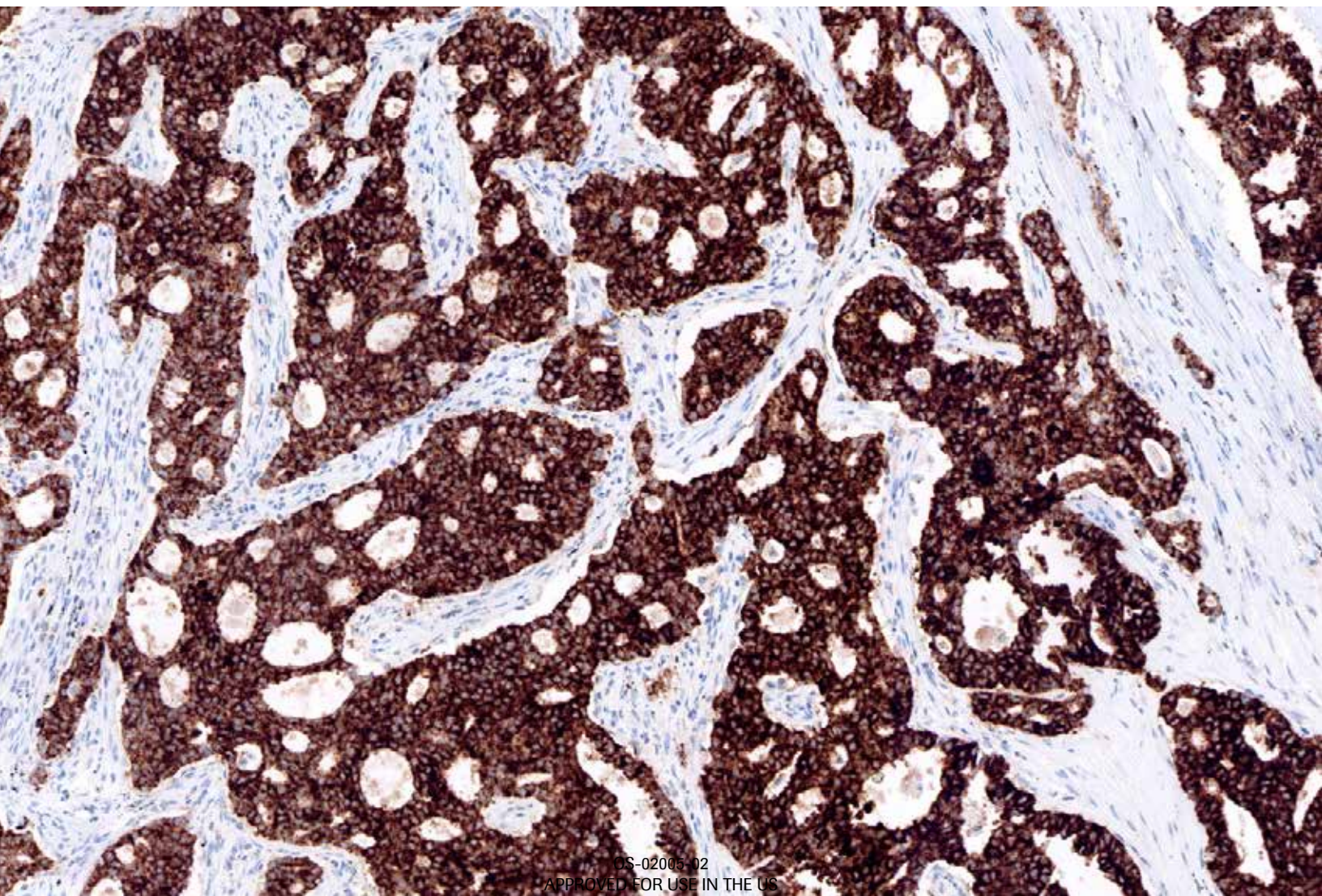


# **VENTANA ALK (D5F3) CDx Assay Interpretation Guide for Non-Small Cell Lung Carcinoma (NSCLC)**





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## Introduction

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The anaplastic lymphoma kinase (ALK) protein is a member of the insulin receptor superfamily of receptor tyrosine kinases.<sup>1</sup> ALK is a type I membrane glycoprotein that is normally expressed only in the nervous system.<sup>2</sup> ALK resides at chromosome 2p23 and is constructed mainly of 2 large introns and 26 exons.<sup>1</sup> The molecular pathogenesis of ALK begins with chromosomal rearrangements that partner the 3' coding sequences for the intracellular signaling domain with 5' promoter elements and coding sequences of other genes.

An inversion within chromosome 2p, resulting in the formation of a fusion gene product comprising portions of the echinoderm microtubule associated protein-like 4 (EML4) gene and the ALK gene, was discovered in 2007 in non-small cell lung carcinoma (NSCLC) cell lines and archived clinical specimens.<sup>3</sup> Studies indicated that EML4-ALK inversion events included at least 9 fusion variants, all containing the same portion of the ALK C-terminal kinase domain, rendering them catalytically active.<sup>4-8</sup> Consistent with this, EML4-ALK expression in lung alveolar epithelial cells in transgenic mice was a potent oncogenic factor.<sup>9</sup> The fusion renders ALK in a cytoplasmic localization.

ALK is now recognized as a key oncogenic driver in NSCLC, and although EML4 is the predominant fusion partner, other fusion partner genes have been identified.<sup>10,11</sup> The incidence of ALK gene rearrangements appears to range from 2-7%, translating to approximately 6,000 ALK positive patients/year in the United States and approximately 40,000 patients/year worldwide.<sup>3,4,7</sup> However, there are limitations to this estimation, including a small dataset (1,500 tumor samples) and the different ALK methodologies used across studies. Notably, the vast majority of ALK gene rearrangements were observed in lung adenocarcinoma specimens compared with squamous or small cell histologies.<sup>3-8</sup> There is also evidence that ALK gene rearrangements tend to correlate with patients who are of "never or light" smoking status, although this may not be a statistically significant cofactor.<sup>3,4,7,9</sup> Importantly, ALK gene rearrangements are rarely coincident with EGFR, HER2, or KRAS mutations, demonstrating that ALK positivity is a distinct disease subtype.<sup>9</sup>

Ventana has developed the VENTANA ALK (D5F3) CDx Assay, which is comprised of the VENTANA ALK (D5F3) CDx Assay antibody, the OptiView DAB IHC Detection Kit, and OptiView Amplification Kit, as a fully automated immunohistochemistry (IHC) assay on a VENTANA BenchMark XT or BenchMark ULTRA automated slide stainer. The sensitivity of the IHC assay enables a reproducible, binary scoring system (Positive or Negative for ALK status) for evaluating the staining results (refer to the package insert for VENTANA ALK (D5F3) CDx Assay, Cat. No. 790-4796 / 06687199001). Ventana used a range of human NSCLC tissue specimens in developing the ALK IHC assay from formalin-fixed, paraffin-embedded (FFPE) primary and metastatic NSCLC tumors, such as resection, needle biopsies, bronchial biopsies, and cell blocks from FNA.





## Intended Use

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## Intended Use

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### Intended Use of Product

Refer to VENTANA ALK (D5F3) CDx Assay package insert for the detailed intended use of this product.

Note: Use of this diagnostic with indicated therapies may not be approved in all countries. Please consult your local Roche representative for local approvals.

### Assay Interpretation

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

This product is intended for *in vitro* diagnostic (IVD) use.

### 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) NSCLC sections stained with the VENTANA ALK (D5F3) CDx Assay in accordance with the proposed product labeling.
- Provide photographic images that illustrate the staining patterns that may result from staining of NSCLC tissues with the VENTANA ALK (D5F3) CDx Assay.
- Provide example images of challenging cases to provide guidance in their evaluation.
- Provide guidance in using an ALK-positive control tissue (e.g., appendix), which serves as a tissue control when stained with the VENTANA ALK (D5F3) CDx Assay.



## Clinical Evaluation

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## Clinical Evaluation

### Evaluating the VENTANA ALK (D5F3) CDx Assay in NSCLC

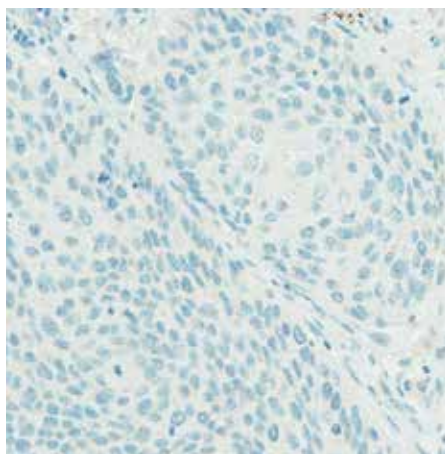
For the VENTANA ALK (D5F3) CDx Assay, each case is stained with the VENTANA ALK (D5F3) CDx Assay and a Rabbit Monoclonal Negative Control Ig antibody. Neoplastic cells labeled with the VENTANA ALK (D5F3) CDx Assay are evaluated for presence or absence of the DAB signal. The matched negative control slide is used to assess non-specific background staining and degree of background staining known to occur due to specific tissue elements (refer to positive and negative case images below). Please note: All cases must be stained with the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit, because some cases are weakly positive for ALK by DAB detection only.

The scoring algorithm for VENTANA ALK (D5F3) CDx Assay is provided below in Table 1. Representative cases are discussed in the images.

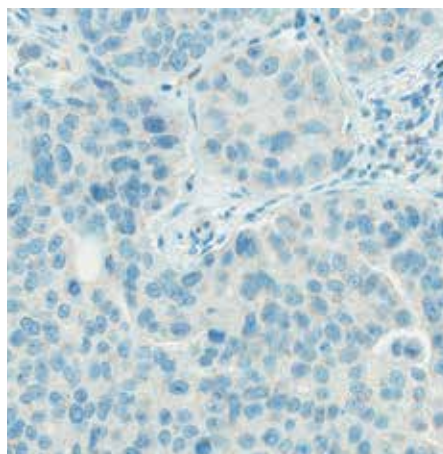
**Table 1: Scoring Criteria for Determination of ALK status in NSCLC.**

Clinical Interpretation	Staining Description
Positive for ALK	<p>Presence of strong granular cytoplasmic staining in tumor cells (any percentage of positive tumor cells). Certain staining elements should be excluded, including:</p> <ul style="list-style-type: none"><li>▪ light cytoplasmic stippling in alveolar macrophages,</li><li>▪ cells of neural origin (nerve and ganglion cells),</li><li>▪ glandular epithelial staining, and</li><li>▪ scattered lymphoreticular cells within lymphocytic infiltrate.</li></ul> <p>Some background staining also may be observed within normal mucosa in NSCLC (including mucin) and in necrotic tumor areas, which should be excluded from the clinical evaluation.</p>
Negative for ALK	Absence of strong granular cytoplasmic staining in tumor cells.

