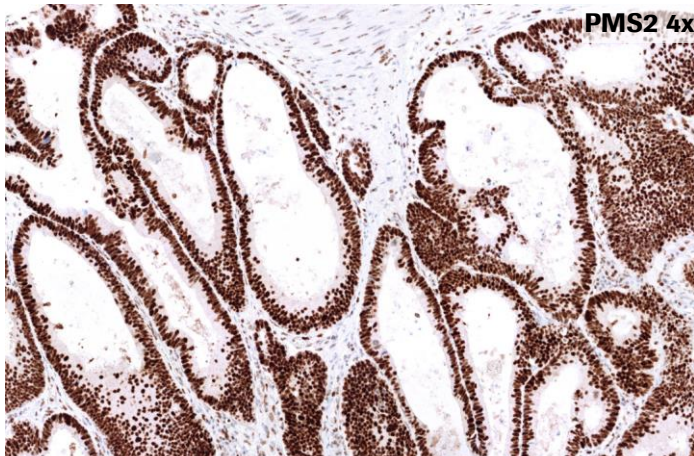
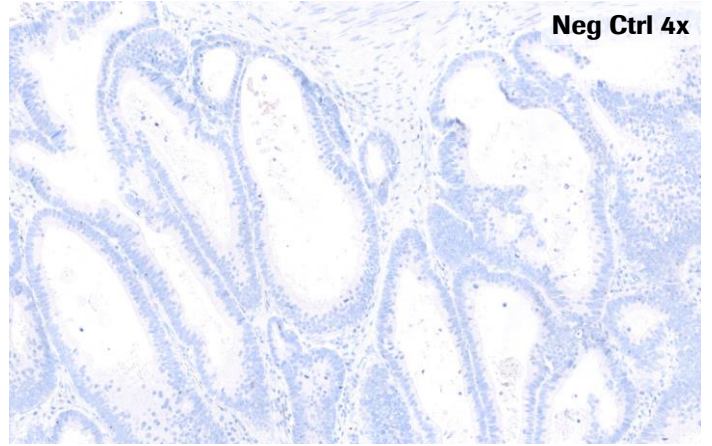
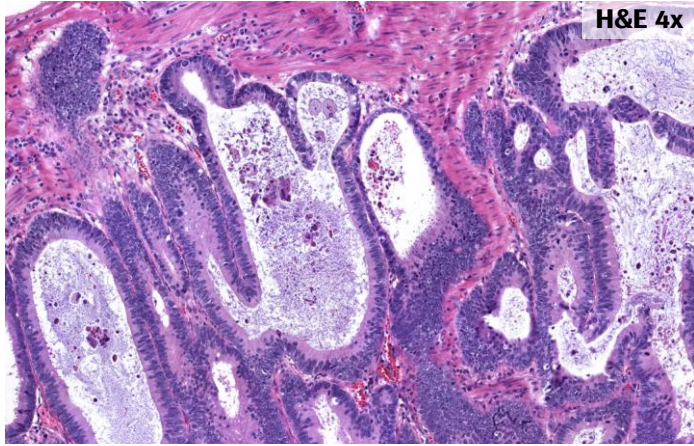


Figure 4: **PMS2 Intact Status** tissue exhibits strong nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

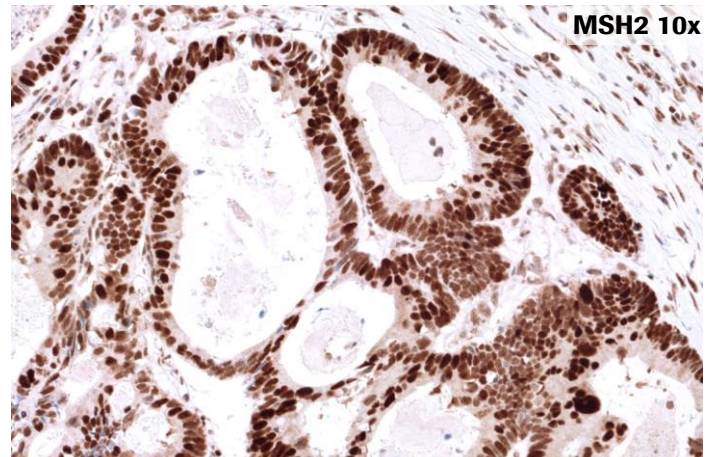
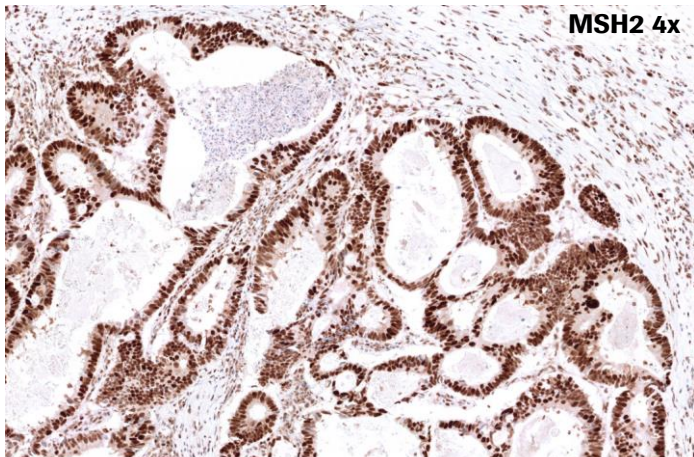
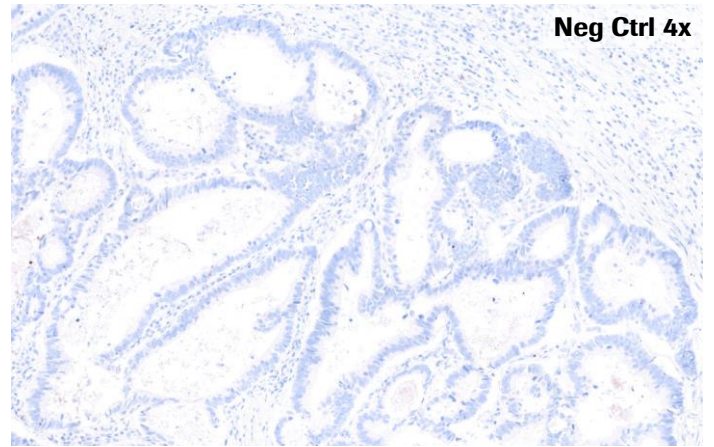
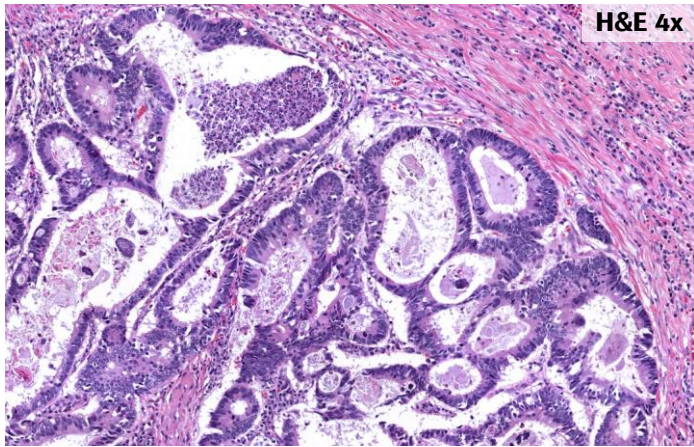


Figure 5: MSH2 Intact Status tissue exhibits strong nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

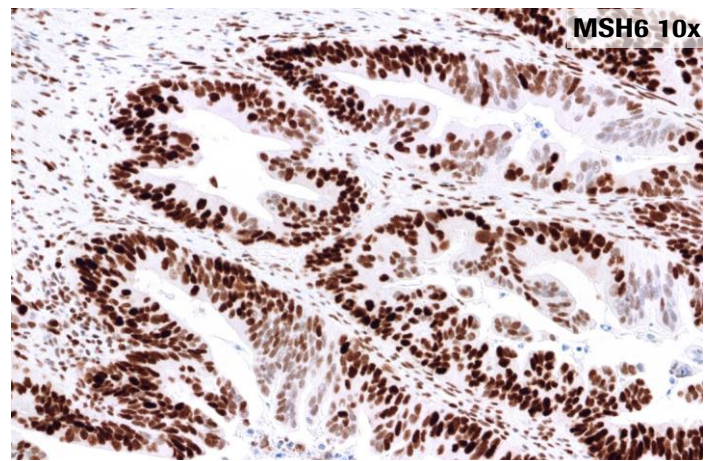
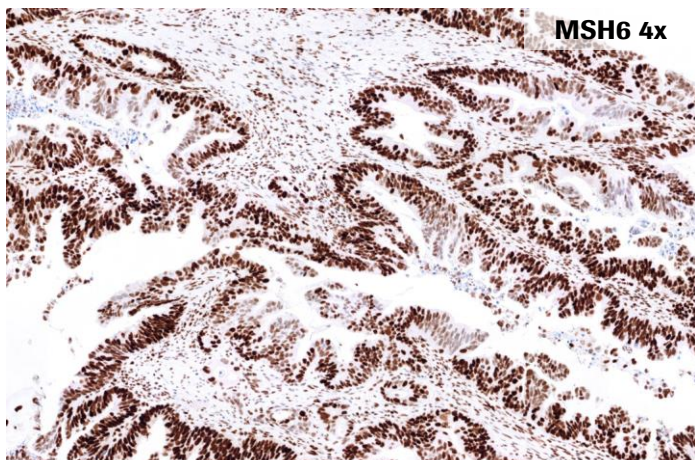
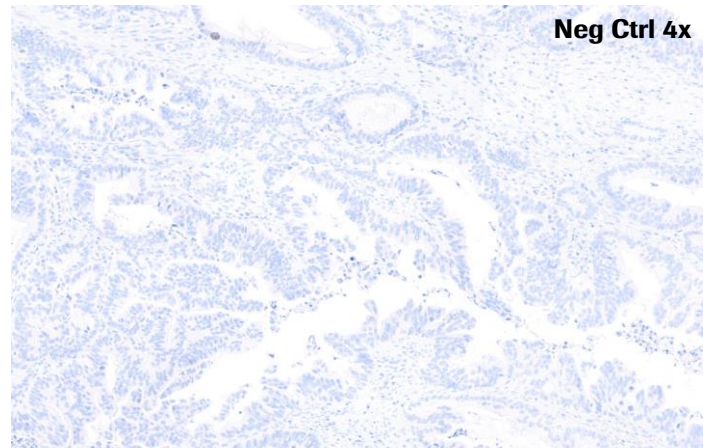
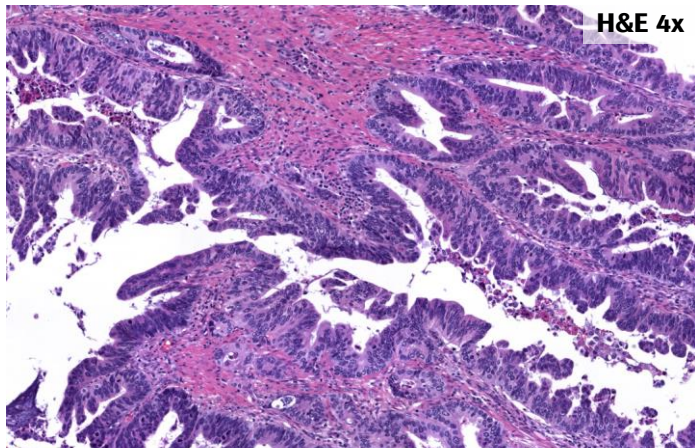


Figure 6: MSH6 Intact Status tissue exhibits strong nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

Cases with MMR Status of Loss

CRC Cases with loss of MMR protein expression

A loss status staining result is characterized by the absence of detectable nuclear signal in the presence of appropriately stained internal controls.

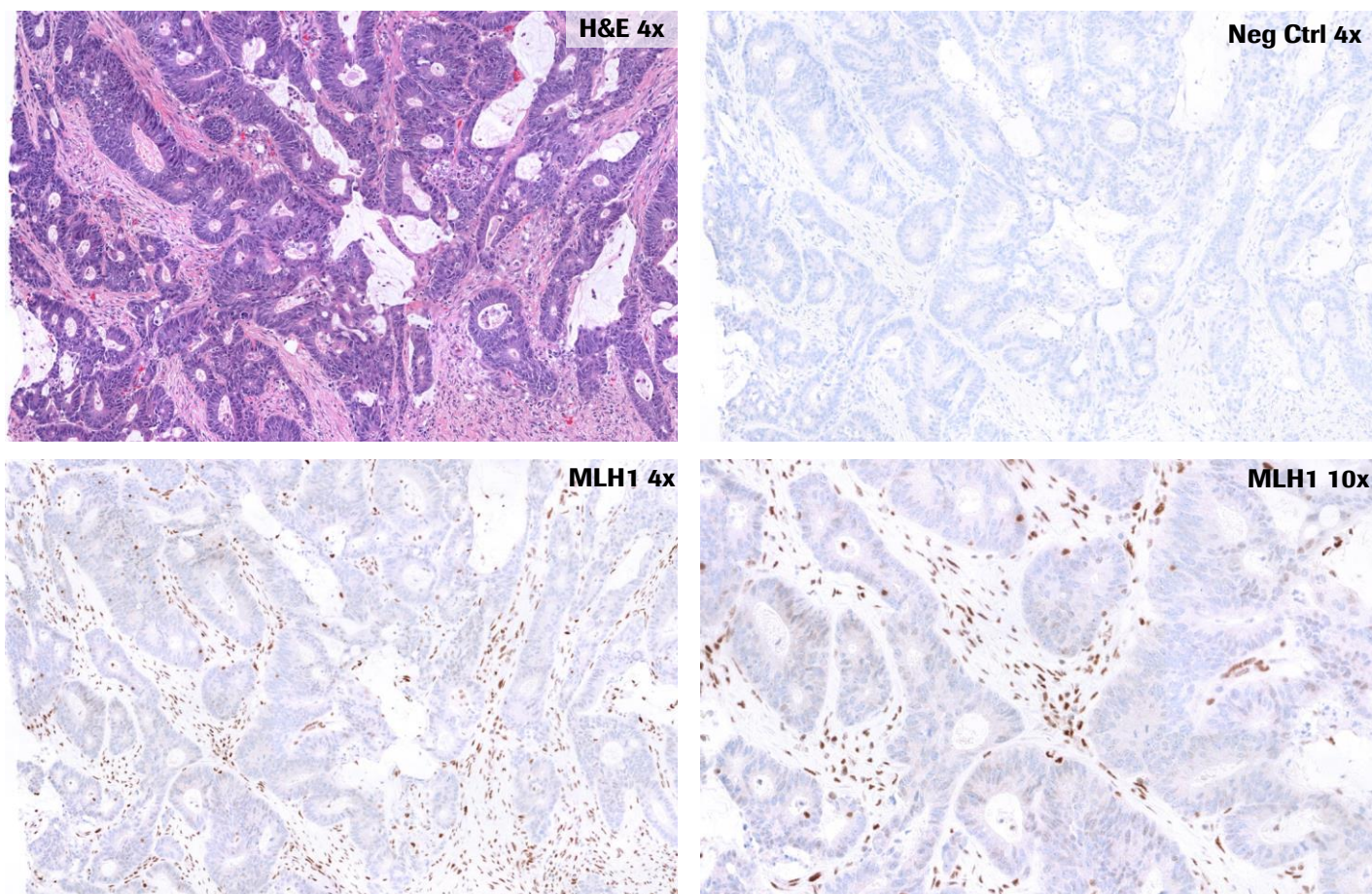


Figure 7: MLH1 Loss Status tissue exhibits no nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