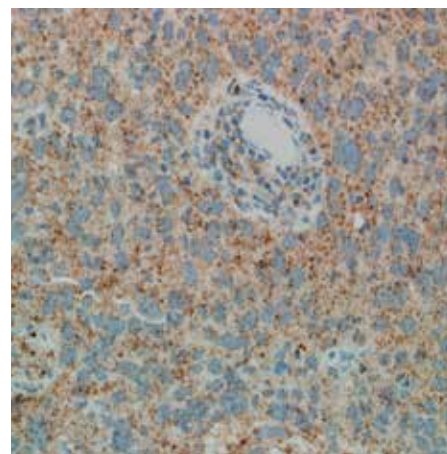
## Clinical Diagnosis Negative



No staining

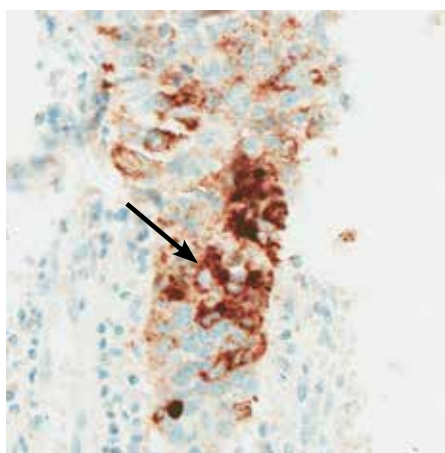


Light speckling in tumor cells

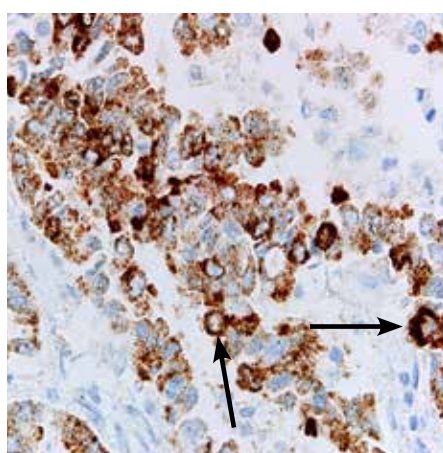


Non-specific diffuse granular staining

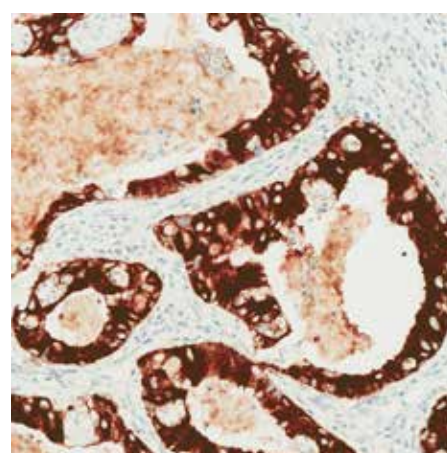
## Clinical Diagnosis Positive



Few strong cytoplasmic staining tumor cells



Strong cytoplasmic staining tumor cells

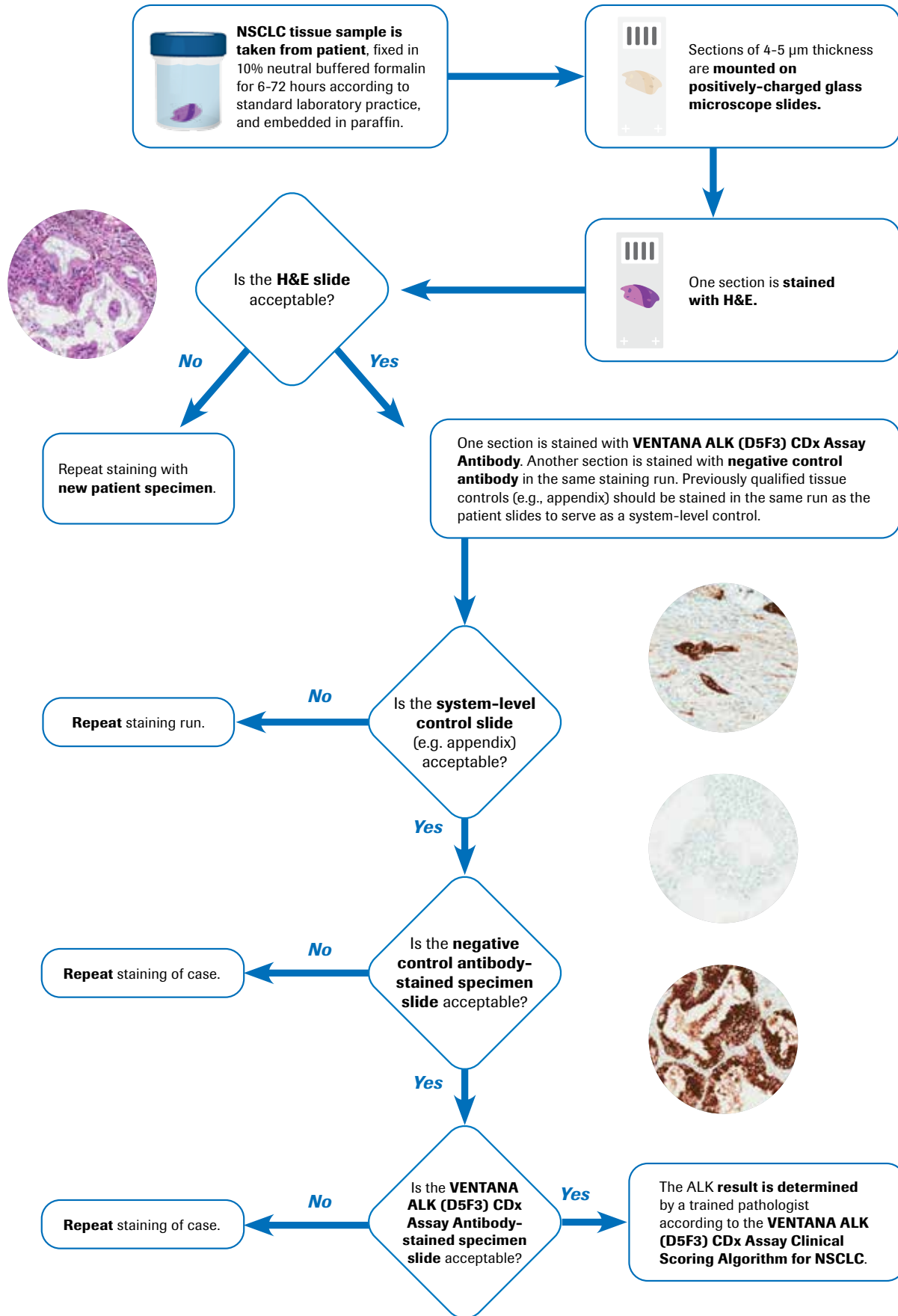


Homogeneously strong cytoplasmic staining within tumor cells

Staining requires three serial sections from each case, one tissue section for hematoxylin and eosin (H&E) staining, a second tissue section for isotype Rabbit Monoclonal Negative Control Ig antibody staining and a third tissue section for VENTANA ALK (D5F3) CDx Assay staining. If the H&E evaluation indicates that the patient specimen is inadequate, then a new specimen should be obtained and stained with the VENTANA ALK (D5F3) CDx Assay.

Pre-qualified NSCLC tissues positive and negative for ALK per the scoring algorithm in Table 1 may be used as system-level run controls. Alternately, pre-qualified appendix tissue exhibiting strong ALK staining of the ganglion cells and absence of strong granular staining in lymphoid, myenteric plexus, and glandular cells of the appendix may also be used as system-level run controls. Both positive and negative elements must be stained appropriately as defined by the scoring algorithm for NSCLC (Table 1) or acceptance criteria for appendix (Table 2) on each run for the run to be considered valid. Specimen slides stained using VENTANA ALK (D5F3) CDx Assay should be assessed by a trained pathologist. If the tissue control slide stained using the VENTANA ALK (D5F3) CDx Assay is not acceptable, staining of patient samples should be repeated. A non-evaluable slide stained using VENTANA ALK (D5F3) CDx Assay would mean that determination of reactivity is not possible due to necrosis, absent tissue, or artifacts.

# Specimen Flow



## System-Level Control

Either appendix tissue with positive and negative staining elements or ALK-positive and ALK-negative NSCLC tissues are recommended for use as system-level run control tissues. Ganglion cells of the appendix show strong cytoplasmic granular staining for ALK. Additionally, strong cytoplasmic granular staining has also been observed in nerve of the muscular layer of the appendix. Absence of strong granular cytoplasmic staining in glandular epithelial cells, muscle and lymphoid tissue of the appendix (scant or rare staining of lymphoreticular cells may be observed in lymphoid aggregates) should be assessed as negative per the acceptance criteria found in Table 2. ALK-positive and ALK-negative NSCLC tissues sourced for use as system-level run controls should be evaluated based on the ALK Scoring Algorithm for NSCLC found above in Table 1.

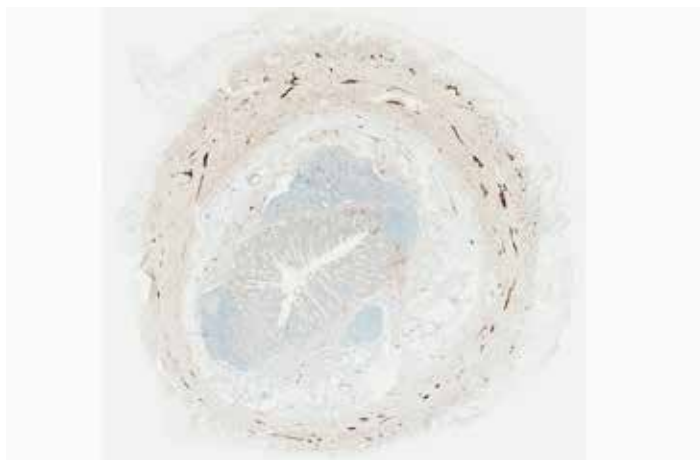
A positive control tissue should be a fresh autopsy/biopsy/surgical specimen that is fixed and processed in the same manner as the patient specimens and should be run for each set of test conditions with every VENTANA ALK (D5F3) CDx Assay performed. This tissue

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 may not be used as a control for fixation or tissue preparation. Positive strong granular cytoplasmic staining of ganglion cells and absence of strong granular cytoplasmic staining in the glandular epithelium, muscle, and lymphoid tissue in the appendix control specimen confirms that VENTANA ALK (D5F3) CDx Assay was applied and the instrument functioned properly. The positive tissue control should only be used to monitor performance and it should not be used to aid the clinical diagnosis of patient samples. In areas of marked inflammation in appendix, an increase in specific staining of neural/neuroendocrine structures and histiocytes in the lymphoid tissue may be observed when stained with the VENTANA ALK (D5F3) CDx Assay. This may be due to reactive hyperplasia of neural structures or drop out of other normal structures due to the inflammation. These structures were confirmed to be neural/neuroendocrine structures and histiocytes by additional antibody stains (S100, Synaptophysin, and CD68).

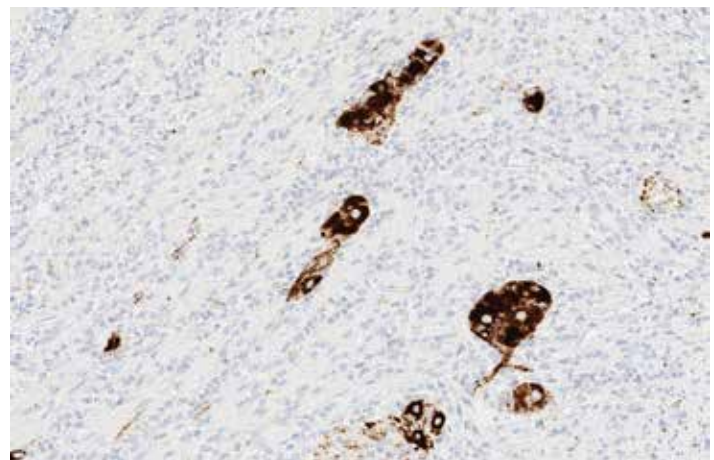
**Table 2: Acceptance Criteria for ALK staining in appendix control tissue**

Acceptable	Unacceptable
Presence of strong granular cytoplasmic staining in ganglion cells. <i>(See note)</i>	Absence of strong granular cytoplasmic staining in ganglion cells.
Absence of strong granular cytoplasmic staining in glandular epithelial cells, muscle and lymphoid tissue (scant or rare staining of lymphoreticular cells may be observed in lymphoid aggregates).	Excessive non-specific background staining of glandular epithelial cells, muscle, or lymphoid tissue that interferes with scoring.

*Note: The nerve in appendix muscular layers shows positive staining.*

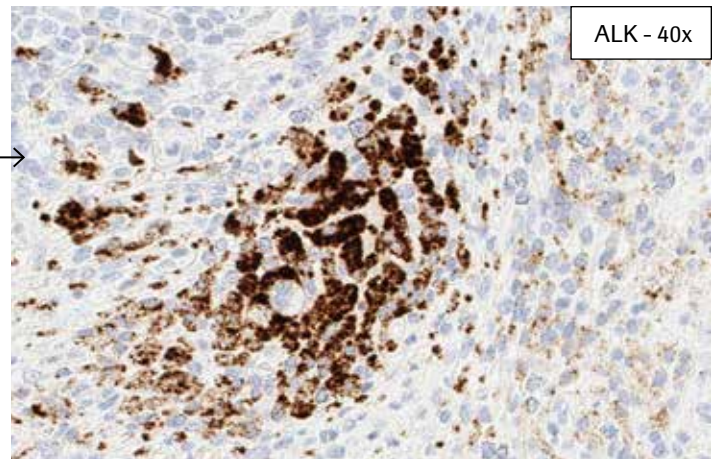
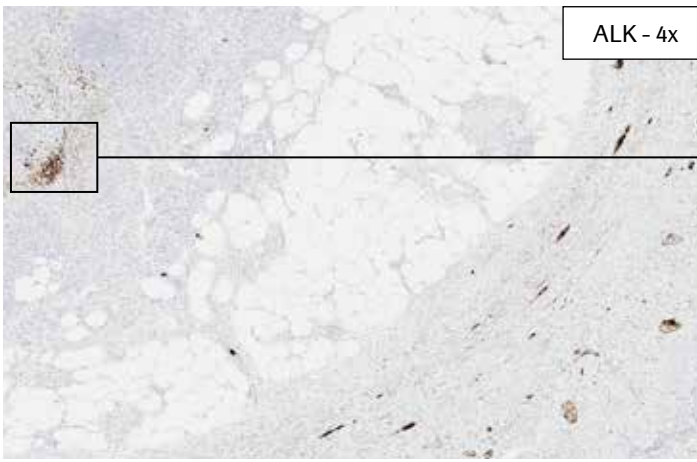


Appendix stained with the VENTANA ALK (D5F3) CDx Assay (Acceptable).

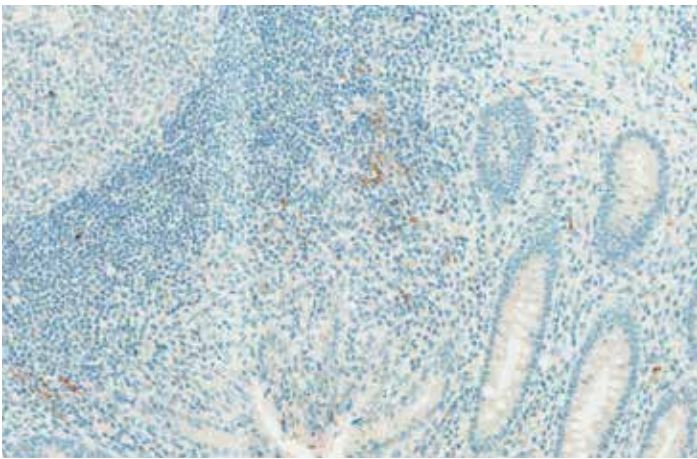


Strong granular cytoplasmic staining on the ganglion cell of the appendix and lack of excessive non-specific staining of the muscle layer (Acceptable).

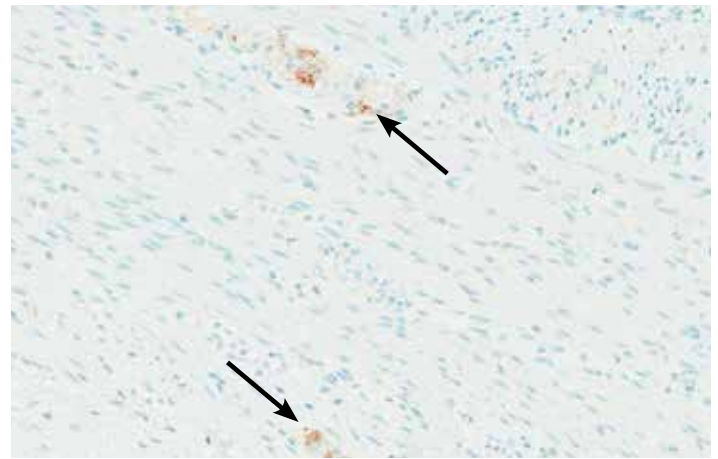




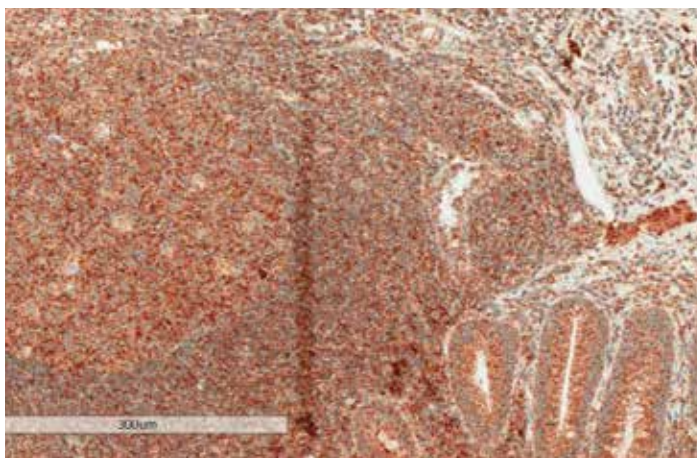
An increase in specific staining of neural structures may be observed in markedly inflamed appendix when stained with the VENTANA ALK (D5F3) CDx Assay. This staining should be excluded when evaluating negative-staining elements of the appendix tissue (Acceptable). Box inset shows area of 40x magnification.



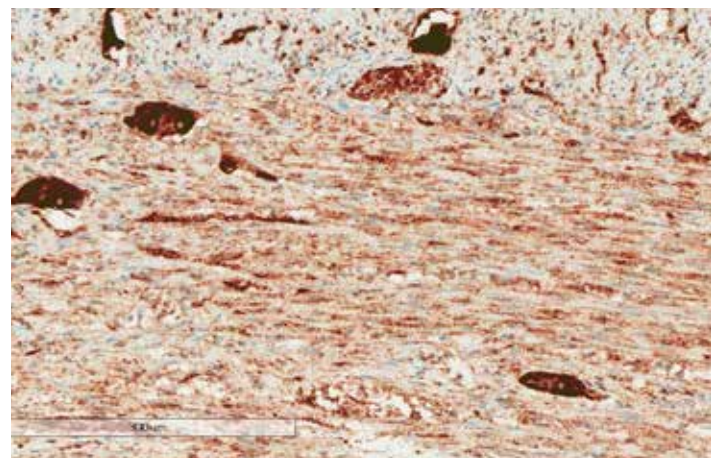
Lack of strong granular cytoplasmic staining in the lymphoid tissue and glandular epithelium (Acceptable).



Arrows indicate lack of strong granular cytoplasmic staining of the ganglion cells of the appendix (Not acceptable).



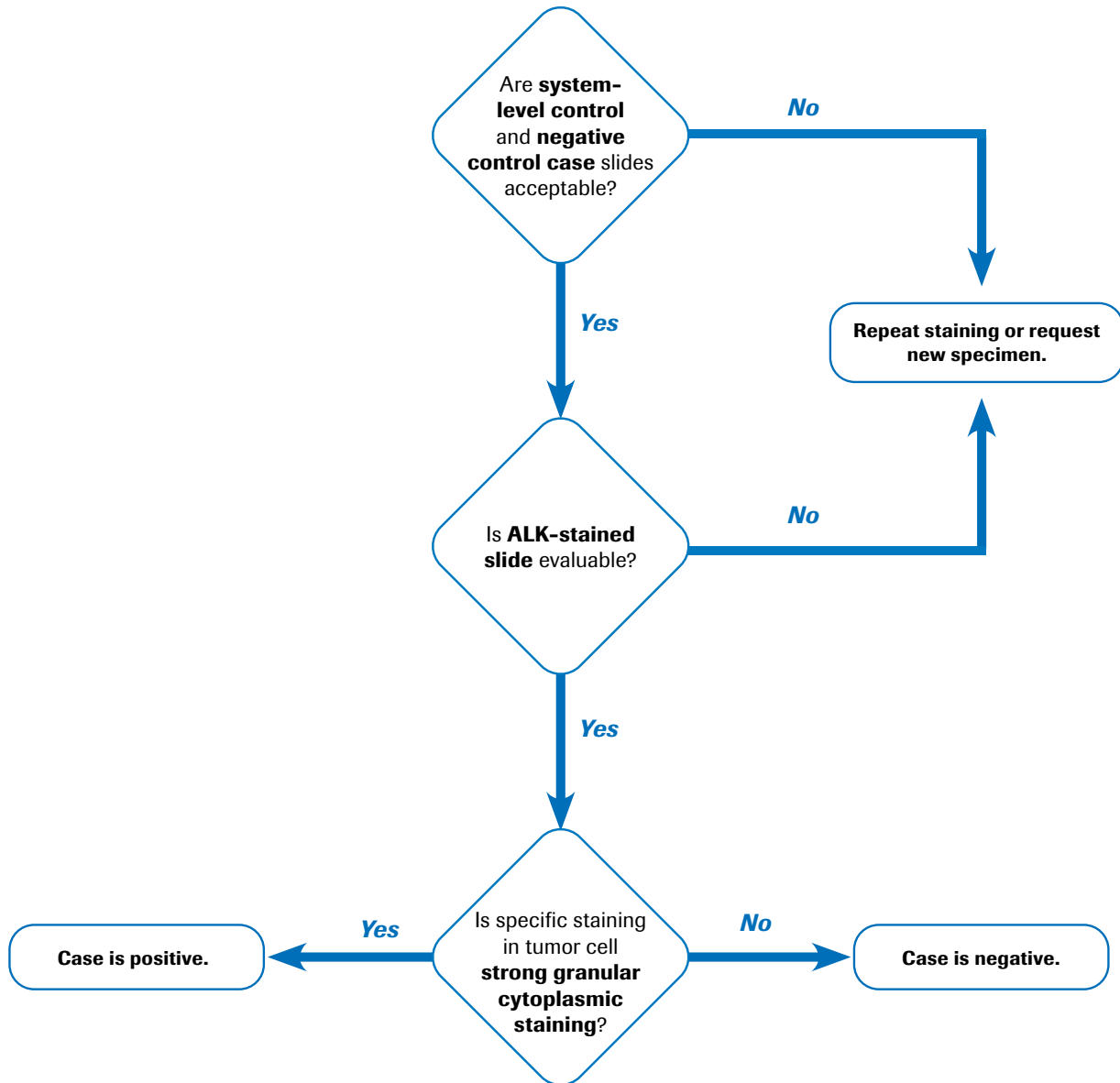
Inappropriate non-specific staining on the lymphoid tissue and glandular epithelium (Not acceptable).



Inappropriate non-specific staining of the muscle layer of the appendix (Not acceptable).

## Decision Tree

Slides stained with VENTANA ALK (D5F3) CDx Assay should be evaluated using the approach noted in the figure below.