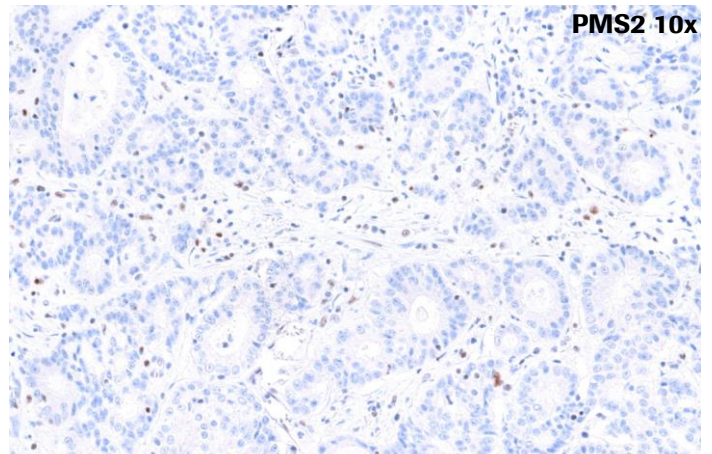
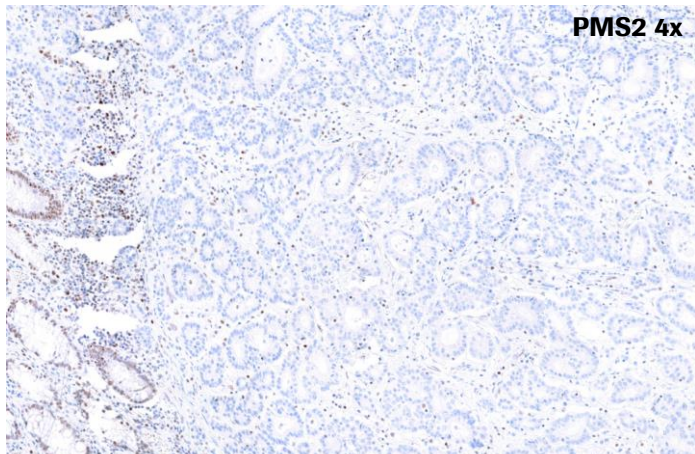
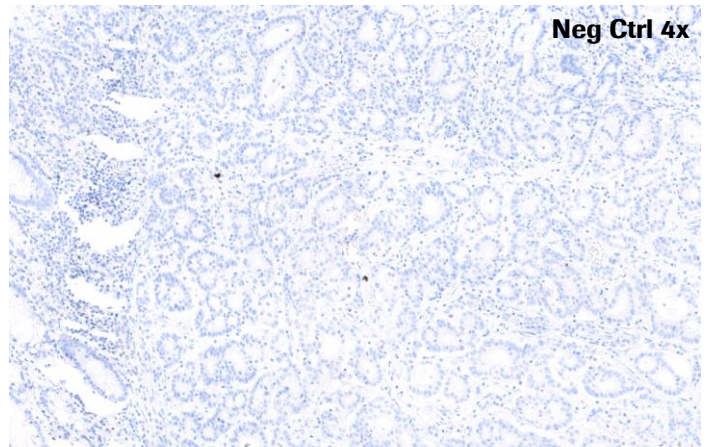
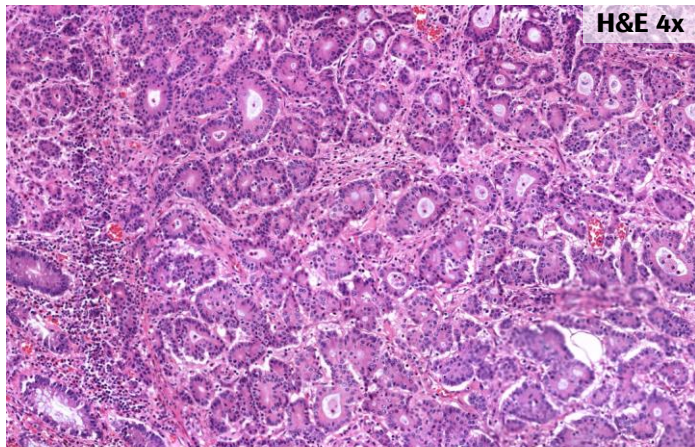


Figure 8: PMS2 Loss Status tissue exhibits no nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

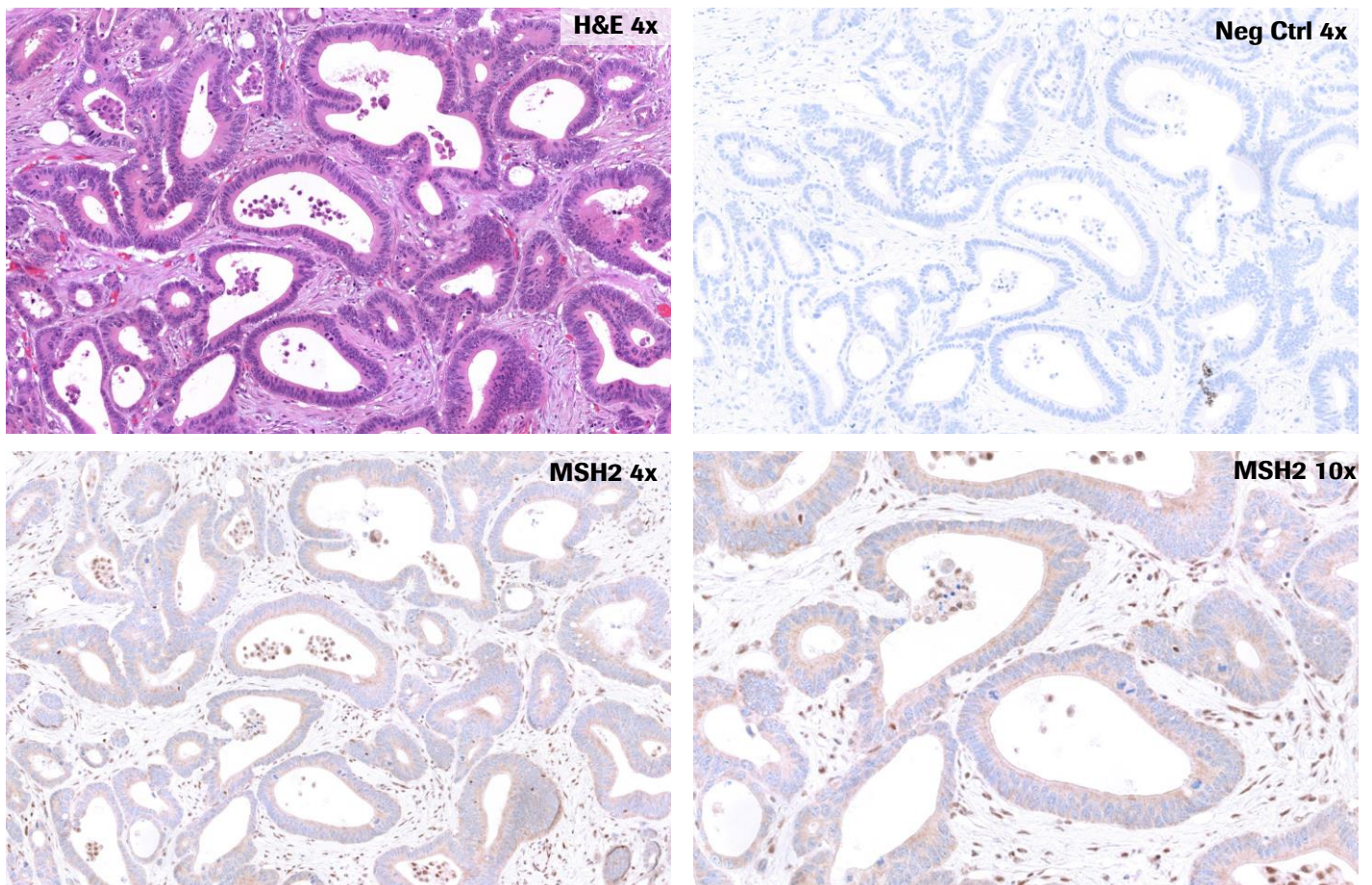


Figure 9: MSH2 Loss Status tissue exhibits no nuclear staining in tumor cells but weak cytoplasmic staining in the presence of appropriately stained internal controls. Cytoplasmic staining should be disregarded in the interpretation of status.

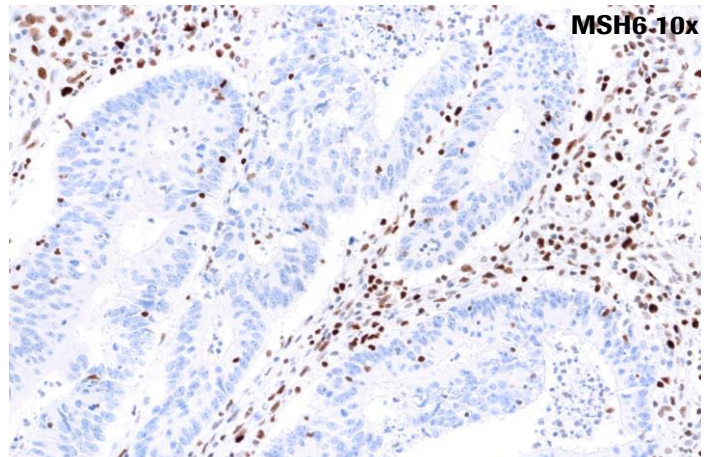
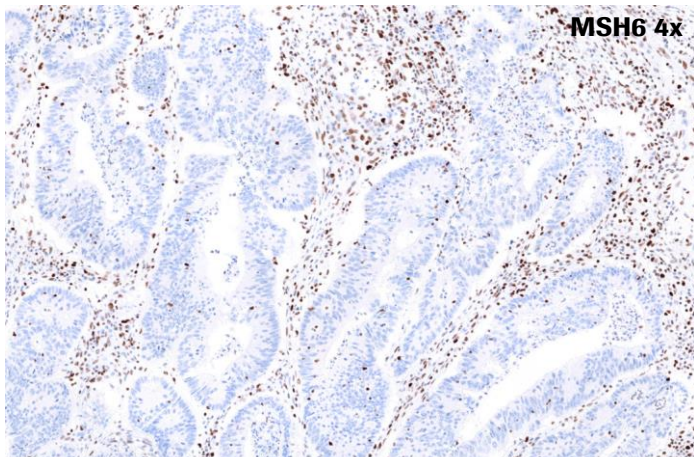
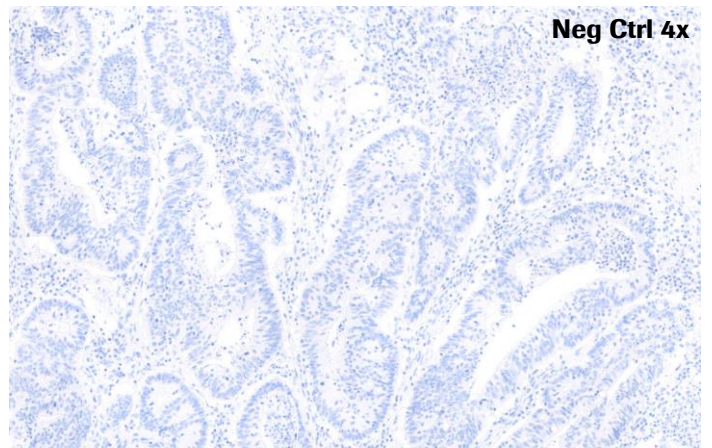
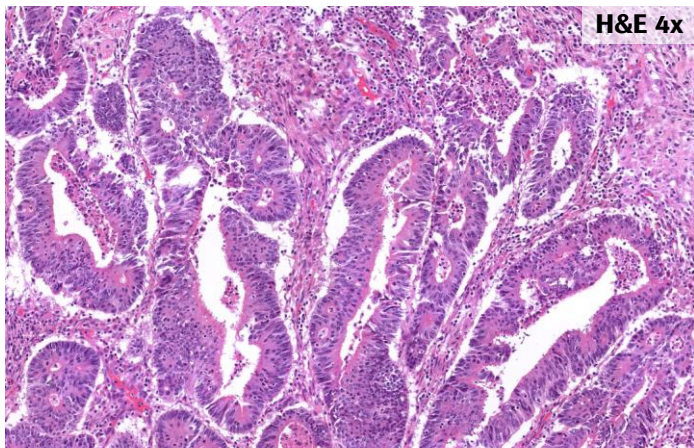


Figure 10: MSH6 Loss Status tissue exhibits no nuclear staining in the tumor cells in the presence of appropriately stained internal controls.

Challenging Cases

CRC cases are categorized as Intact or Loss for an MMR IHC assay according to the presence or absence of staining over the entire tumor area. The staining can vary in the level of intensity and this intensity may vary throughout the tumor; however, this does not impact MMR status.

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

- **Non-specific background**

Some specimens may exhibit non-specific background staining for reasons that are not well understood. For this reason, evaluation of a MMR IHC slide must include a comparison of the slide to the negative reagent control slide to determine the level of non-specific background staining. Cytoplasmic staining, if present, should be disregarded in MMR IHC interpretation.

- **Focal Staining**

Some specimens may exhibit focal staining in the tumor cells and staining intensity may vary from weak to strong. Based on the MMR IHC scoring algorithm, focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls should be categorized as Loss.

- **Punctate Staining**

Some specimens may exhibit discrete punctate staining within a few nuclei of the tumor; the staining intensity may vary from weak to strong. This staining pattern should be ignored and if a case has only this type of staining pattern, it should be given a Clinical Status of Loss.

- **Tissue or Staining Artifact**

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

Some challenging cases are shown on the following pages.

Non-specific background staining

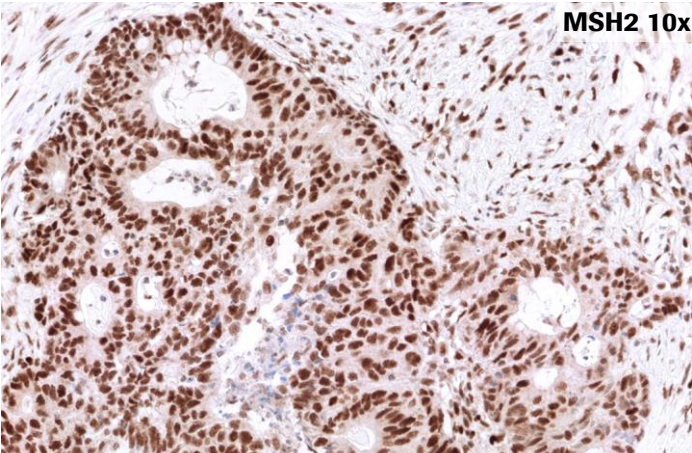
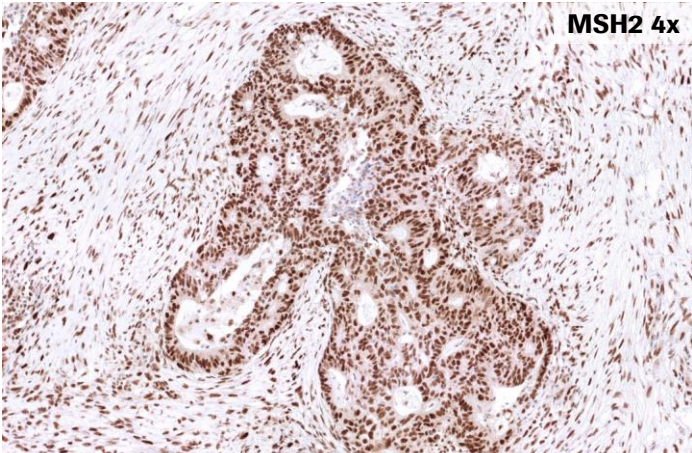
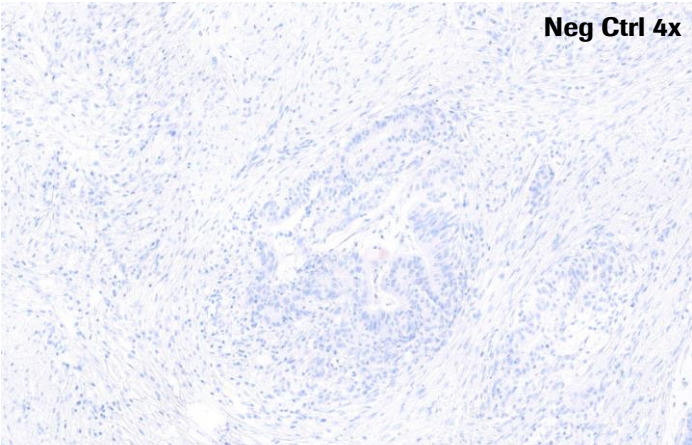
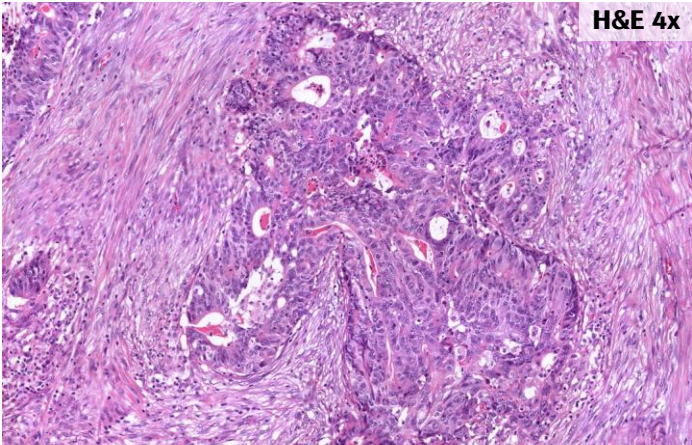


Figure 11: MSH2 Intact Status tissue exhibits strong nuclear staining in the presence of appropriately stained internal controls; cytoplasmic background staining is seen in the tumor cells which should be disregarded.