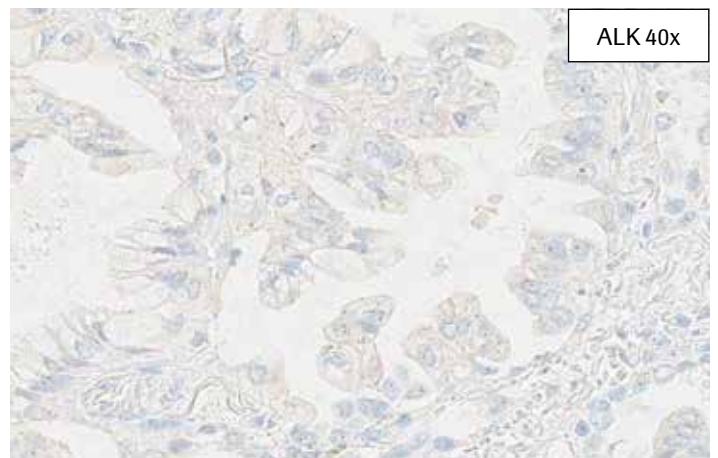
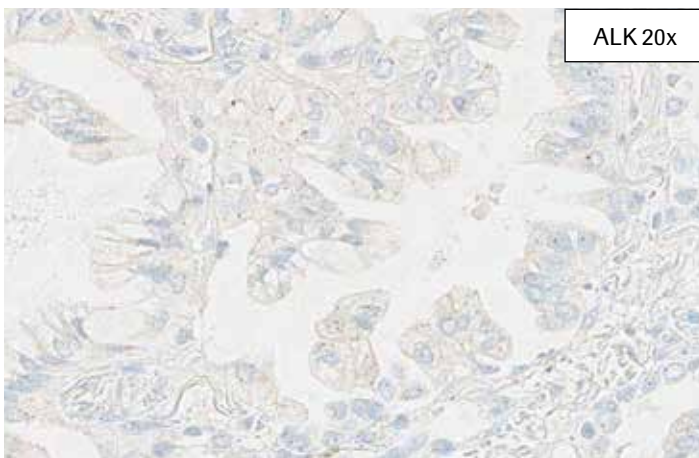
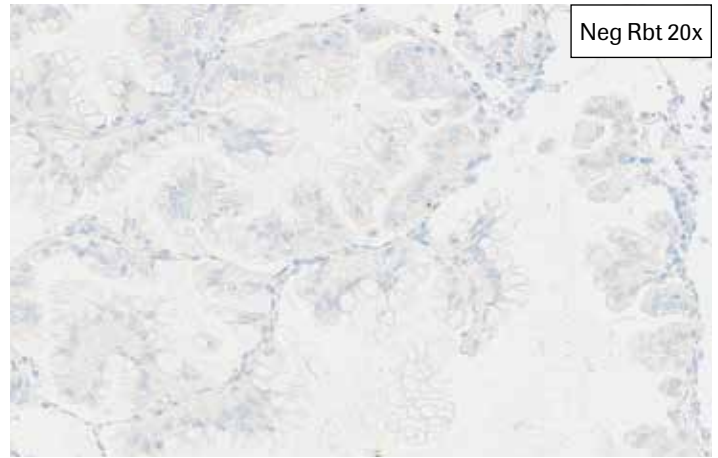
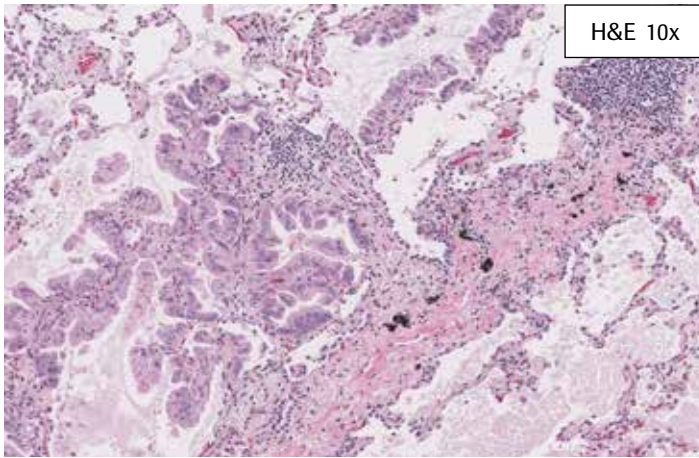


## Negative Cases

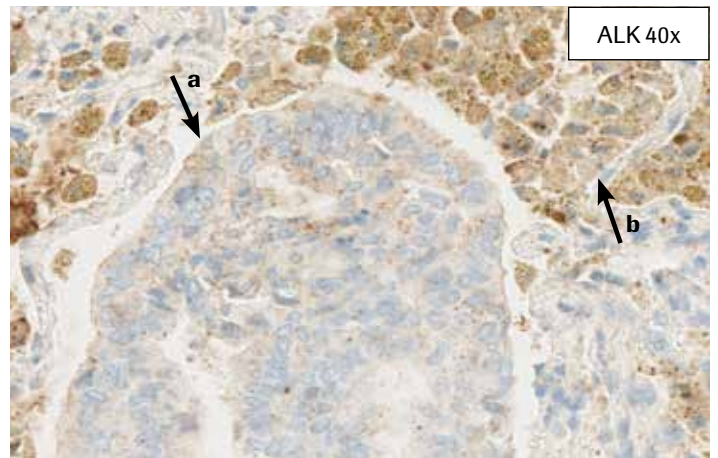
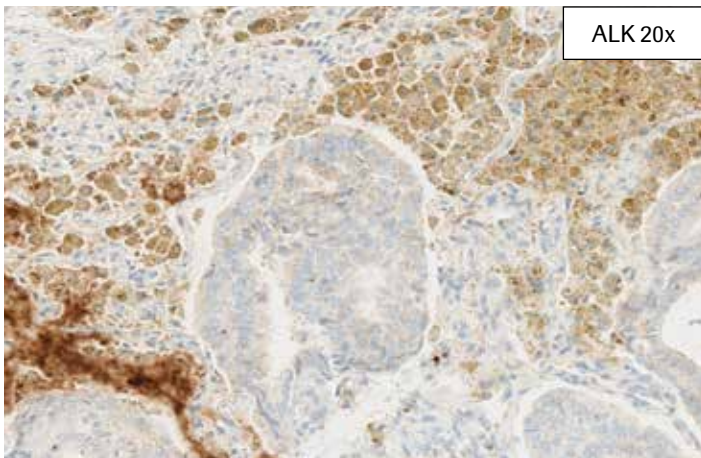
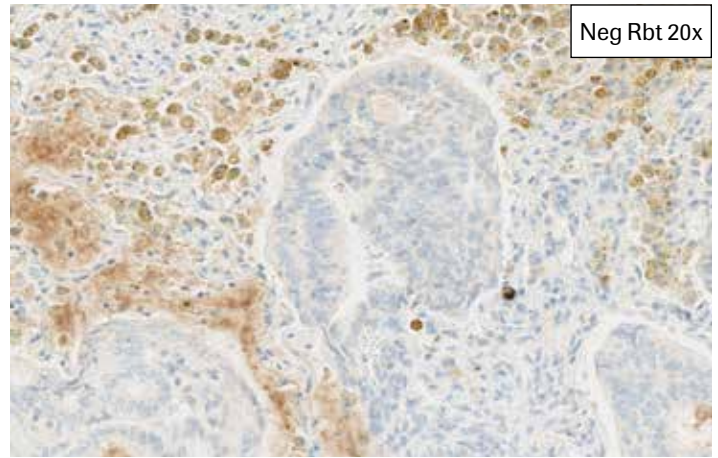
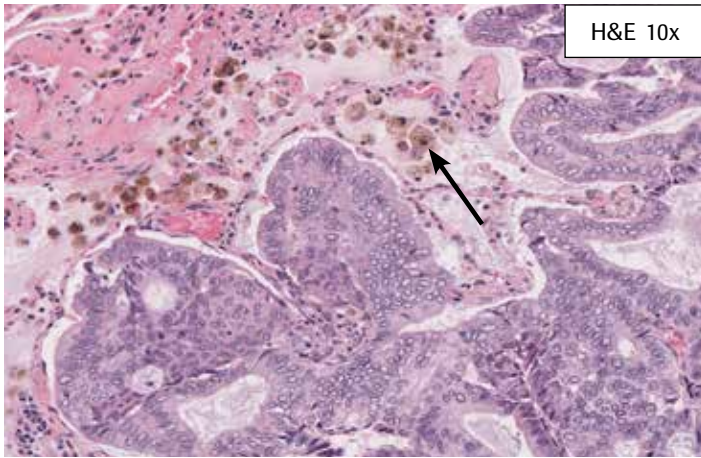
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## Negative Cases

A case is assigned a Negative Clinical Diagnosis for ALK status if no strong granular cytoplasmic staining is observed in any tumor cell.

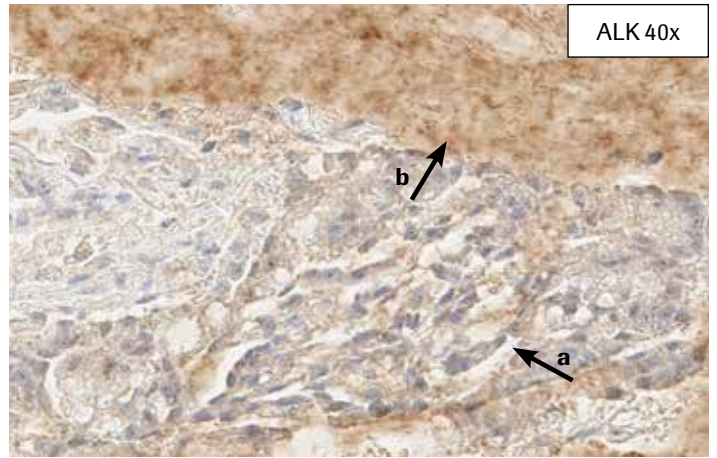
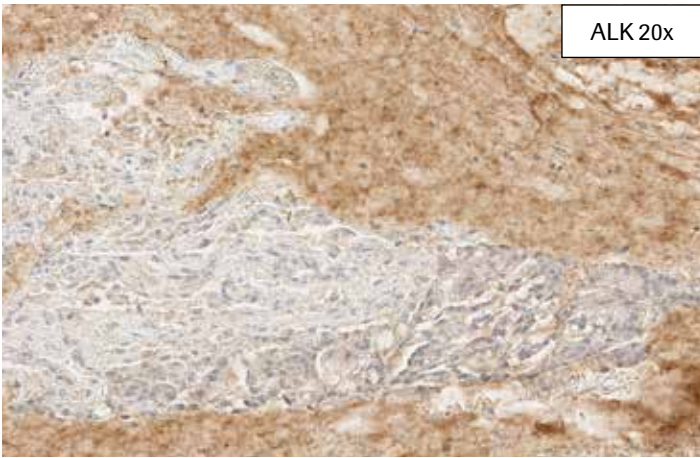
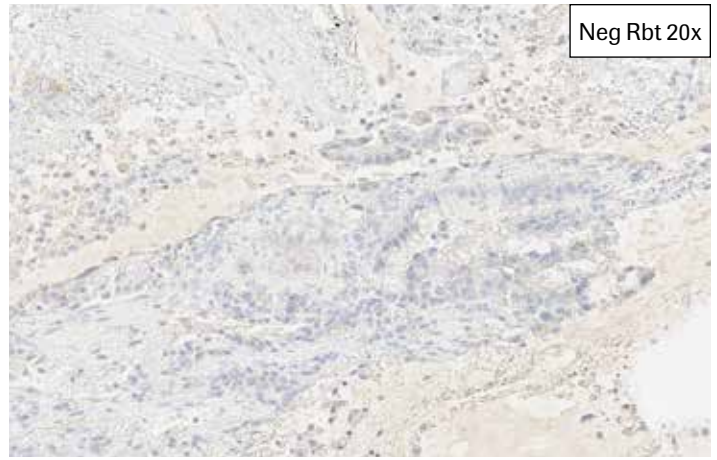
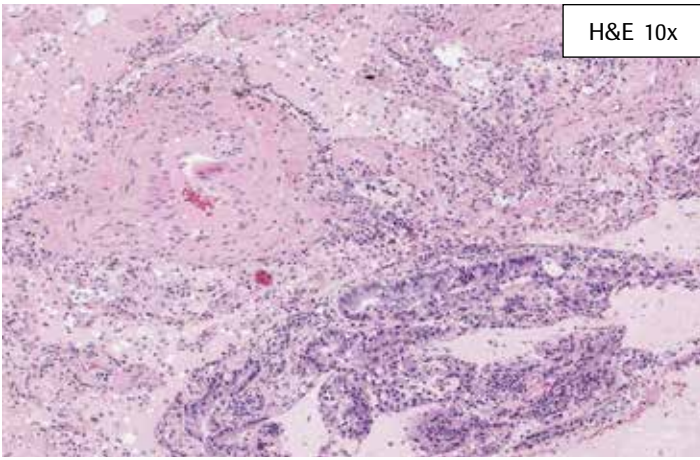


**Negative Case 1** exhibits no detectable ALK staining relative to the negative control antibody-stained slide. This case is assigned a Negative Clinical Diagnosis for ALK status.



**Negative Case 2** exhibits non-specific diffuse granular ALK staining in the tumor cells (a) and staining in the alveolar macrophages (b). No strong granular cytoplasmic staining is observed in tumor cells. This case is assigned a Negative Clinical Diagnosis for ALK status.





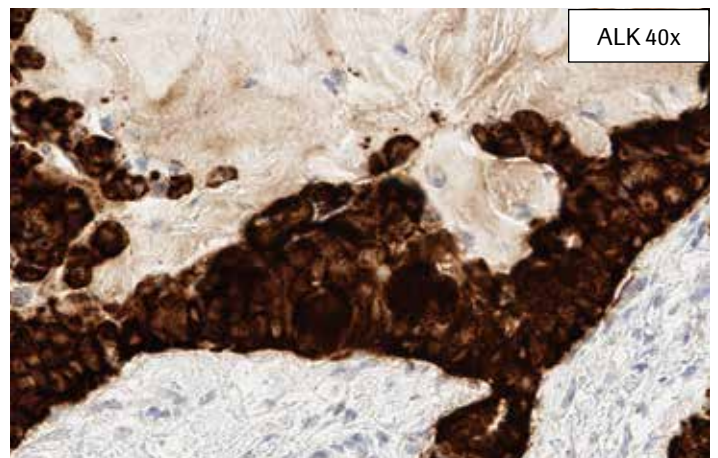
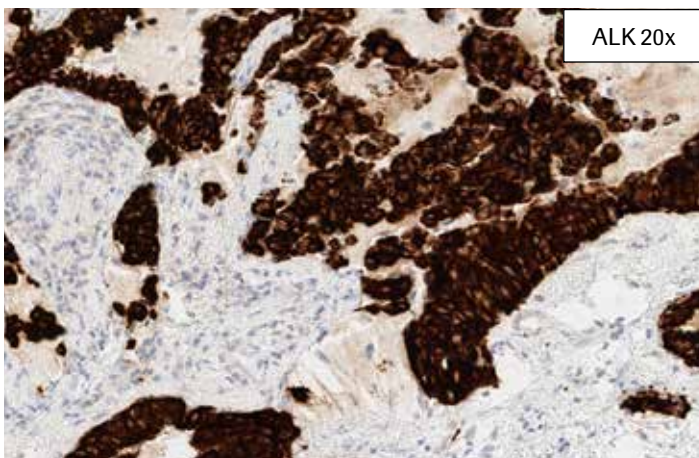
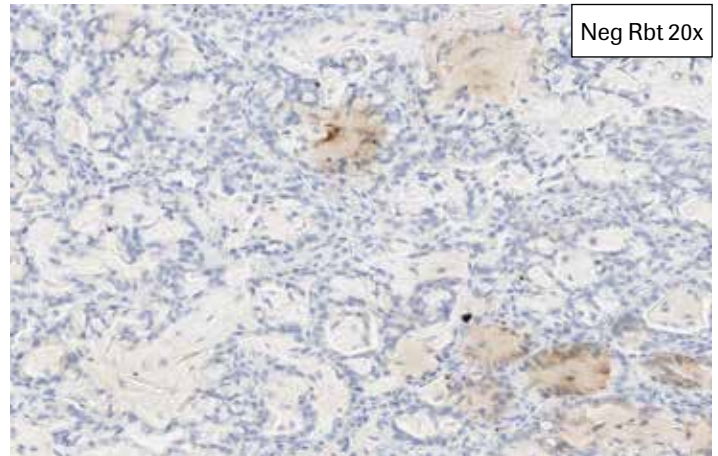
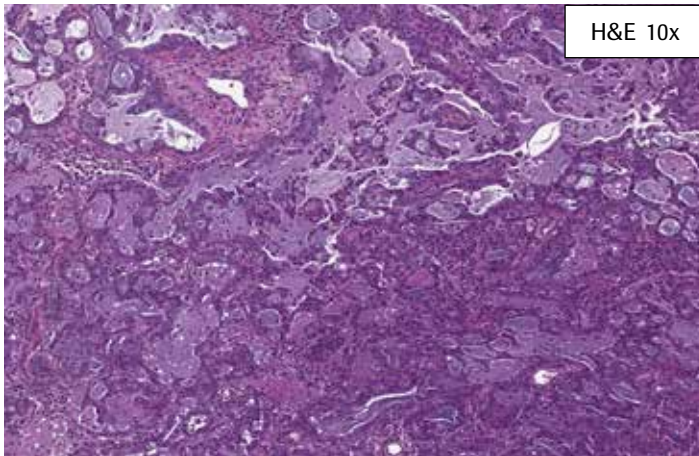
**Negative Case 3** exhibits weak cytoplasmic ALK staining in tumor cells (a) and granular non-specific background staining of necrotic tissue (b). This case is assigned a Negative Clinical Diagnosis for ALK status

## Positive Cases

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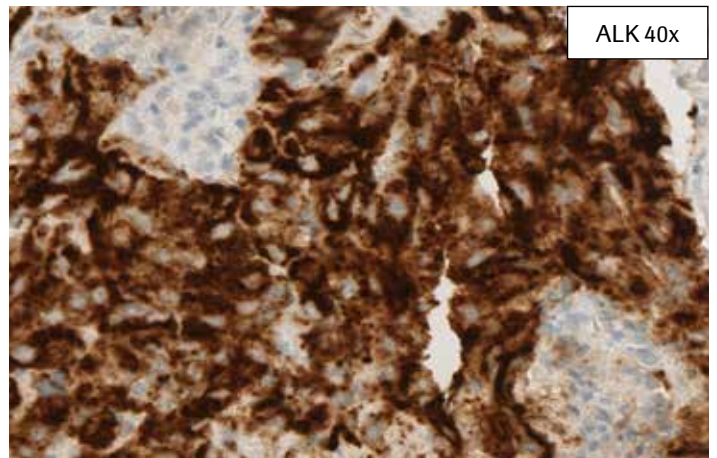
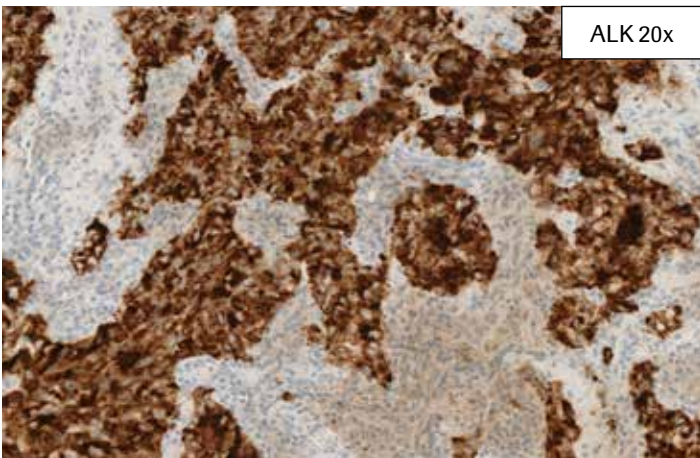
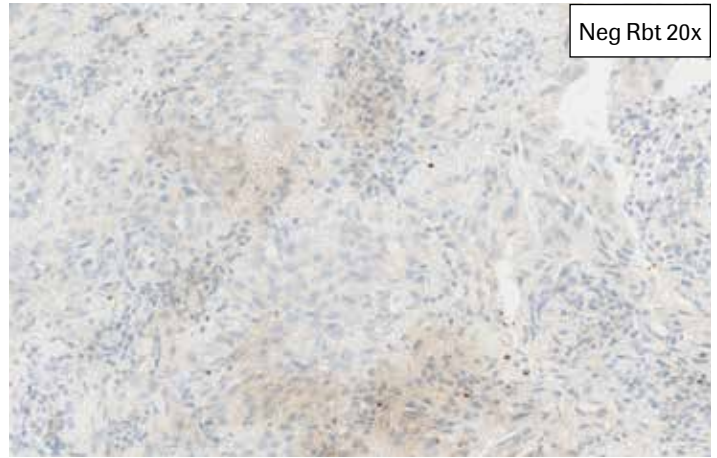
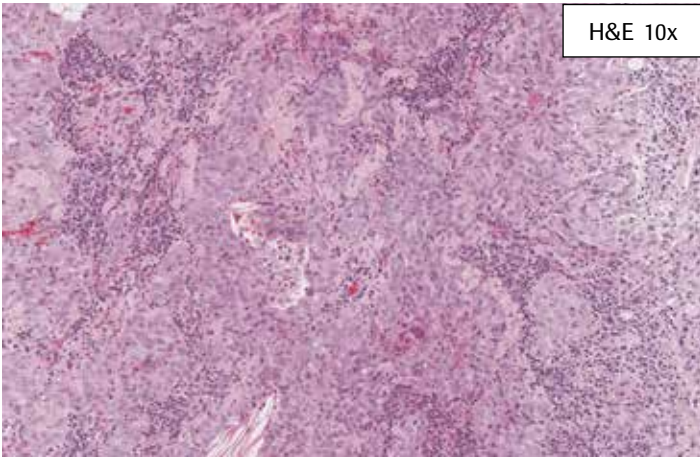
## Positive Cases

A case is assigned a Positive Clinical Diagnosis for ALK status if strong granular cytoplasmic staining of the tumor cell is observed.

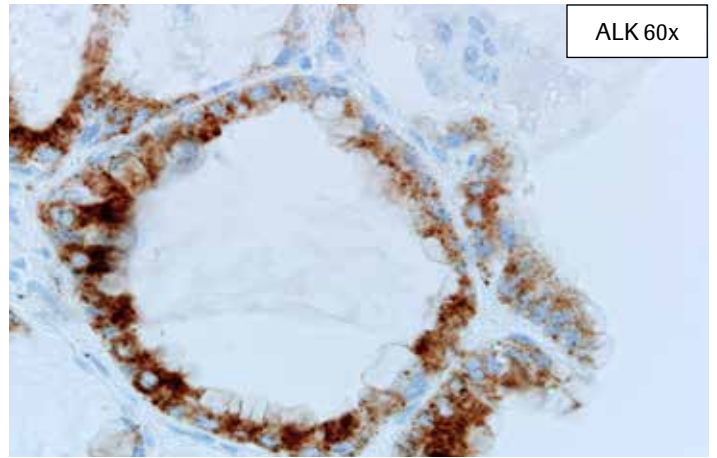
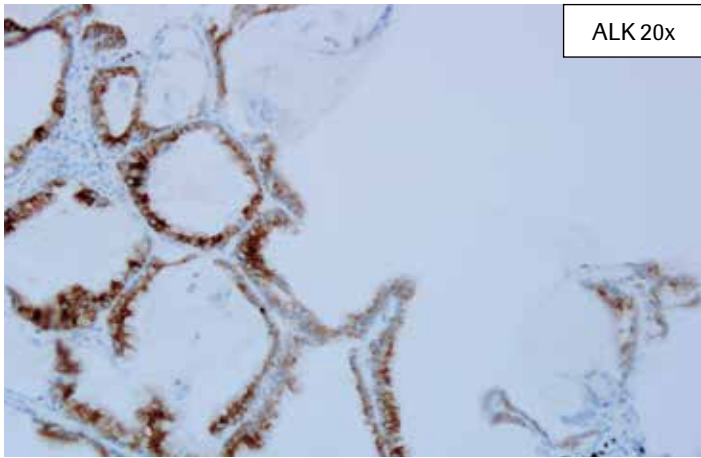
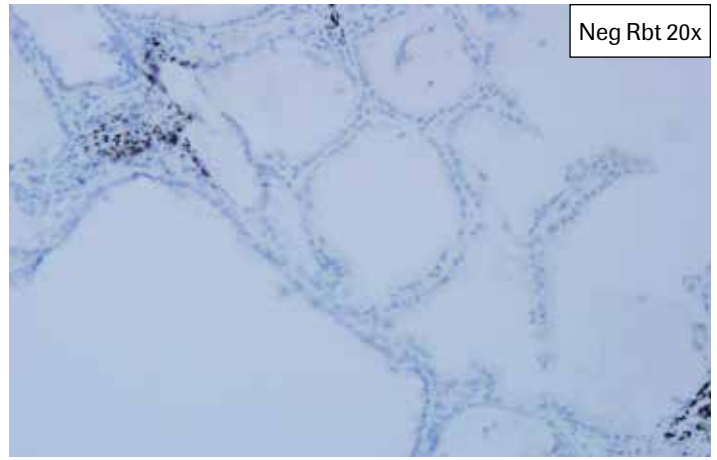
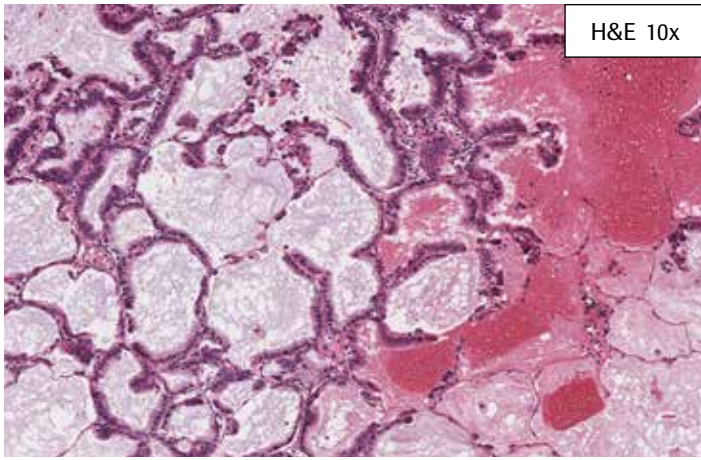