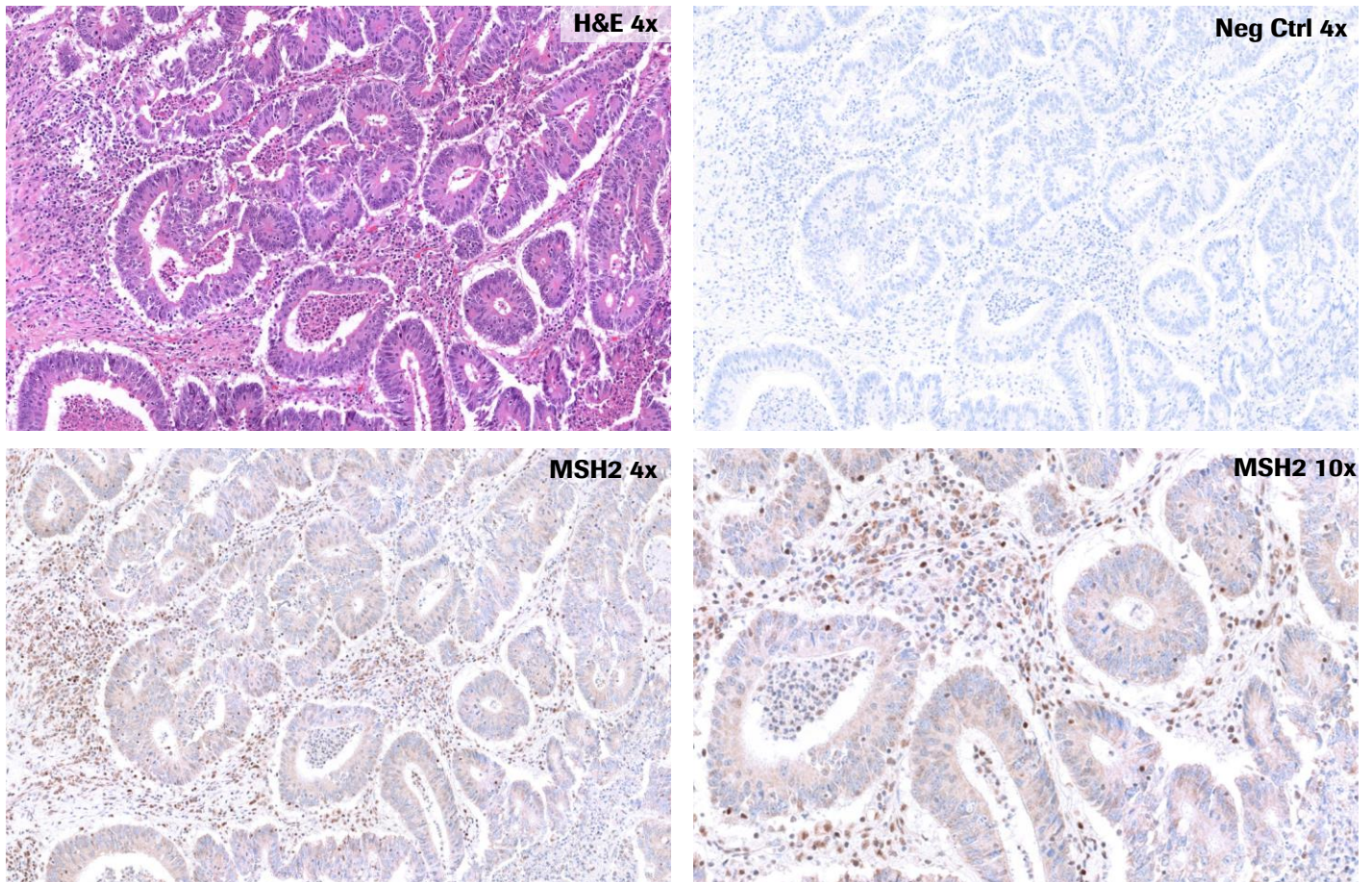


Figure 12: MSH2 Loss Status tissue exhibits pale tan cytoplasmic background in the tumor cells; however, no nuclear staining is present in the tumor in the presence of the appropriately stained internal positive controls. Cytoplasmic staining should be disregarded in the interpretation of status.

Focal Staining

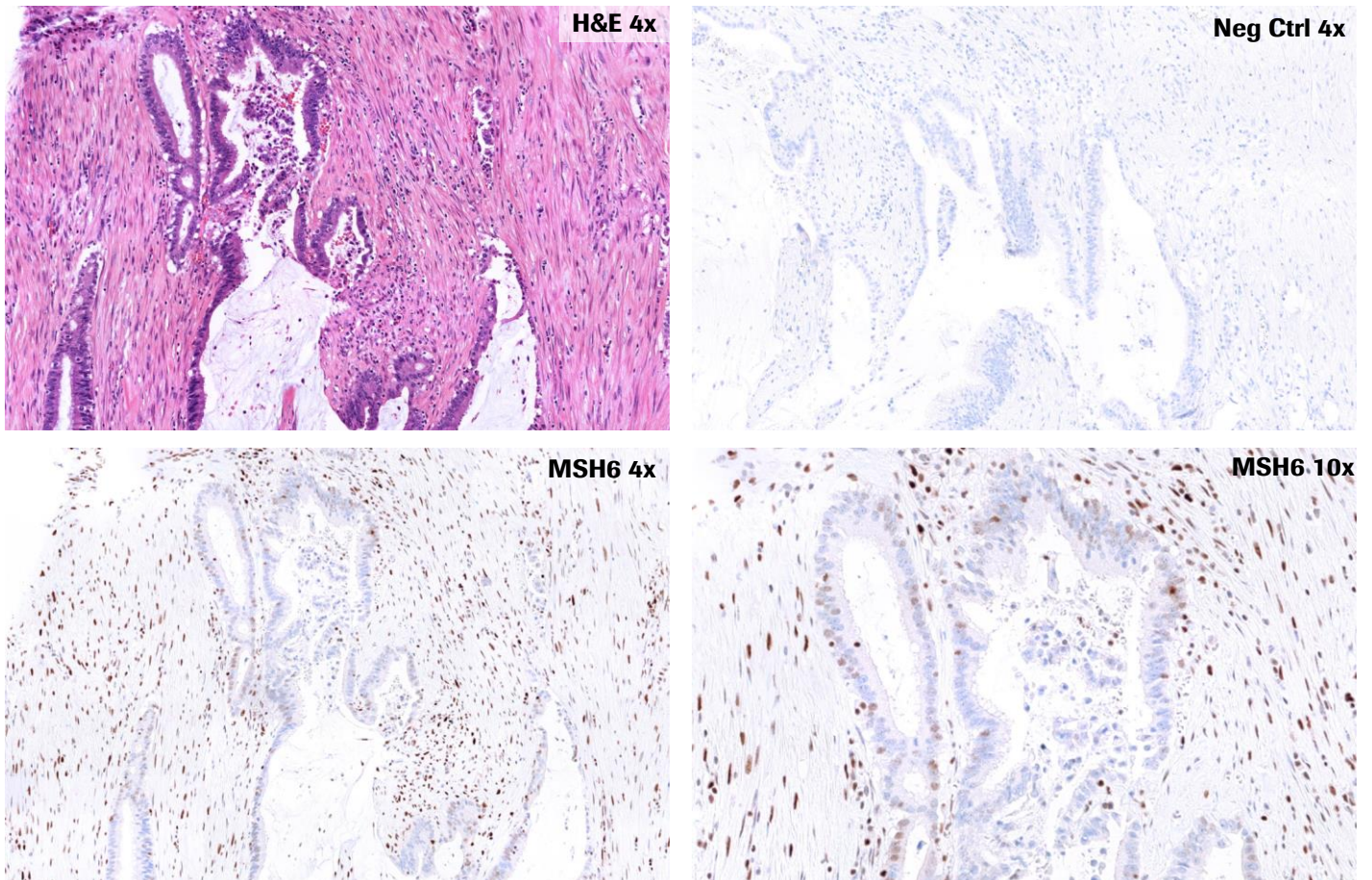


Figure 13: MSH6 Loss Status tissue exhibits focal weak equivocal nuclear staining in the viable tumor cells in the presence of appropriately stained internal positive controls. Also note that the intensity of the focal nuclear staining is less than that of the internal positive controls and not confluent around the gland.

Punctate Staining

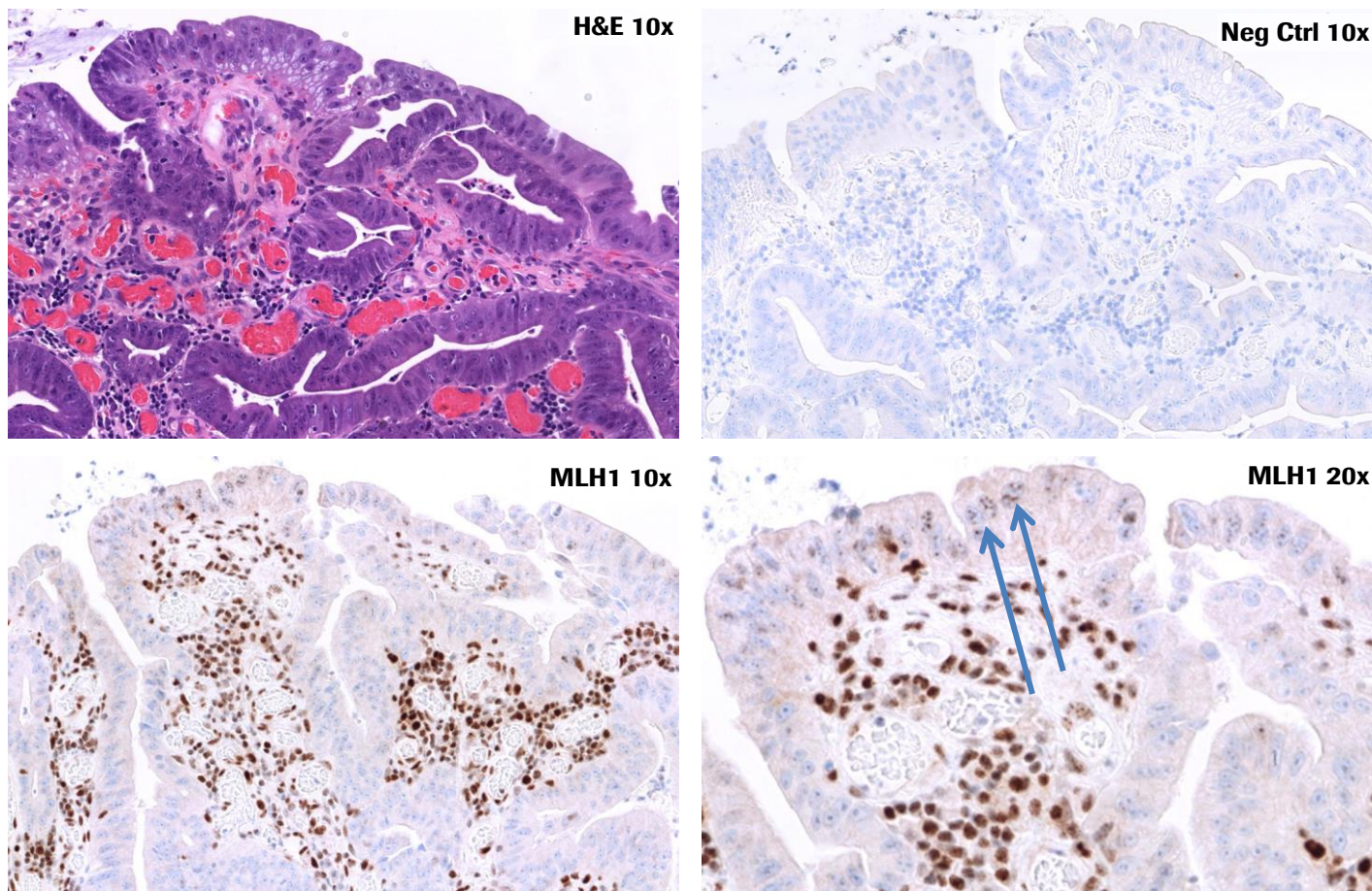


Figure 14: MLH1 Loss Status tissue tumor exhibits strong punctate staining in the nuclei of a few tumor cells however, there are no cells in the tumor that exhibit unequivocal nuclear staining in the presence of appropriately staining internal positive controls.