**Positive Case 1** exhibits a homogeneous strong granular cytoplasmic staining throughout the tumor. This case is assigned a Positive Clinical Diagnosis for ALK status.



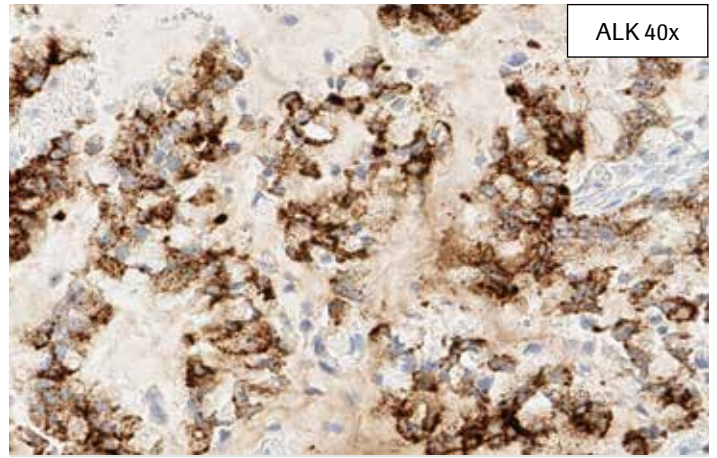
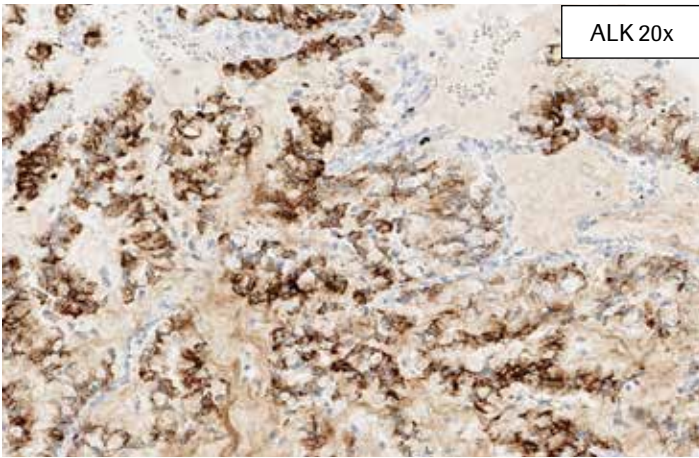
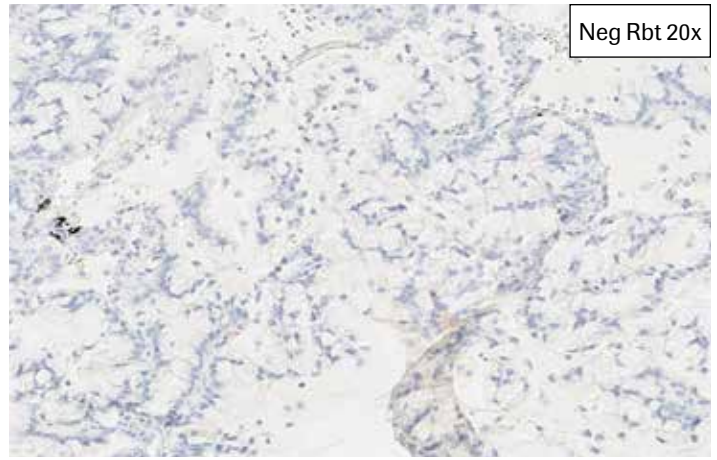
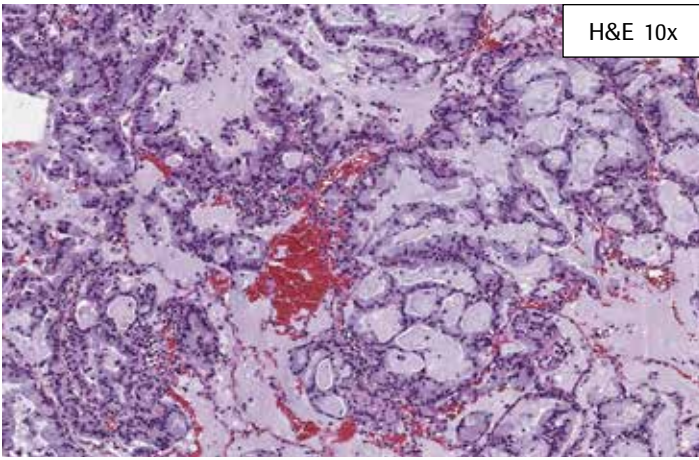


**Positive Case 2** exhibits a homogeneous strong granular cytoplasmic staining throughout the tumor. This case is assigned a Positive Clinical Diagnosis for ALK status.



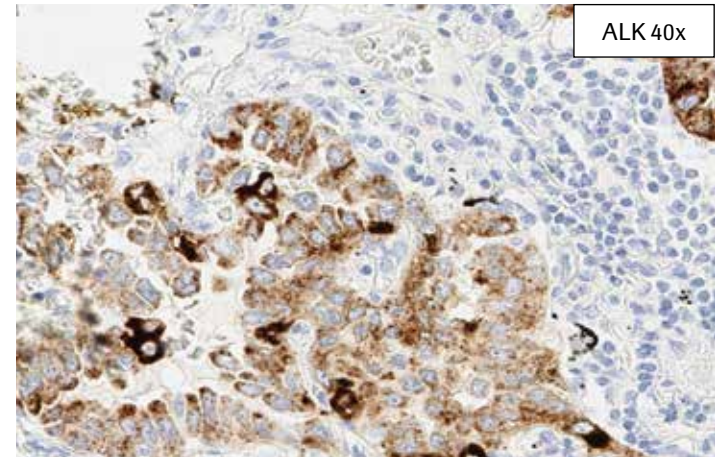
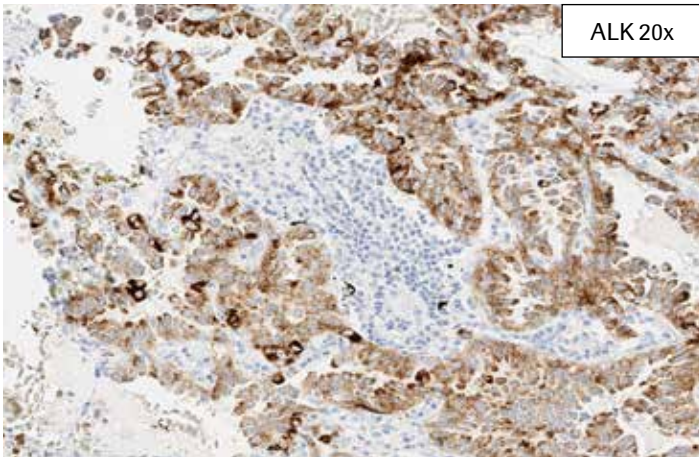
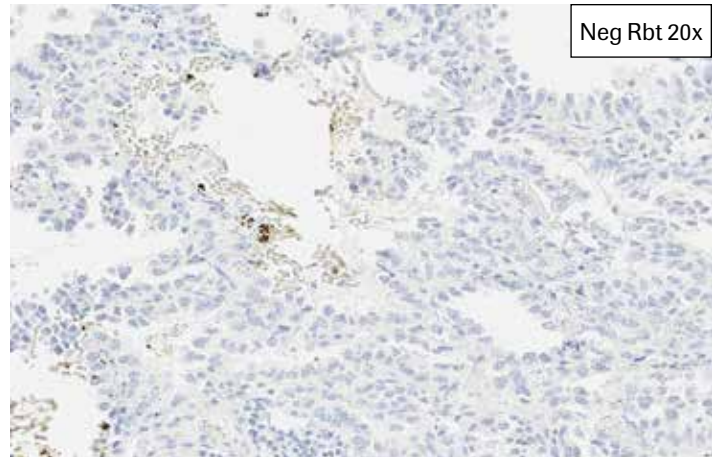
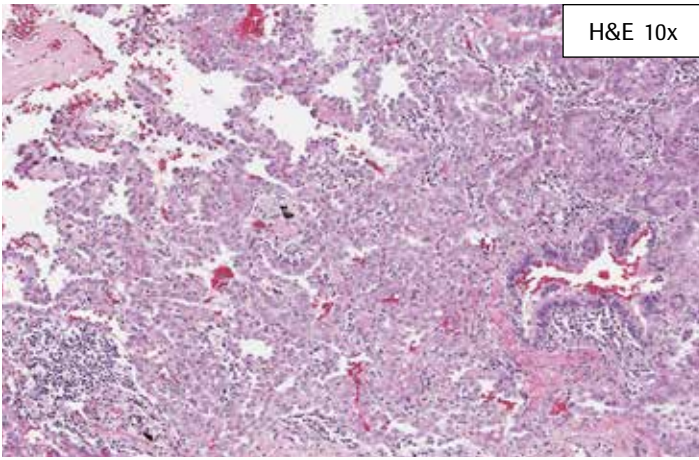
**Positive Case 3** exhibits heterogeneous granular cytoplasmic staining throughout sections of the tumor with strong granular cytoplasmic staining in some tumor cells. This case is assigned a Positive Clinical Diagnosis for ALK status.