Clinical Evaluation of VENTANA anti-BRAF V600E IHC Assay

Evaluating Staining Patterns and Intensities

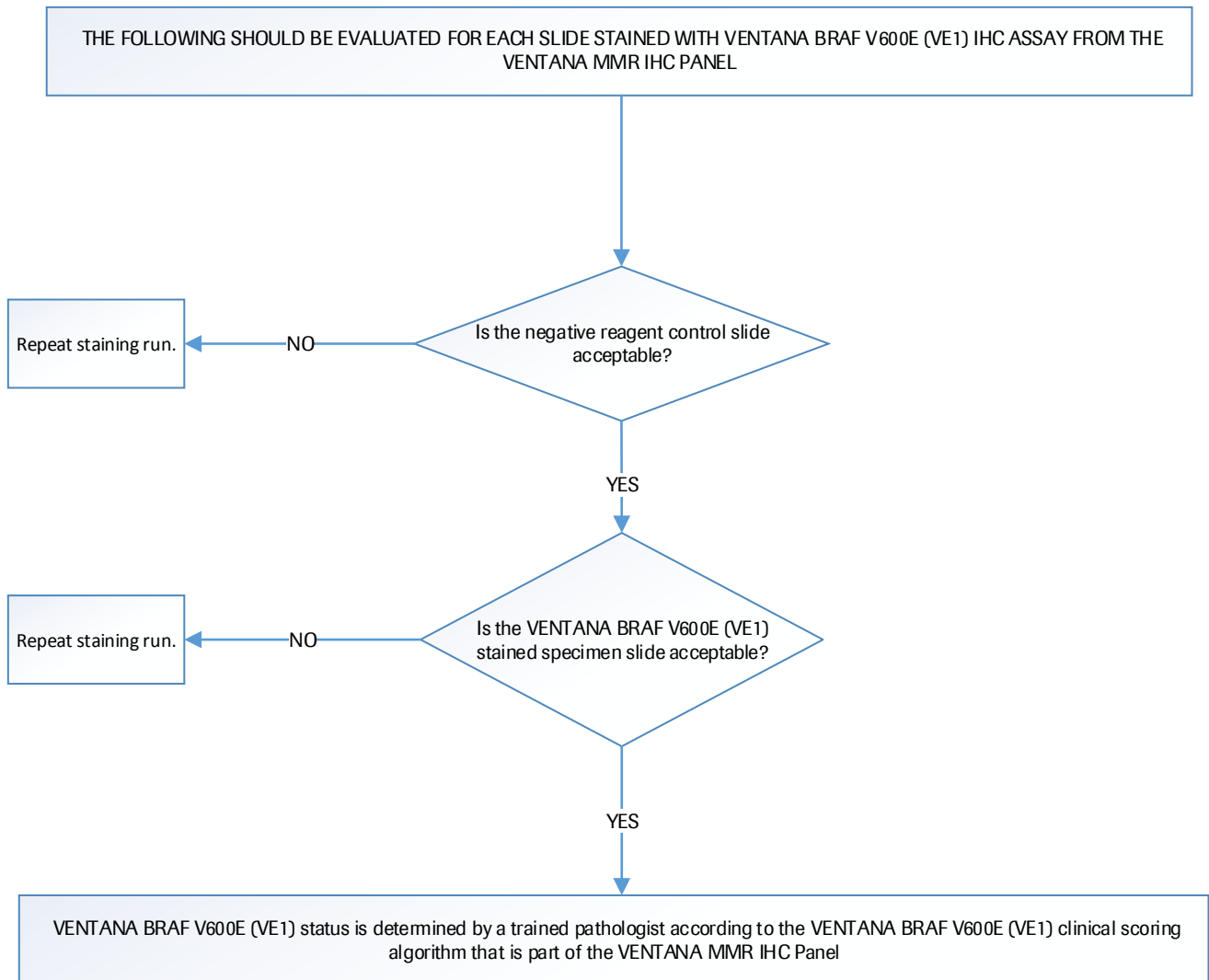
In colorectal carcinoma, neoplastic cells labeled with the VENTANA anti-BRAF V600E (VE1) antibody are evaluated for the presence or absence of the diaminobenzidine (DAB) signal. The immunohistochemical staining in colorectal carcinoma follows a cytoplasmic staining pattern

- **Positive** signal is characterized by cells that exhibit unequivocal cytoplasmic staining; the intensity may range from weak to moderate.
- **Negative** signal intensity is characterized by an absence of any detectable signal. Negative cases may still exhibit pale grey or tan cytoplasmic discoloration.

The signal may be distributed homogeneously, having a uniform level of intensity throughout the neoplastic portions of the tumor or distributed heterogeneously having more than one intensity level. An isotype-matched negative control antibody is used to evaluate the presence of background in test samples and establish a staining intensity baseline.

The VENTANA anti-BRAF V600E (VE1) IHC assay requires one serial tissue section for hematoxylin and eosin (H&E) staining, a second serial tissue section for negative reagent control antibody staining and a third serial tissue section for VENTANA anti-BRAF V600E (VE1) antibody staining. A CRC that is positive for BRAF V600E mutation by VENTANA anti-BRAF V600E (VE1) antibody IHC may be used as a system-level control. If the H&E evaluation indicates that the patient specimen is inadequate (for example, if fewer than 50 viable tumor cells are present), then a new specimen should be obtained. Repeat staining of a specimen should be carried out on unstained slides if (1) the system-level positive control slide does not exhibit acceptable staining; (2) the negative reagent control antibody case slide does not exhibit acceptable staining; or (3) the BRAF V600E stained case slide is not evaluable. If the last of these slides is not interpretable due to artifacts, edge effects, necrosis, lack of tissue, or any other reason, then the slide cannot be used for clinical evaluation. If controls are acceptable and the VENTANA anti-BRAF V600E (VE1) antibody IHC slide is evaluable, the slide can be evaluated by a trained pathologist as described in the Scoring Criteria.

Specimen Flow for VENTANA anti-BRAF V600E IHC Assay



Morphology and Background Acceptability Criteria

Tissue morphology and background acceptability are assessed for each patient case using the criteria described below in Table 5.

Table 5: Morphology and Background Acceptability Criteria

	Acceptable	Unacceptable
Morphology	Cellular elements of interest are visualized, allowing clinical interpretation of the stain	Cellular elements of interest are not visualized, compromising clinical interpretation of the stain
Background	Background does not interfere with clinical interpretation of the stain	Background interferes with ability to interpret the stain

System-level Control

A known system level control tissue fixed and processed in the same manner as the patient specimens should be run with every VENTANA anti-BRAF V600E (VE1) antibody staining protocol performed. The control tissue should be a fresh biopsy/surgical specimen prepared and fixed as soon as possible in a manner identical to patient specimens. This tissue is 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. A positive tissue with moderate staining is more suitable for quality control than one that stains strongly; it can be used to detect minor levels of reagent degradation or out-of-specification issues that might be instrument-related. System-level control tissue may be run on the same slide as the patient case or on a separate slide run at the same time as the patient case.

A positive system-level control would be a pre-qualified case of CRC that is positive for BRAF V600E mutation by VENTANA anti-BRAF V600E (VE1) antibody IHC. The staining of the CRC tissue case that has the BRAF V600E mutation will confirm that all the reagents were applied and the instrument functioned properly. The positive tissue control should be used only to monitor performance; it should not be used to aid the clinical diagnosis of patient samples.

A negative system-level control would be a pre-qualified case of CRC that is negative for VENTANA anti-BRAF V600E (VE1) antibody IHC negative. The negative tissue control should be used only to monitor performance; it should not be used to aid the clinical diagnosis of patient samples.

Control tissue on each VENTANA anti-BRAF V600E (VE1) antibody stained slide will be judged as acceptable or unacceptable by the reviewing pathologists according to criteria outlined in the Table 6.

Table 6: Staining Criteria for a Positive System-Level Control

	Acceptable	Unacceptable
CRC tissue	Unequivocal cytoplasmic staining of any intensity in viable tumor cells above background.	No staining or equivocal cytoplasmic staining in viable tumor cells.
Fibroblasts and lymphocytes in normal epithelium	No staining or equivocal cytoplasmic staining in fibroblasts and lymphocytes in normal epithelium.	Unequivocal cytoplasmic staining in the fibroblasts and lymphocytes in the normal epithelium.

Scoring Algorithm for the VENTANA anti-BRAF V600E IHC Assay

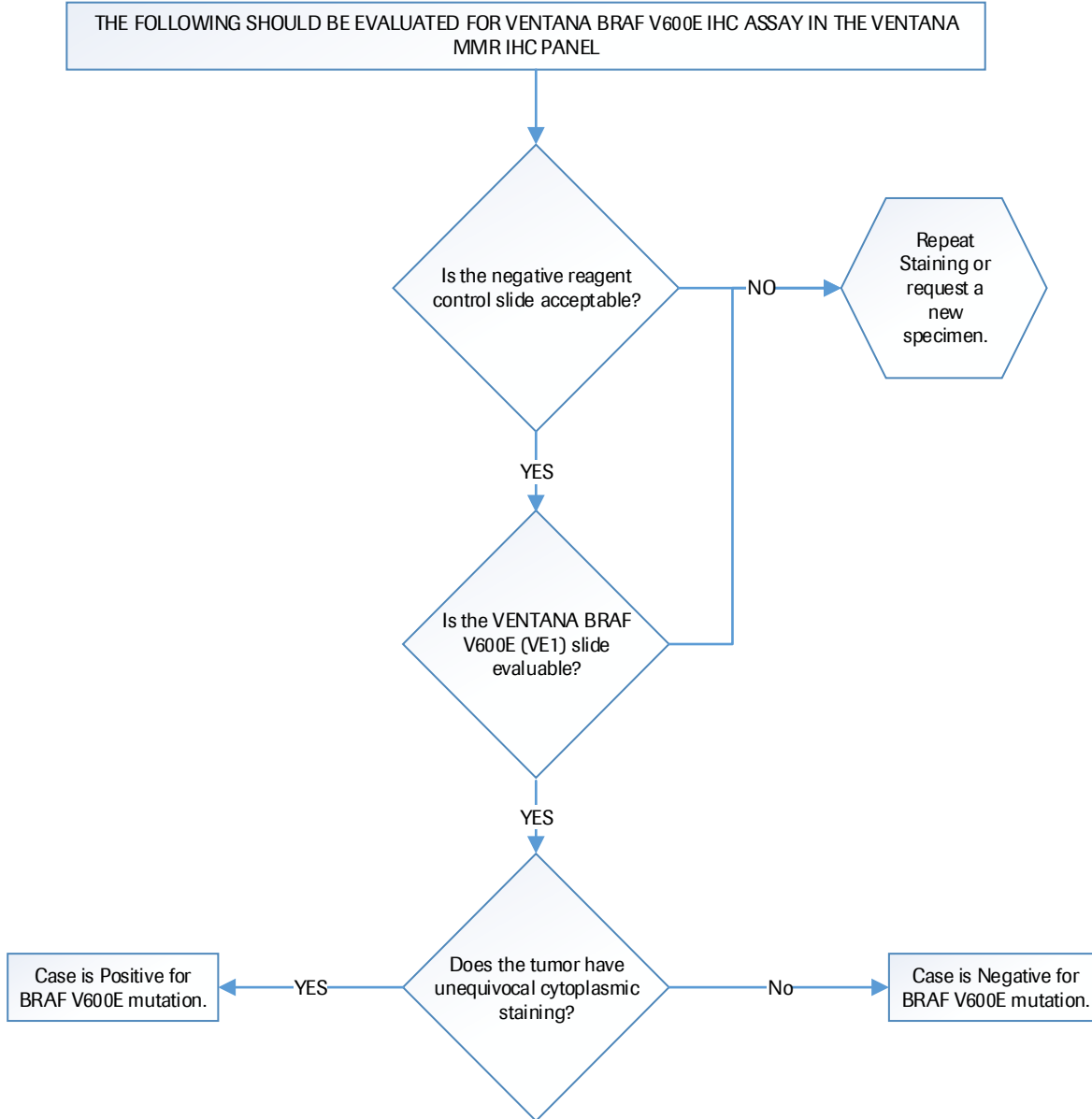
VENTANA anti-BRAF V600E (VE1) antibody IHC status is assigned by a trained pathologist based on his or her evaluation of the intensity of specific staining for VENTANA anti-BRAF V600E (VE1) as illustrated in Table 7 below. A Positive status is assigned to cases with positive staining and a Negative Status is assigned to cases with absent staining.

Clinical interpretation of colorectal carcinoma cases stained with VENTANA anti-BRAF V600E (VE1) antibody should be based on the criteria noted in the Table 7. Images of various Negative and Positive staining patterns are provided after Table 7.

Table 7: Clinical Interpretation Criteria for the VENTANA anti-BRAF V600E (VE1) IHC Assay

Positive for BRAF V600E mutation	Negative for BRAF V600E mutation
Unequivocal cytoplasmic staining of any intensity in viable tumor cells above background.	No staining or equivocal cytoplasmic staining in viable tumor cells. Note: Nuclear staining, weak to strong staining of isolated viable tumor cells/or small tumor clusters should be considered negative.

Decision Tree



Cases Negative for BRAF V600E

CRC Cases with BRAF V600E (VE1) Negative Status

A negative staining result is characterized by an absence of any detectable signal. Negative cases may still exhibit pale grey or tan cytoplasmic discoloration.

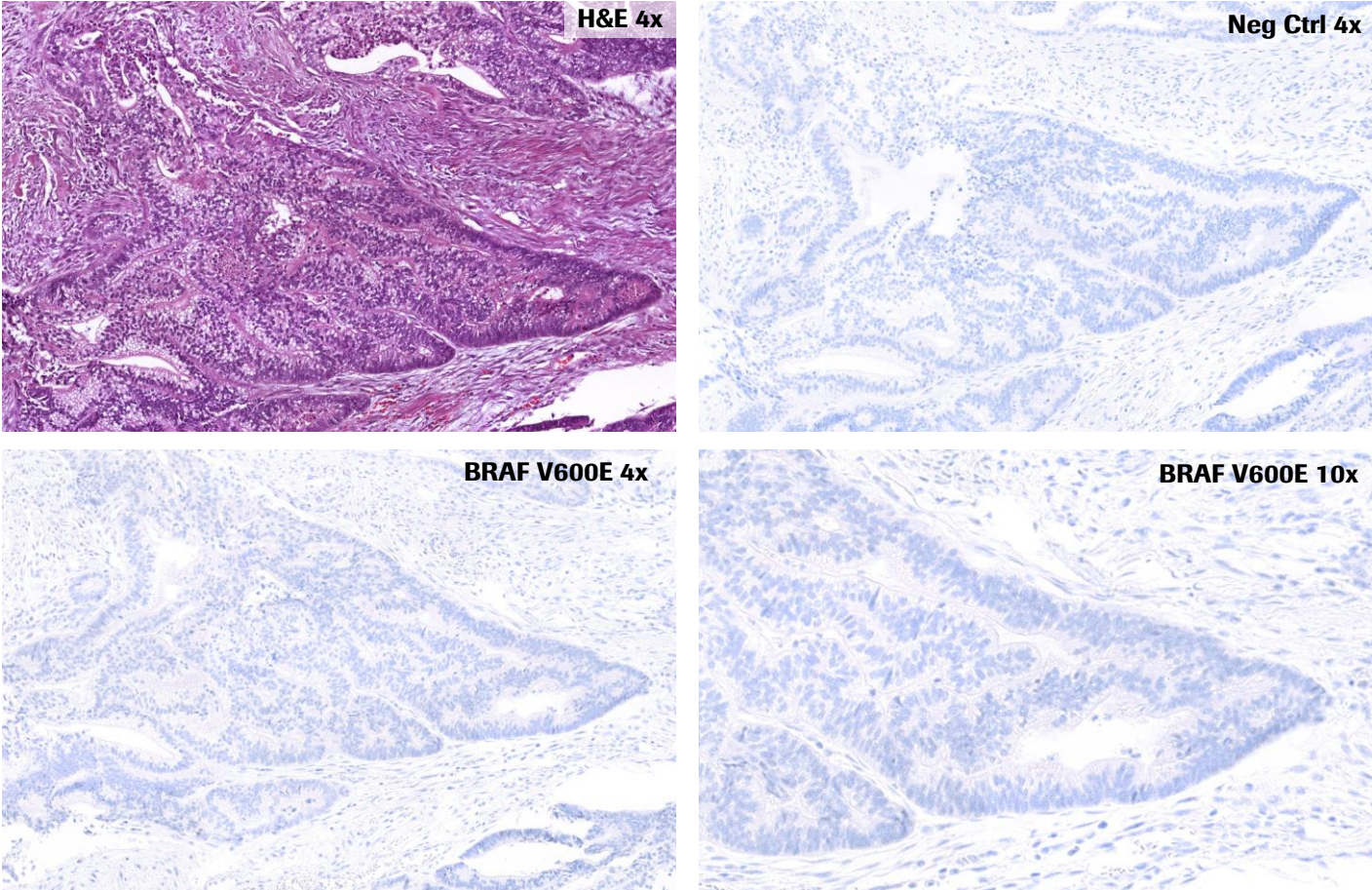


Figure 15: BRAF V600E Negative Status tissue exhibits no cytoplasmic staining in the tumor cells.

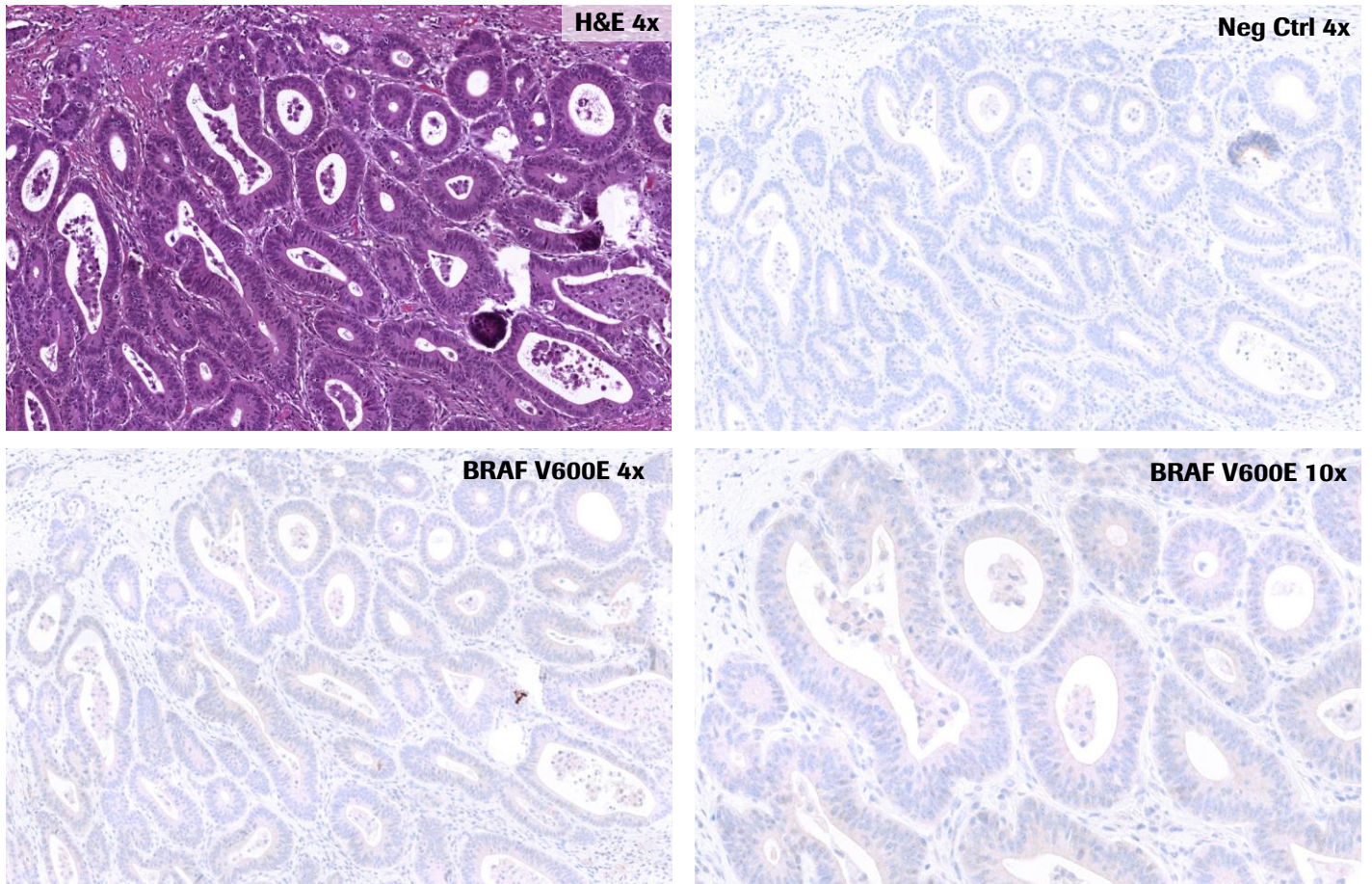


Figure 16: BRAF V600E Negative Status tissue exhibits a light cytoplasmic blush.

Cases Positive for BRAF V600E

CRC Cases with BRAF V600E (VE1) Positive Status

A positive staining result is characterized by cells that exhibit unequivocal cytoplasmic staining; the intensity may range from weak to moderate.

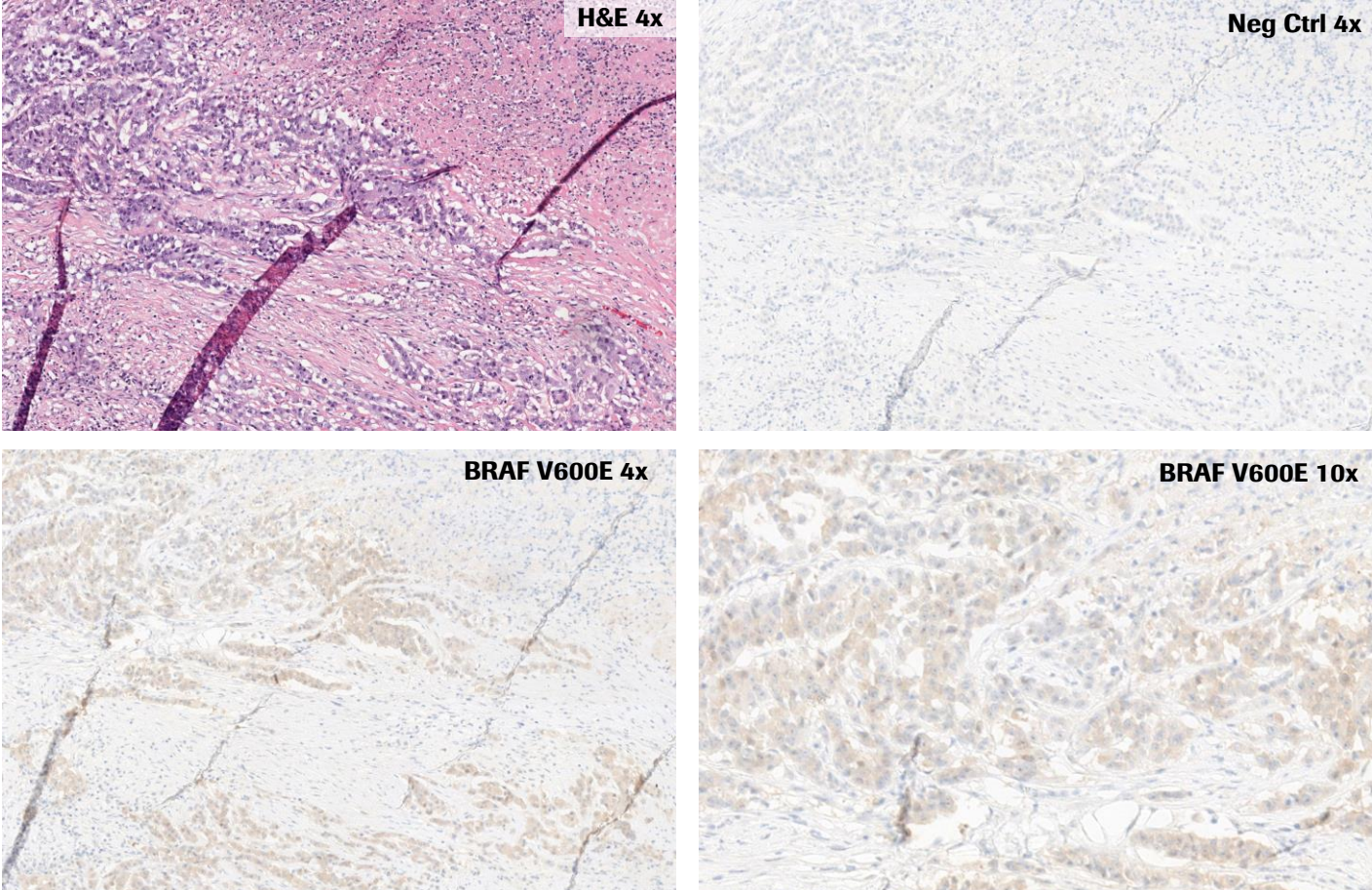


Figure 17: BRAF V600E Positive Status tissue exhibits weak cytoplasmic staining.

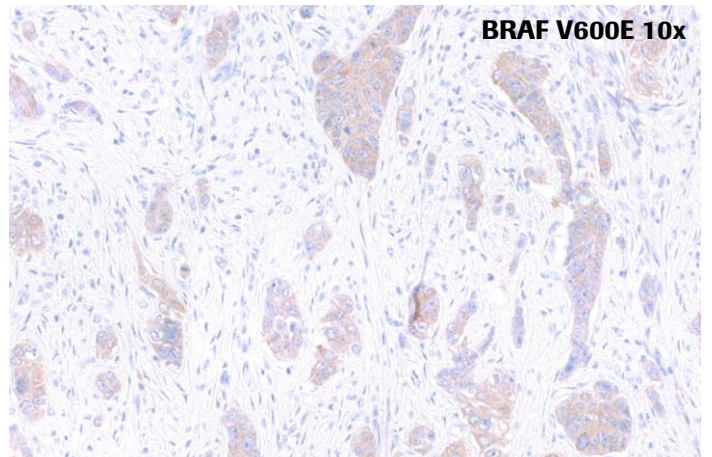
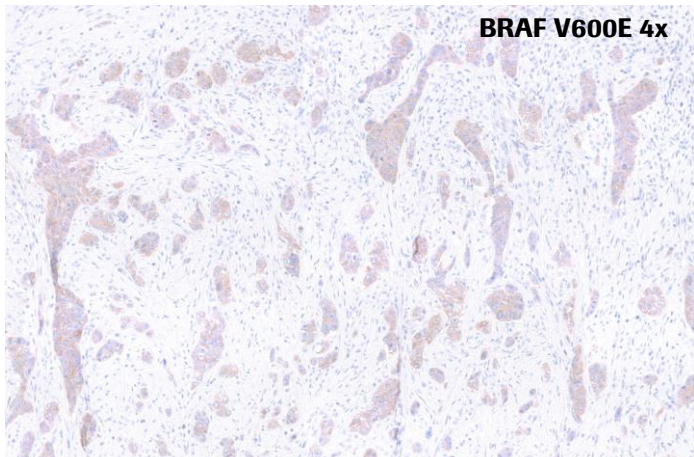
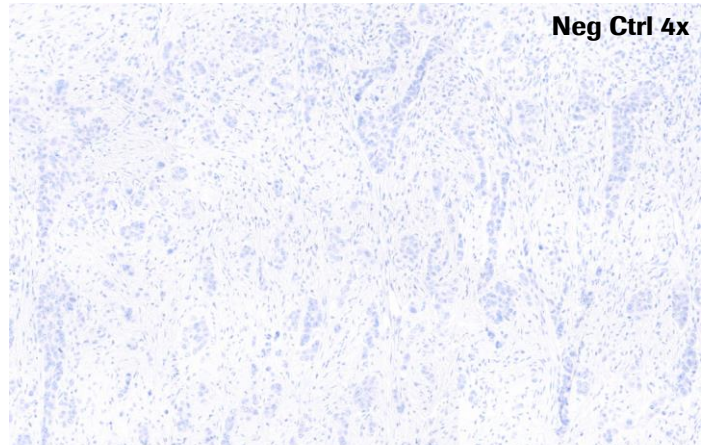
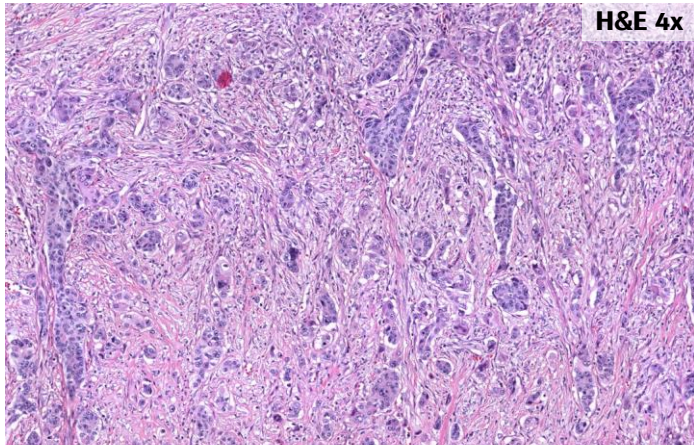


Figure 18: BRAF V600E Positive Status tissue exhibits a weak to moderate cytoplasmic staining.

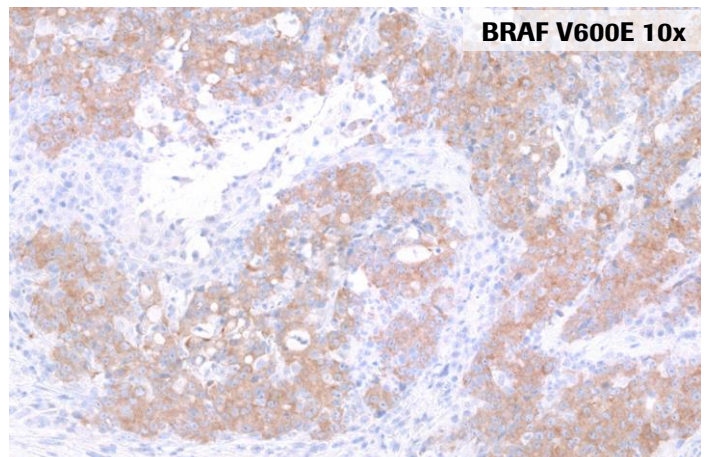
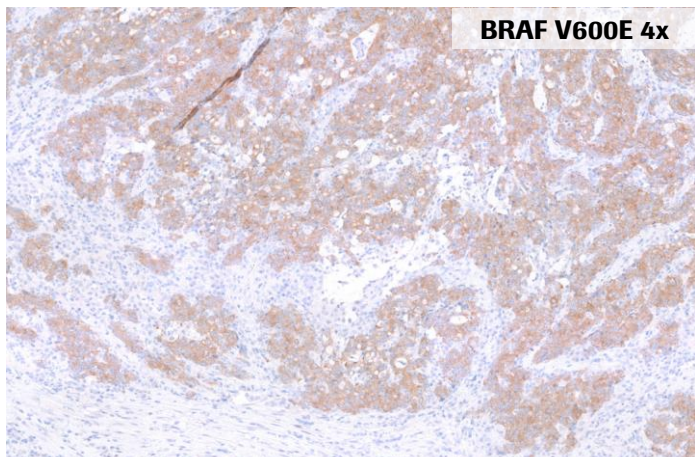
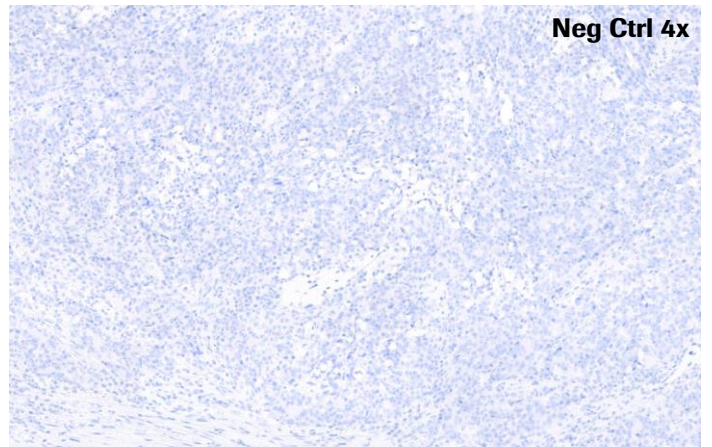
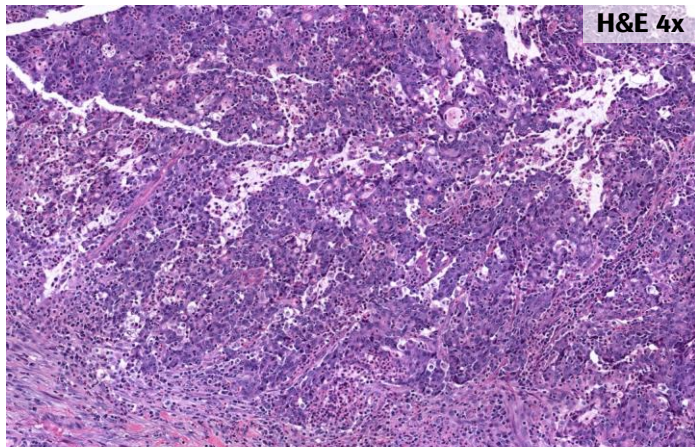


Figure 19: BRAF V600E Positive Status tissue exhibits moderate cytoplasmic staining.