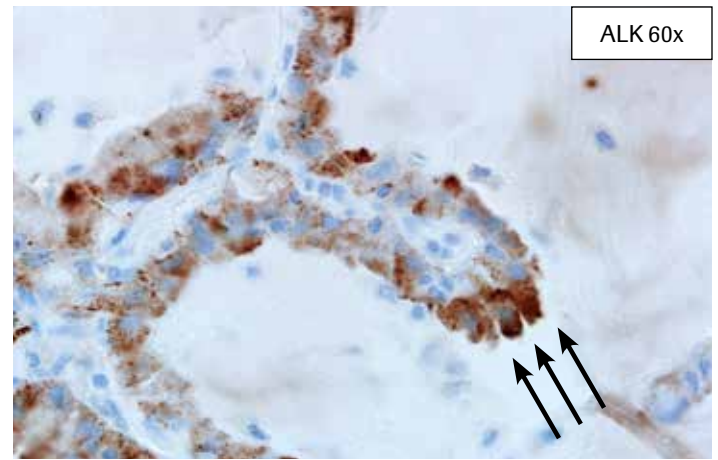
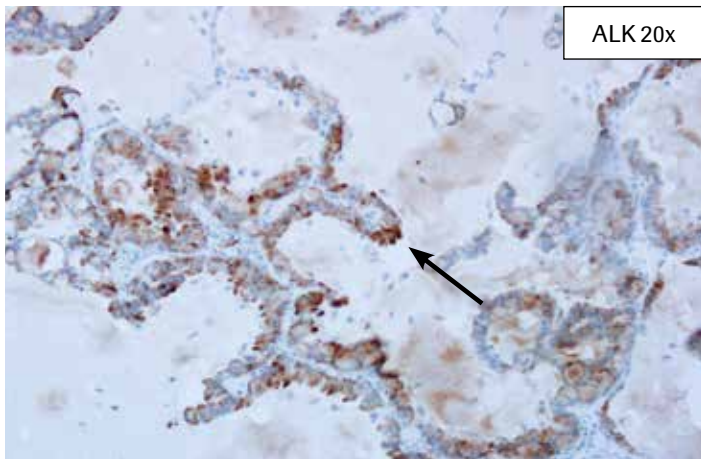
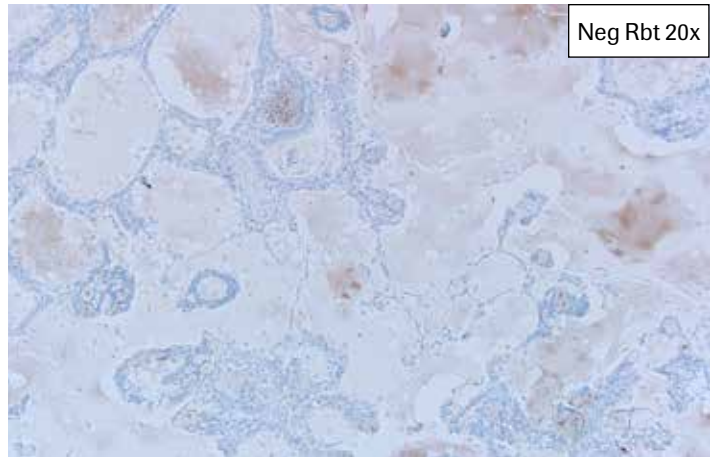
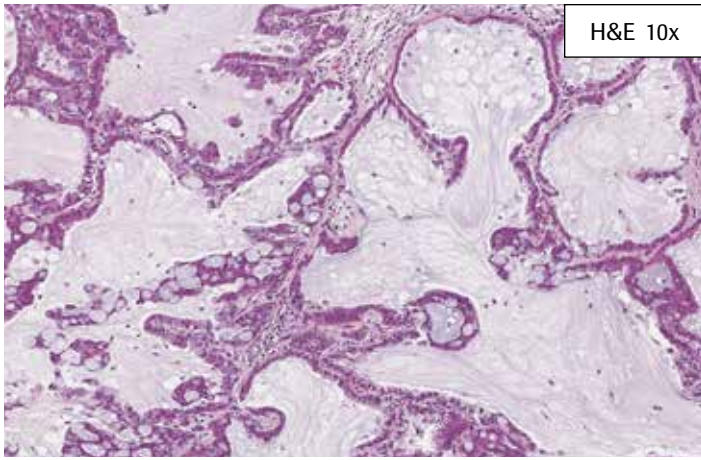


**Positive Case 4** exhibits a heterogeneous granular cytoplasmic staining pattern throughout sections of the tumor with strong granular cytoplasmic staining in some tumor cells. This case is assigned a Positive Clinical Diagnosis for ALK status



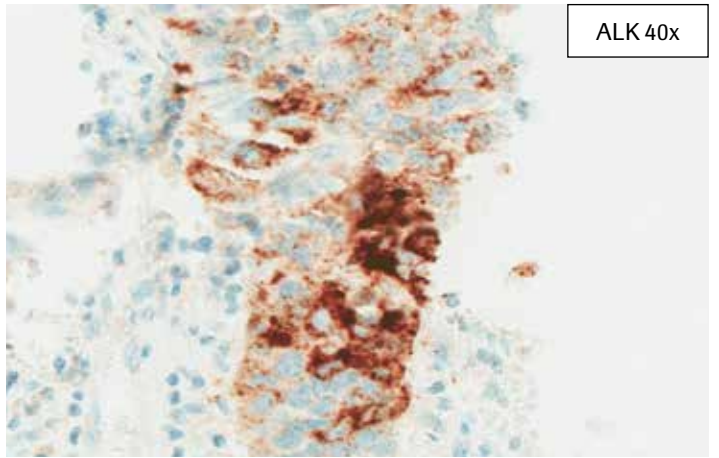
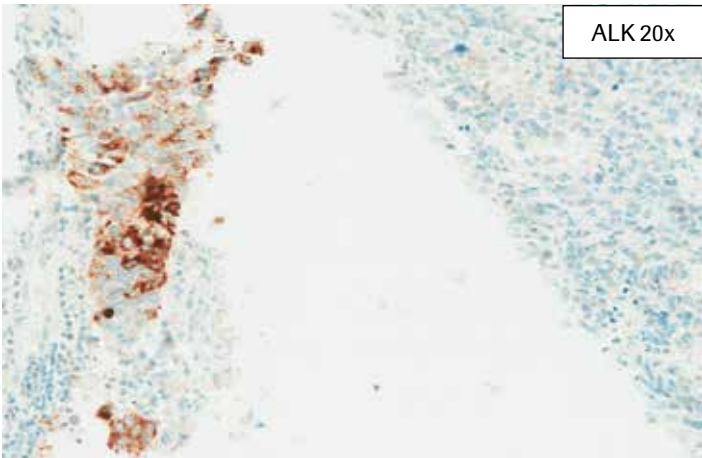
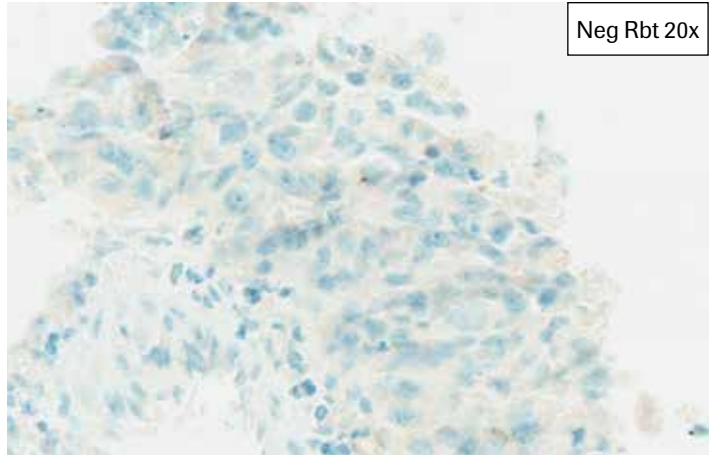
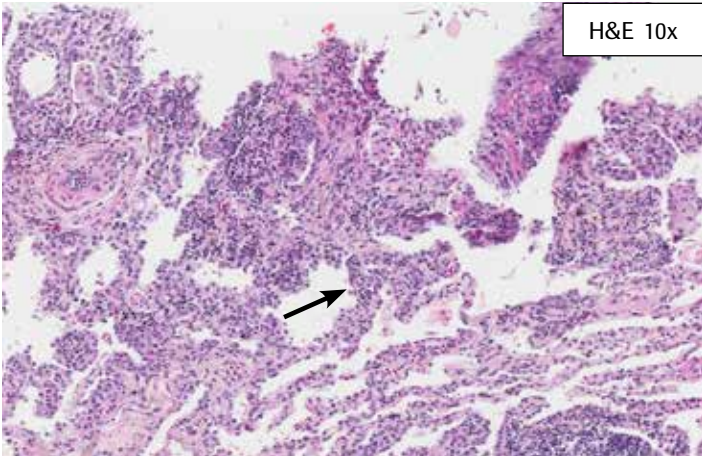


**Positive Case 5** exhibits a heterogeneous granular cytoplasmic staining pattern throughout sections of the tumor with strong granular cytoplasmic staining in few tumor cells. This case is assigned a Positive Clinical Diagnosis for ALK status.

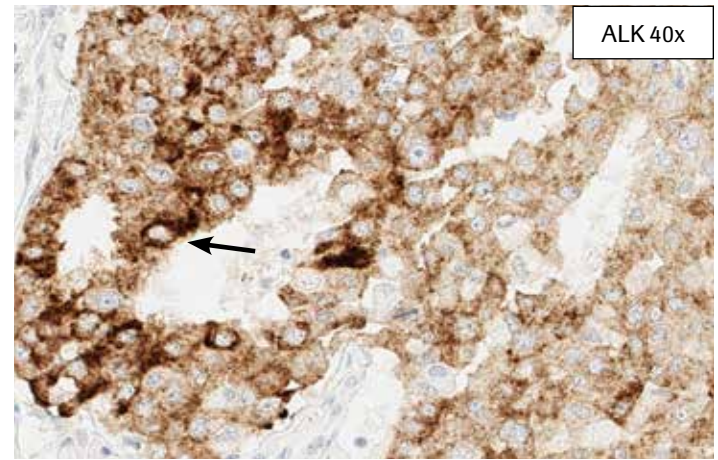
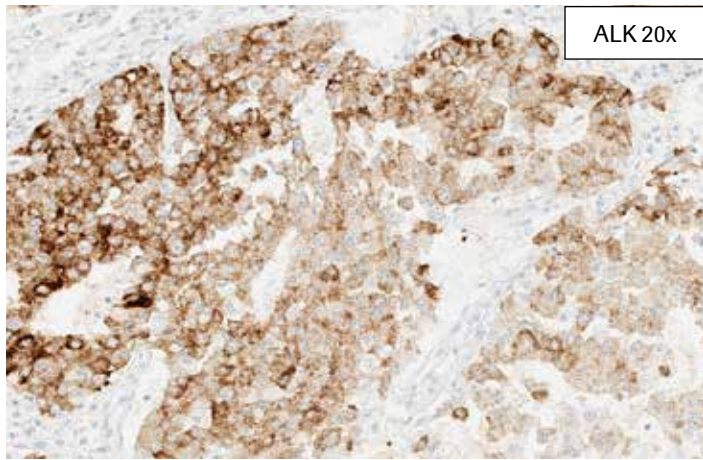
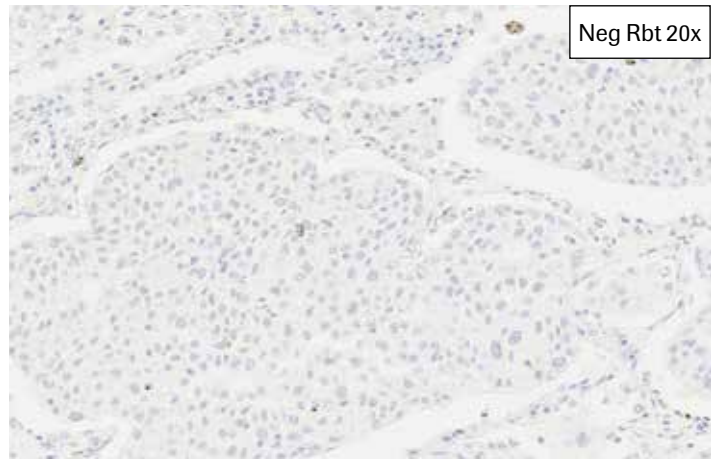
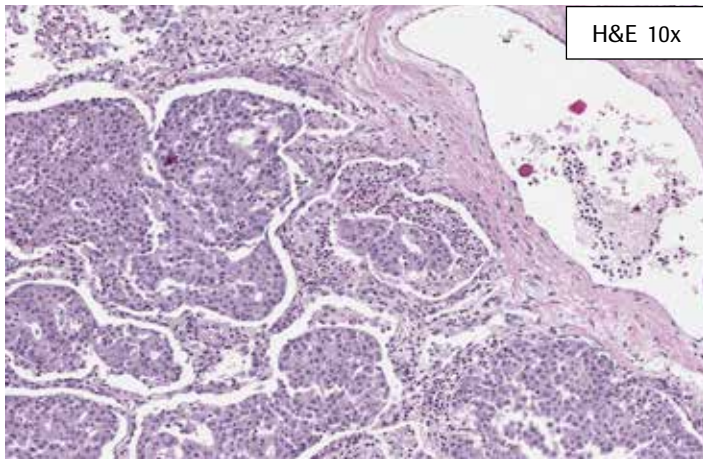


**Positive Case 6** exhibits strong granular cytoplasmic staining (see arrows) in few tumor cells. This case is assigned a Positive Clinical Diagnosis for ALK status.