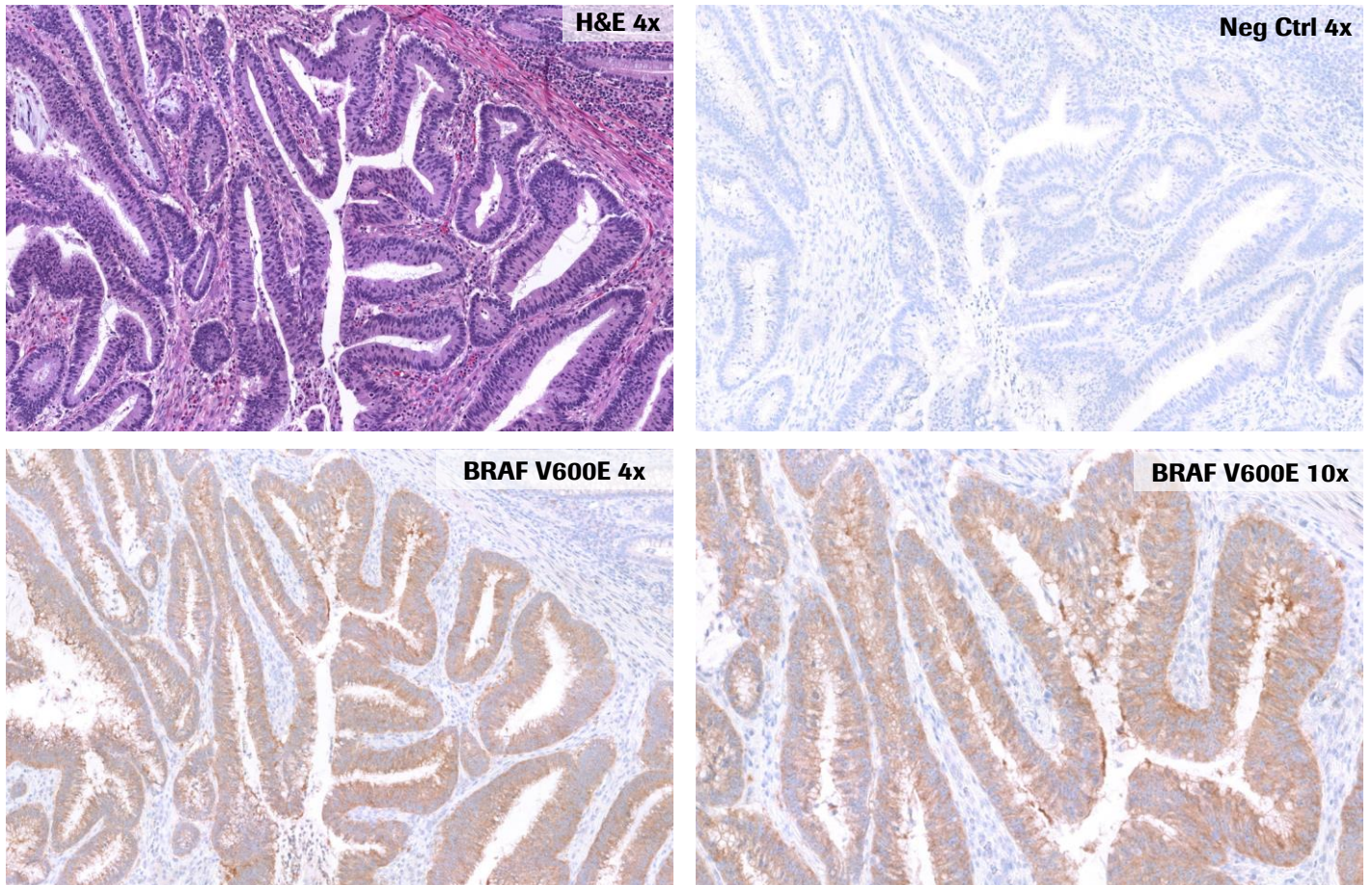


Figure 20: BRAF V600E Positive Status tissue exhibits strong cytoplasmic staining.

Challenging Cases

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

- **Non-specific background**

Some specimens may exhibit non-specific background staining for reasons that are not well understood. VENTANA anti-BRAF V600E (VE1) antibody was found to occasionally exhibit cytoplasmic background staining in smooth muscle and nuclear staining in normal colon epithelial cells, enterocytes, Leydig cells of testis, adrenal gland, pituitary gland and some tumor cells; however, such cases should not be considered as positive for the BRAF V600E mutation. In addition, this antibody also stains cilia in lung. For this reason, evaluation of a VENTANA anti-BRAF V600E (VE1) antibody stained slide must include a comparison to the negative control slide to determine the level of non-specific background staining.

- **Nuclear Staining**

Some specimens may only exhibit nuclear staining. Based on the VENTANA anti-BRAF V600E (VE1) antibody IHC scoring algorithm, these cases should be considered negative.

- **Tissue or Staining Artifact**

Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of VENTANA anti-BRAF V600E (VE1) antibody IHC Clinical Status. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

- **Heterogeneous Staining**

Some cases may show only focal, but unequivocal cytoplasmic staining. These cases are challenging and additional testing may be considered (Fig 25).

Some challenging cases are shown on the following pages.

Non-Specific Background Staining

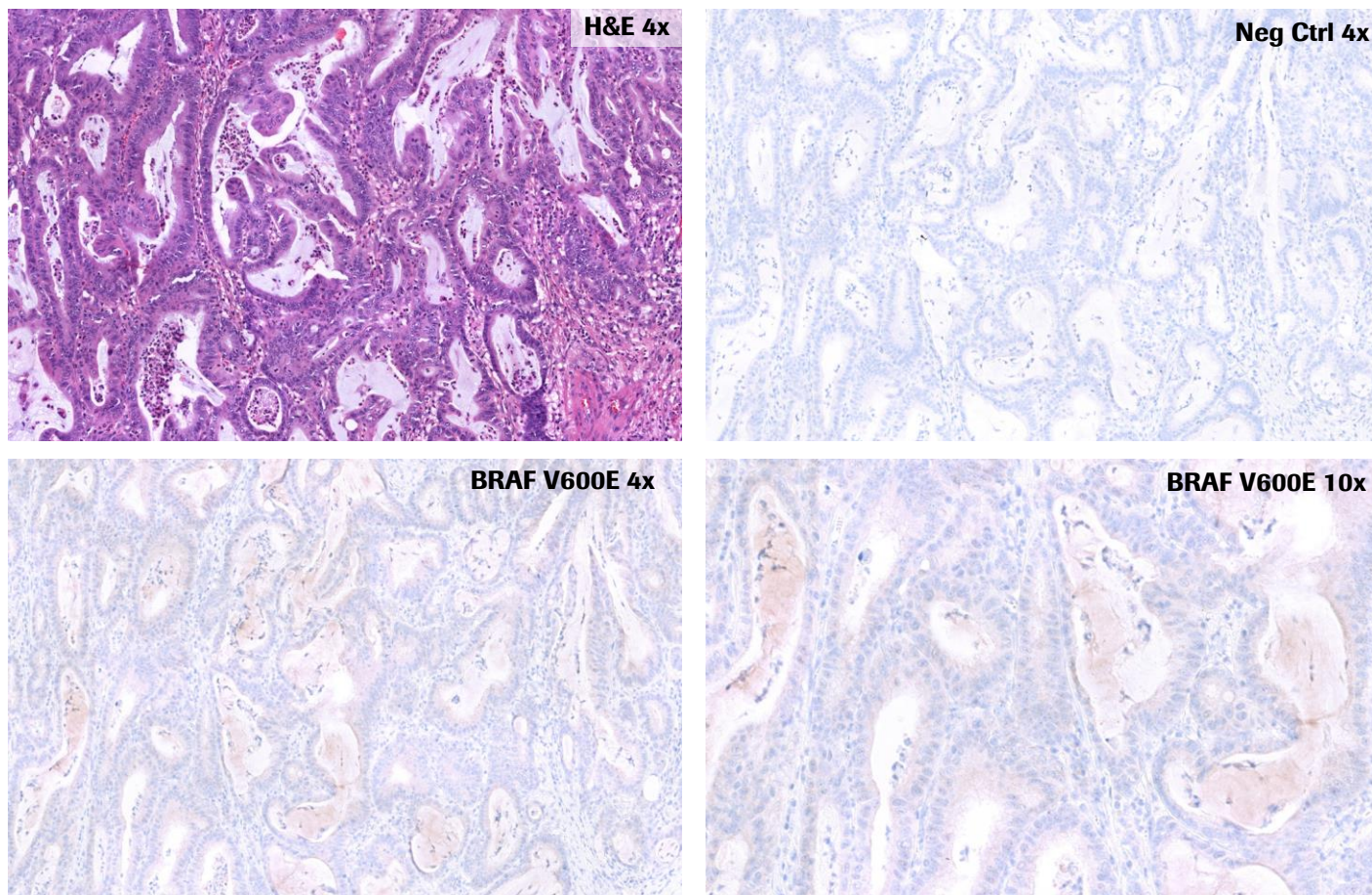


Figure 21: BRAF V600E Negative Status tissue exhibits a weak cytoplasmic blush throughout the entire tumor.

Non-Specific High Background Staining

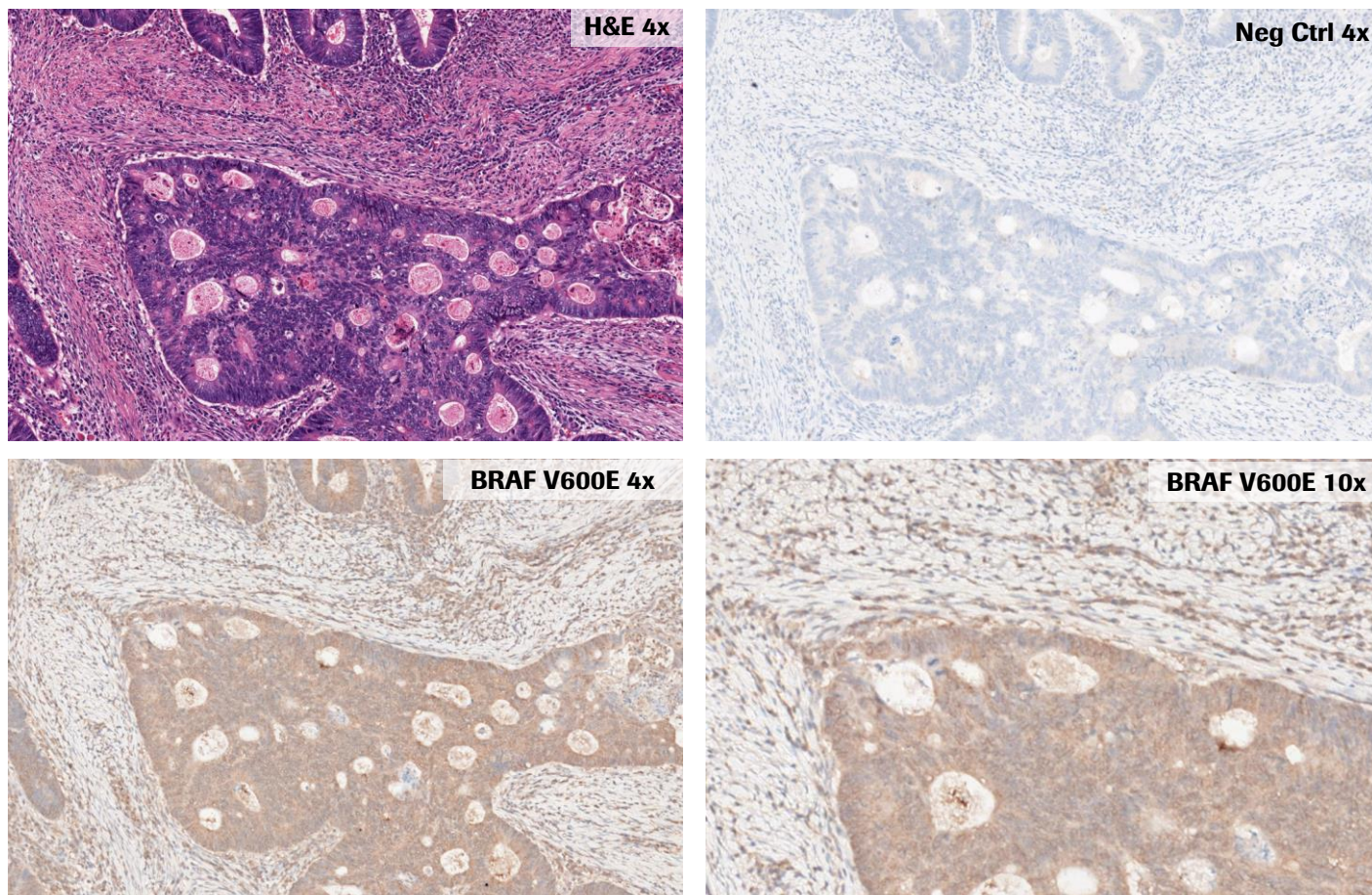


Figure 22: BRAF V600E Positive Status tissue exhibits a high non-specific background outside of the tumor cells.

Nuclear Staining

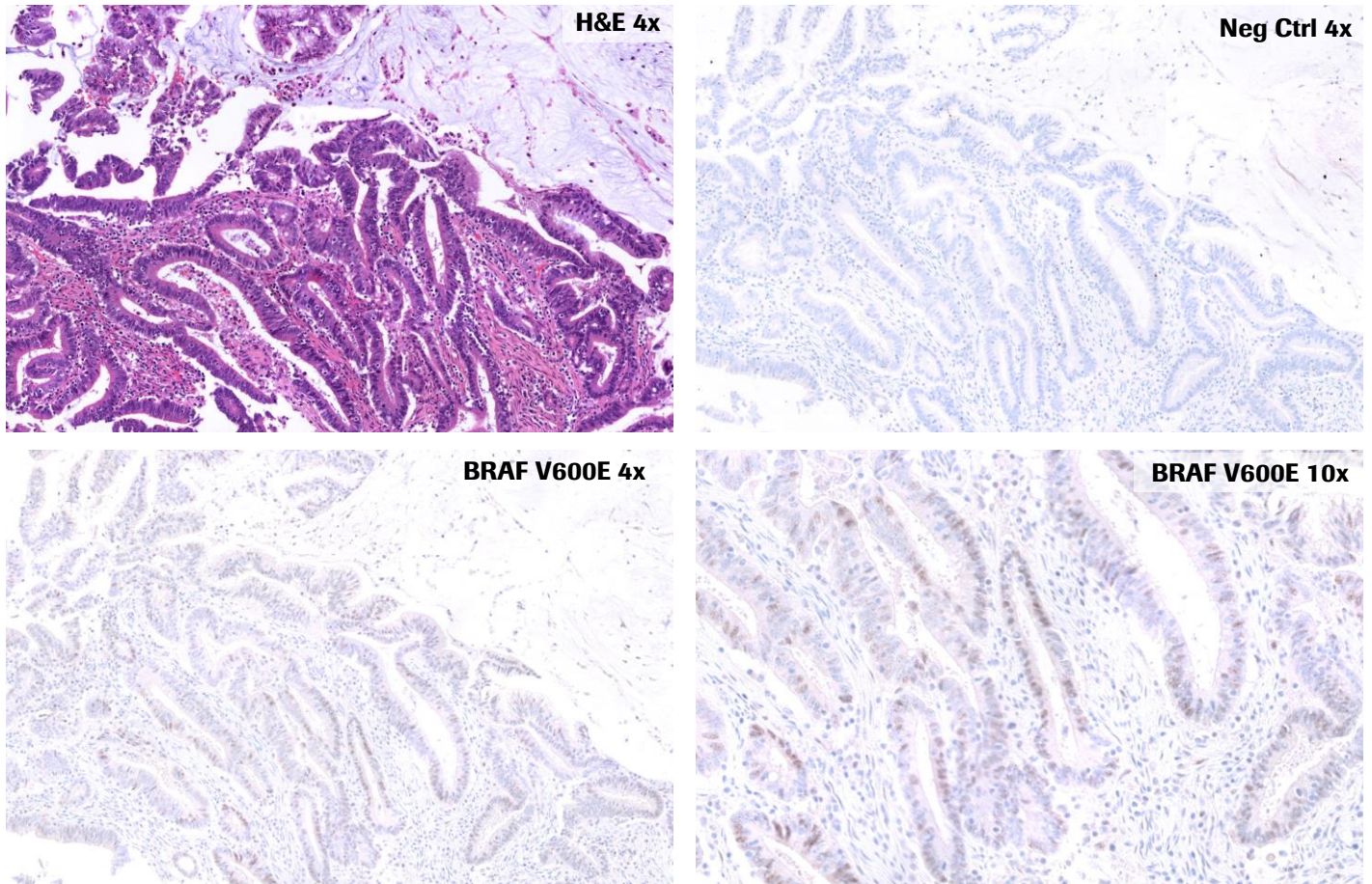


Figure 23: BRAF V600E Negative Status tissue exhibits no cytoplasmic staining in the tumor cells. Moderate nuclear staining is present in some cells, however, based on the interpretation criteria only cytoplasmic staining should be used in determining the status for BRAF V600E.

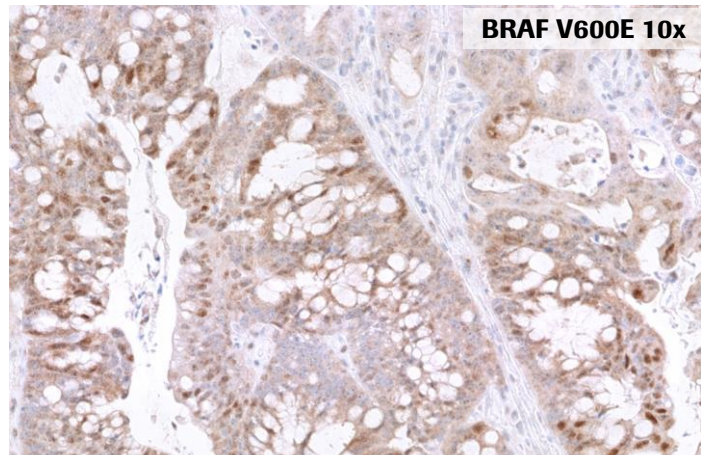
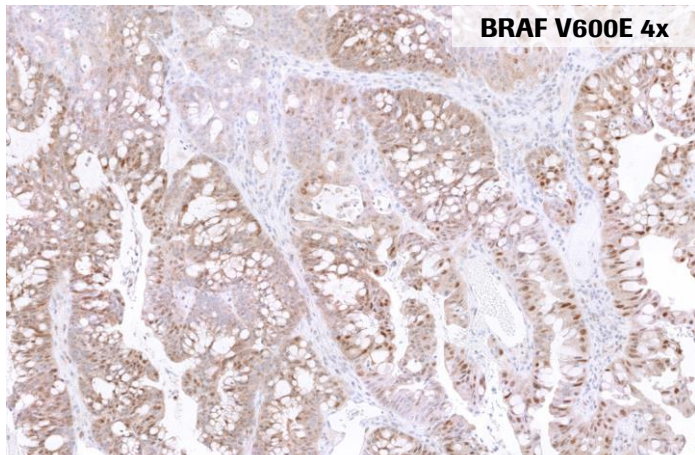
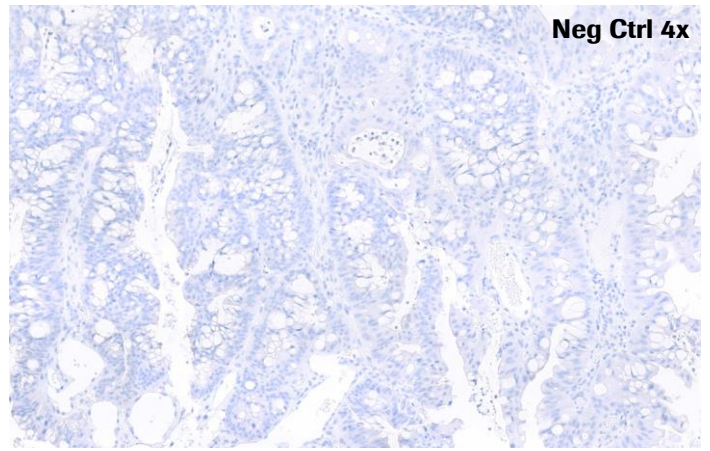
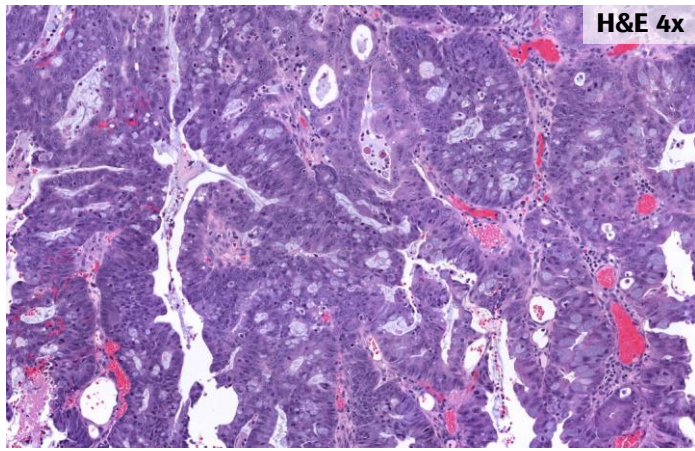


Figure 24: BRAF V600E Positive Status tissue exhibits unequivocal strong cytoplasmic staining within the tumor cells. Moderate nuclear staining is present in some cells, however, based on the interpretation criteria only cytoplasmic staining should be used in determining the status for BRAF V600E.

Focal Staining

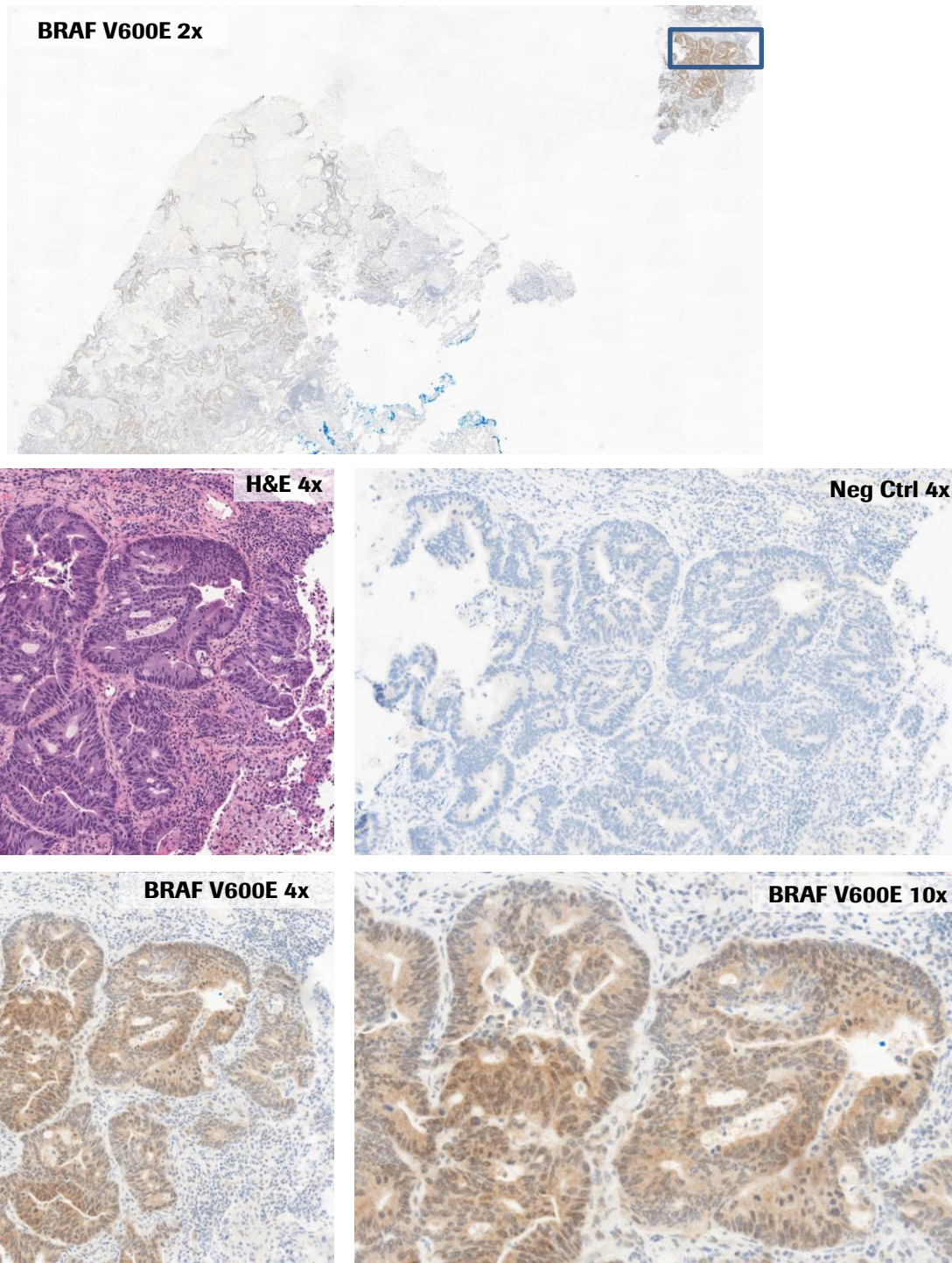


Figure 25: BRAF V600E Positive Status tissue exhibits heterogeneous BRAF V600E staining intensity. Weak equivocal cytoplasmic staining is present in the majority of the tumor, with focal moderate to strong unequivocal cytoplasmic staining.

VENTANA MMR IHC Panel Case Examples

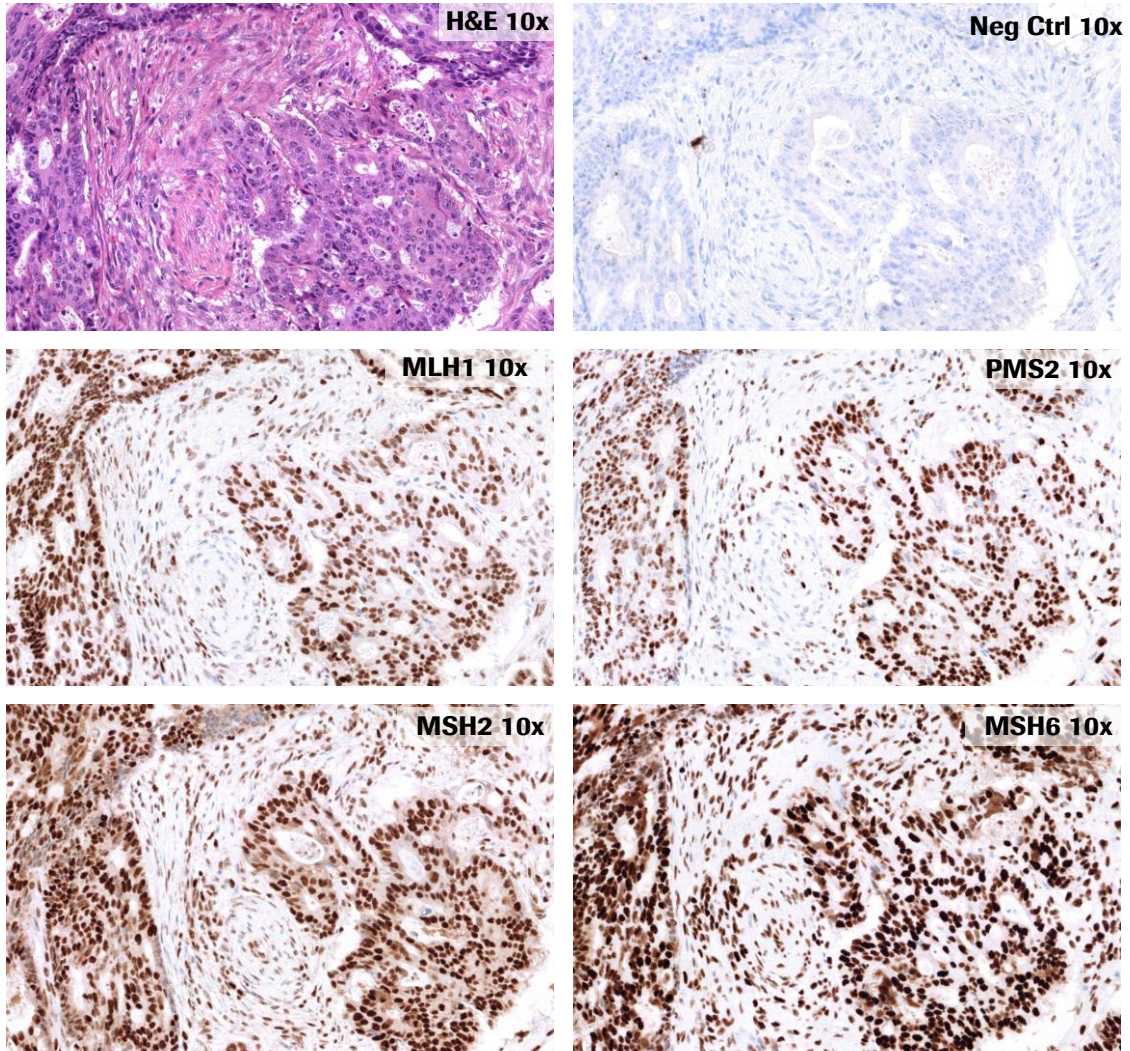


Figure 26: Proficient CRC this case exhibits proficient DNA mismatch repair status (pMMR) for the four MMR markers in the VENTANA MMR IHC Panel.

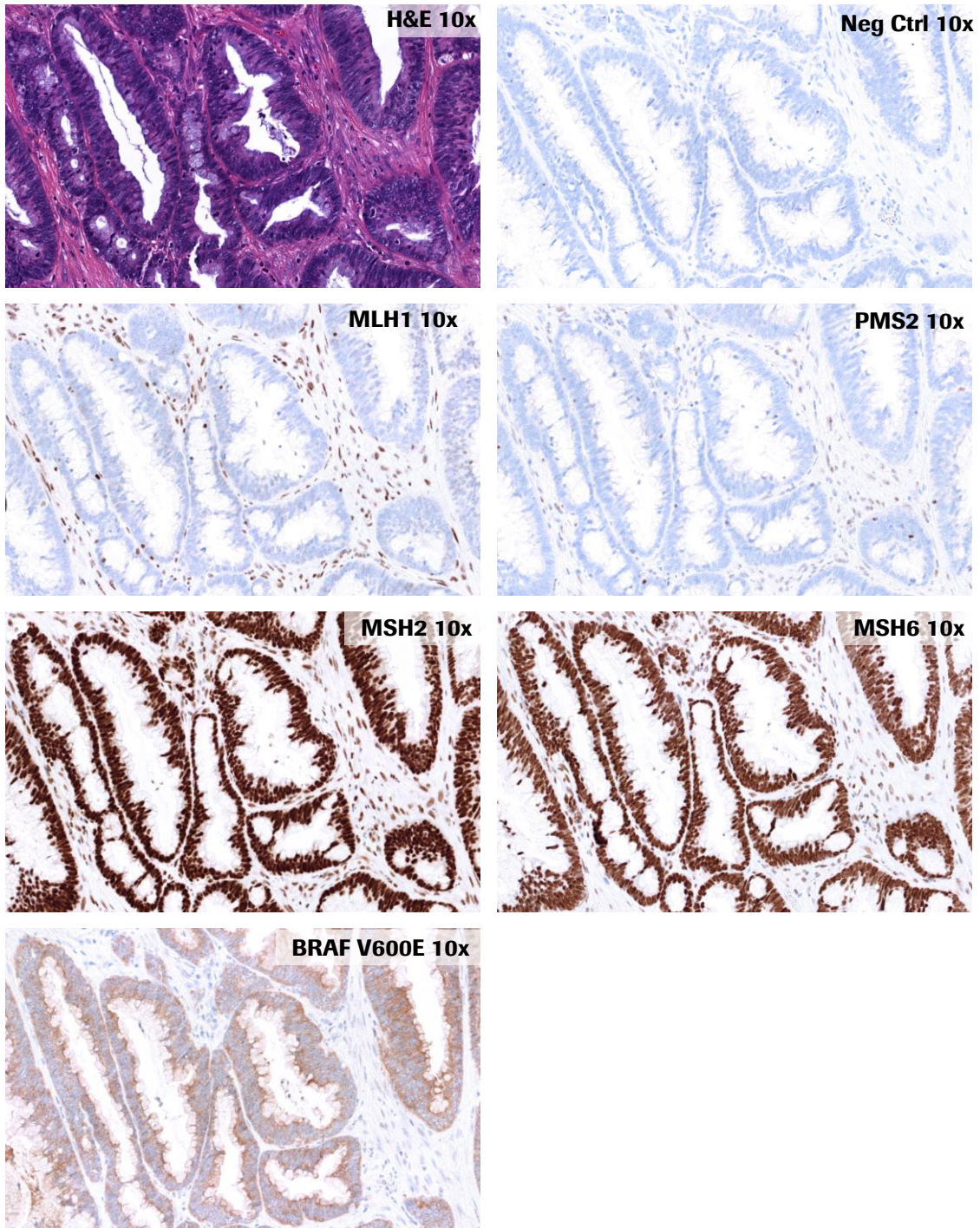


Figure 27: Sporadic CRC this case exhibits deficient DNA mismatch repair status (dMMR) due to loss of MLH1 and PMS2 with intact MSH2 and MSH6. BRAF V600E is positive for this case.

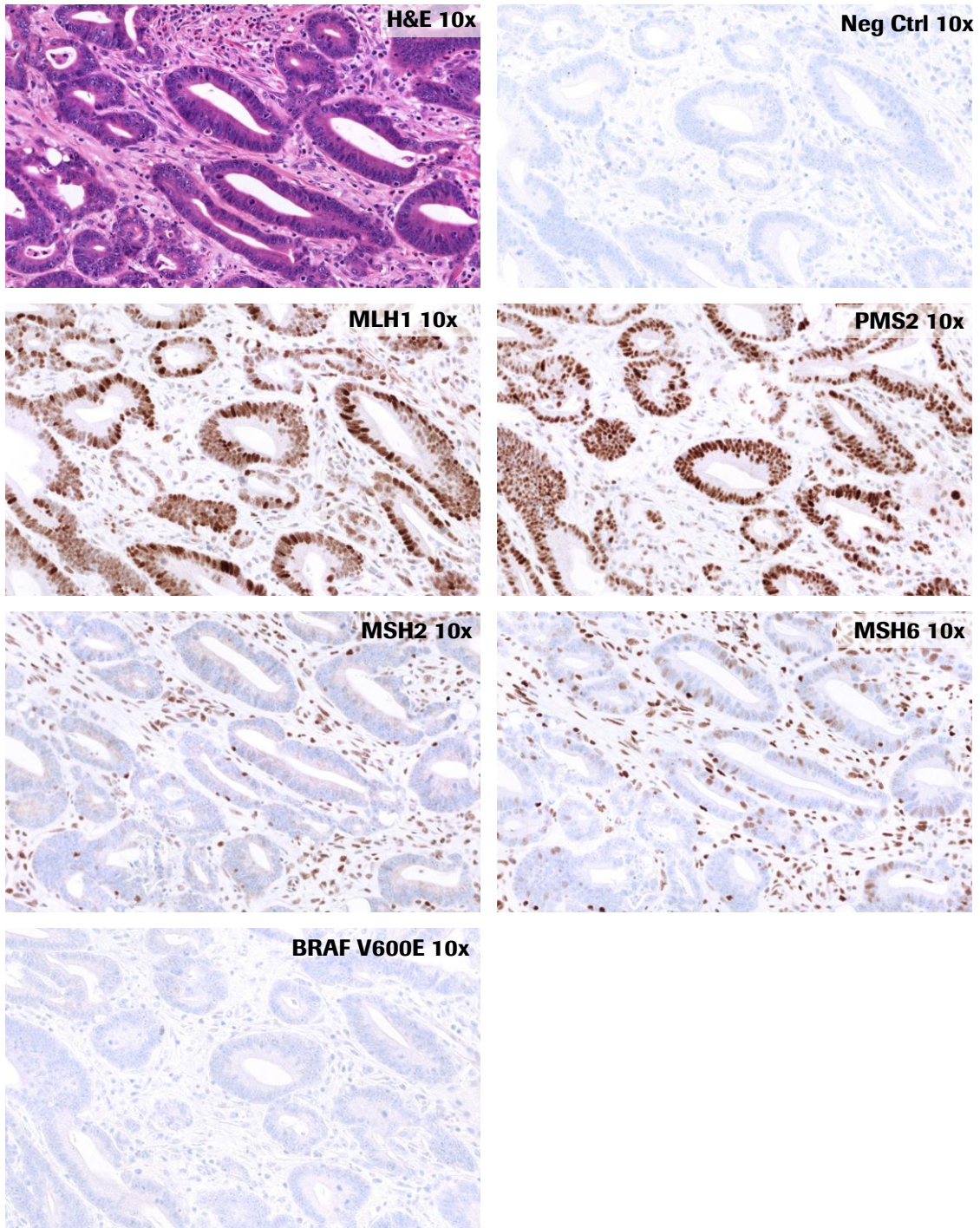


Figure 28: Possible Lynch Syndrome this case exhibits deficient DNA mismatch repair status (dMMR) due to MSH2 and MSH6 loss, with intact MLH1 and PMS2. BRAF V600E is negative for this case.

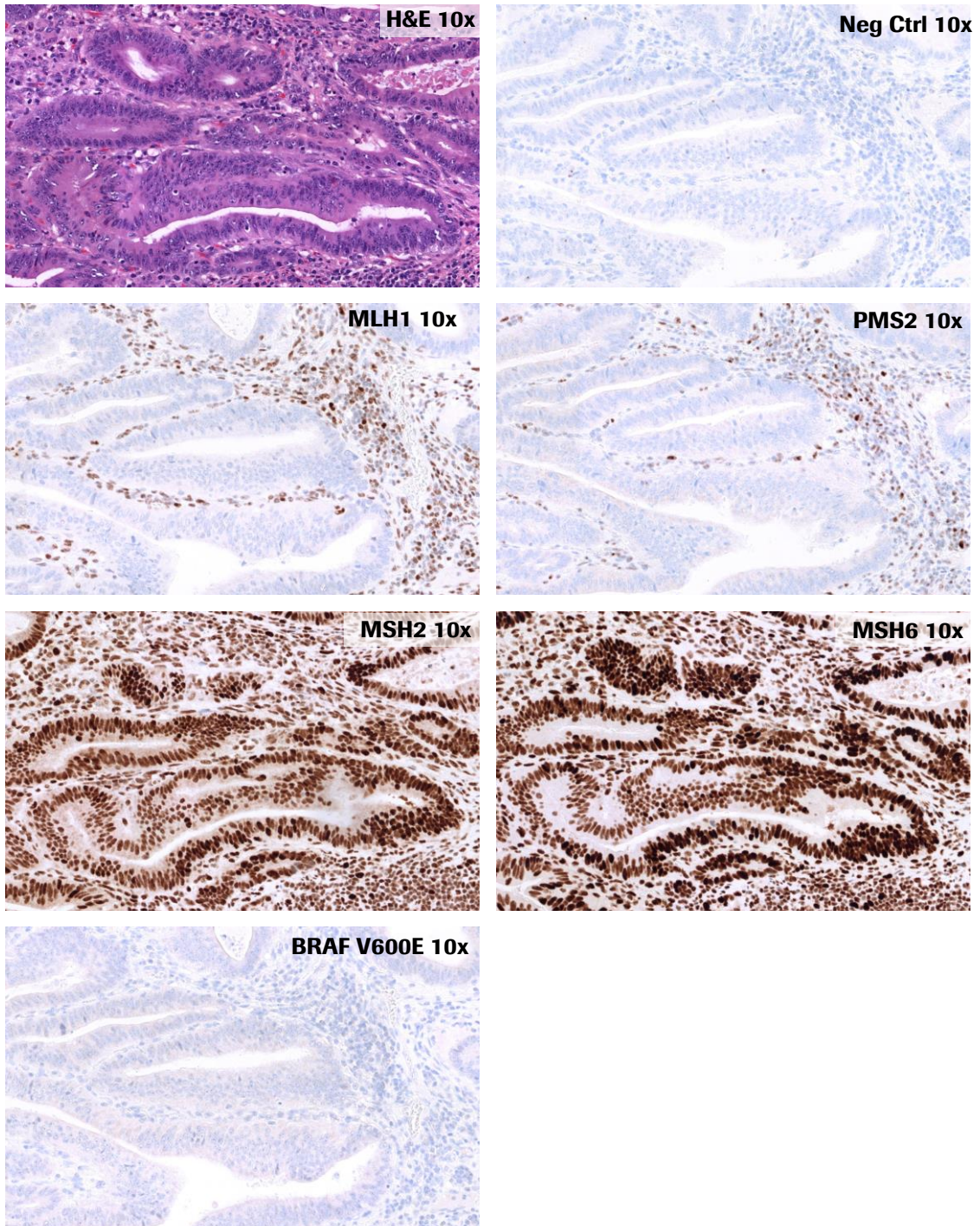


Figure 29: Possible Lynch Syndrome this case exhibits deficient DNA mismatch repair status (dMMR) due to MLH1 and PMS2 loss, with intact MSH2 and MSH6, BRAF V600E is negative for this case.

Cut Slide Stability

Ventana recommends that sections approximately 4 μm in thickness should be cut and mounted on positively charged slides. Slides should be stained within 8 weeks of preparation.

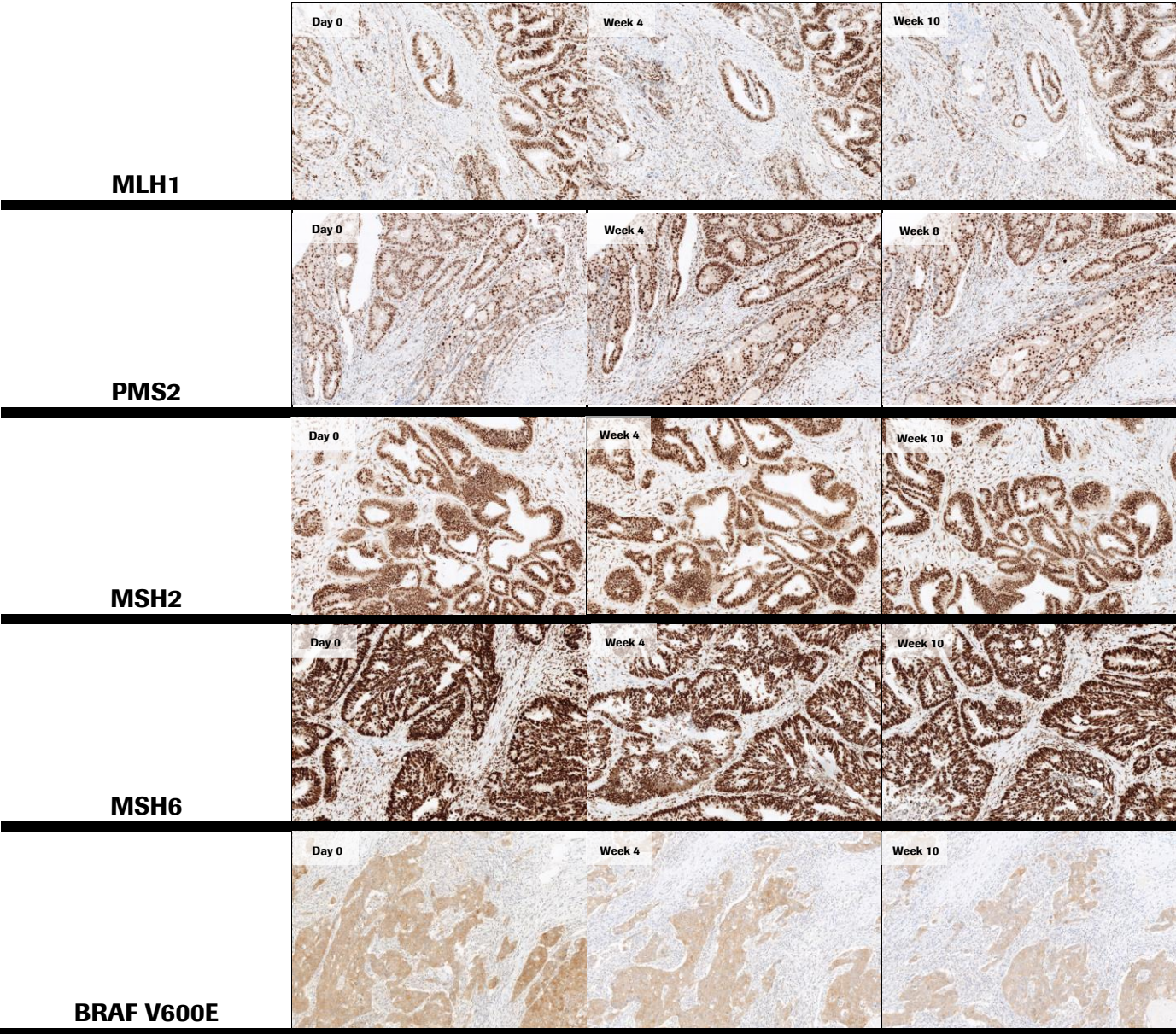


Figure 30: Cut Slide Stability Staining. This panel exhibits cases that have an intact status for MLH1, PMS2, MSH2 and MSH6 and a Positive status for BRAF V600E for the time points indicated in the figure.

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