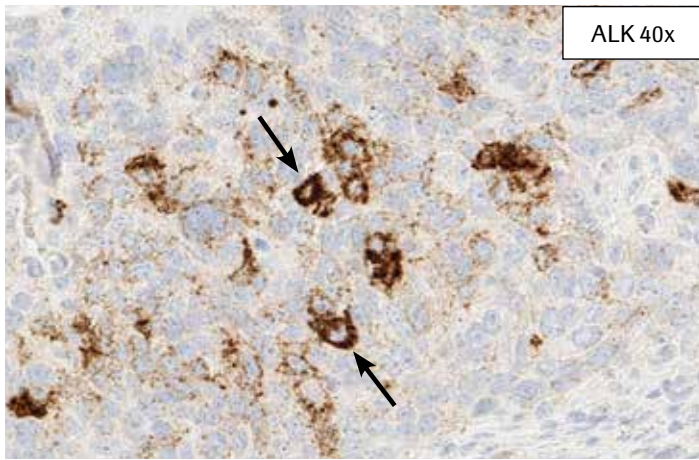
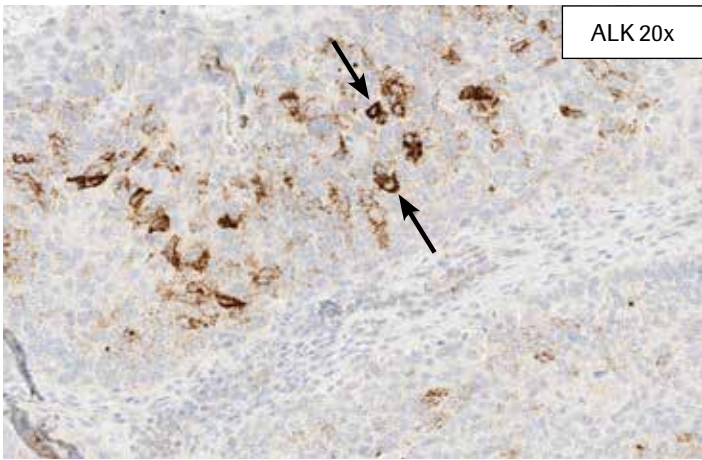
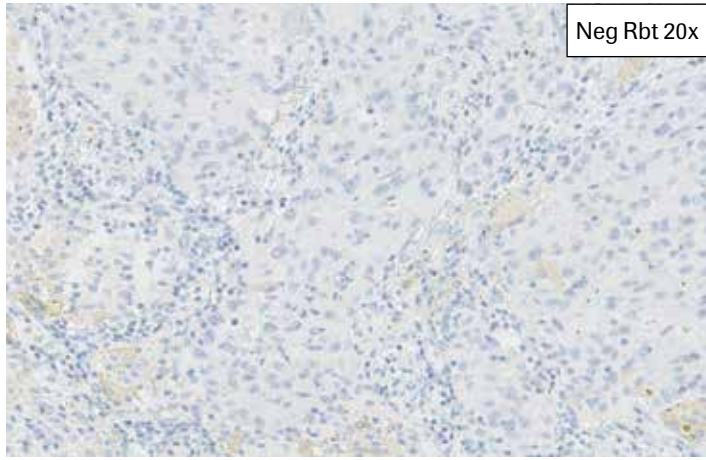
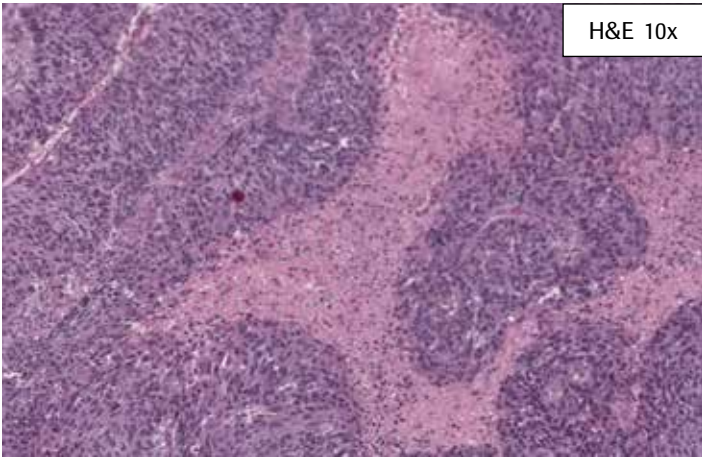


**Positive Case 7** exhibits strong granular cytoplasmic staining in few tumor cells. This case is assigned a Positive Clinical Diagnosis for ALK status.



**Positive Case 8** exhibits strong granular cytoplasmic staining in few tumor cells (see arrow). This case is assigned a Positive Clinical Diagnosis for ALK status.





**Positive Case 9** exhibits strong granular cytoplasmic staining in few tumor cells (see arrows). This case is assigned a Positive Clinical Diagnosis for ALK status.

## Challenging Cases

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## Challenging Cases and Known Staining Artifacts

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While the vast majority of cases stained with VENTANA ALK (D5F3) CDx Assay are clearly positive or negative in their staining results, a few cases have been observed that present a challenge in interpretation.

- **Non-specific background**

A small percentage of negative cases display a weak, diffuse granular cytoplasmic pattern that is detected above background staining observed on the associated Rabbit Monoclonal Negative Control Ig stained slide. Ventana has estimated these cases represent ~1-2% of all cases stained to date with the VENTANA ALK (D5F3) CDx Assay and are negative by FISH.

- **Granular cytoplasmic staining in normal tissue elements**

Granular cytoplasmic staining in alveolar macrophages and benign glandular epithelial cells may be present on both the ALK and negative reagent control-stained slides. Additionally, neuronal tissue elements may stain positive for ALK. This staining should be excluded in slide interpretation as it is present in normal tissue elements but not in the tumor cells.

- **Non-specific staining of mucin**

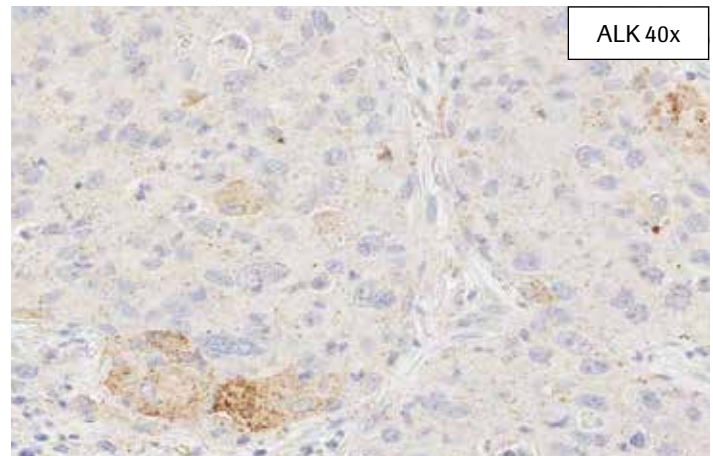
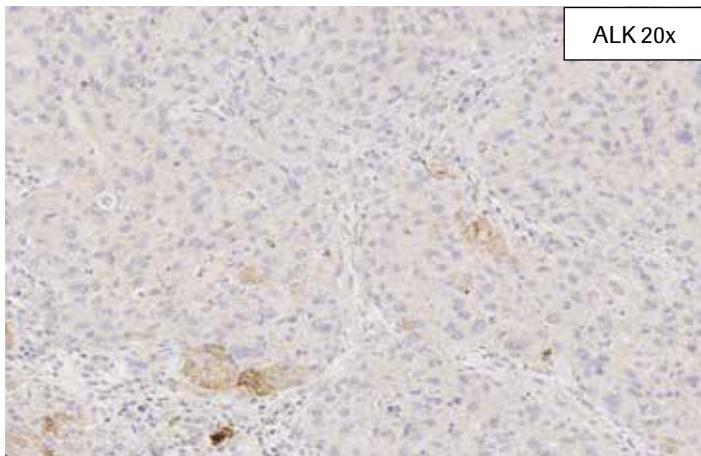
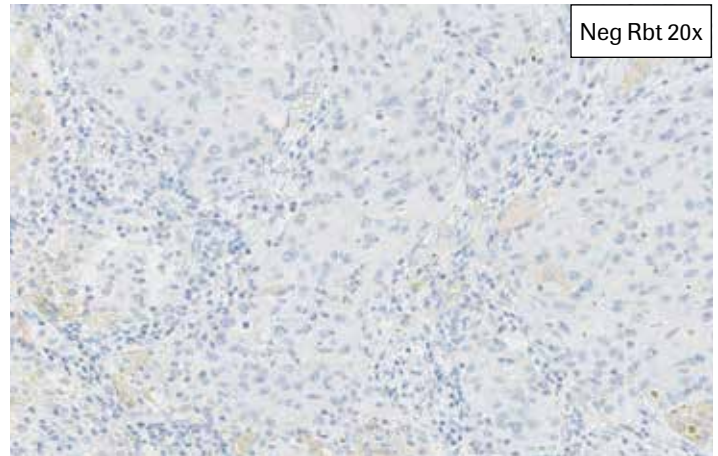
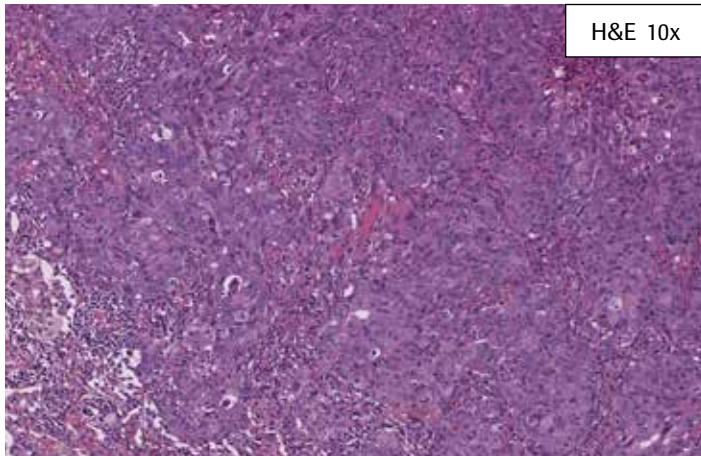
Non-specific staining of mucin should be excluded from slide interpretation providing that the staining does not interfere with interpretation of the slide.

- **Tissue or Staining Artifacts**

Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of an ALK Clinical Diagnosis. These artifacts may include, but are not limited to: fixation gradients and edge effects, DAB trapping, 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.

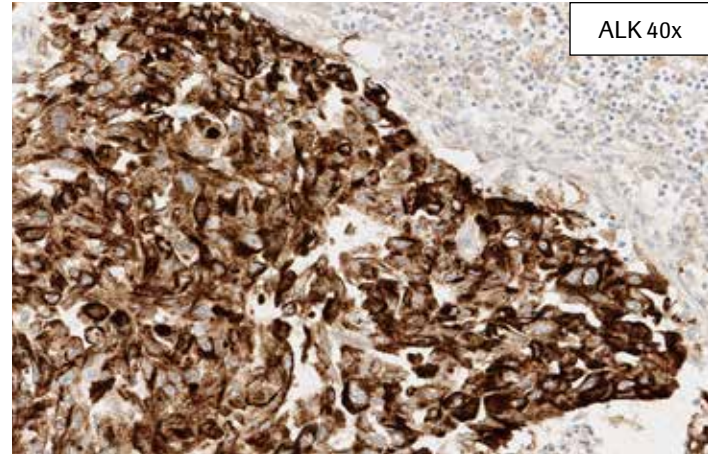
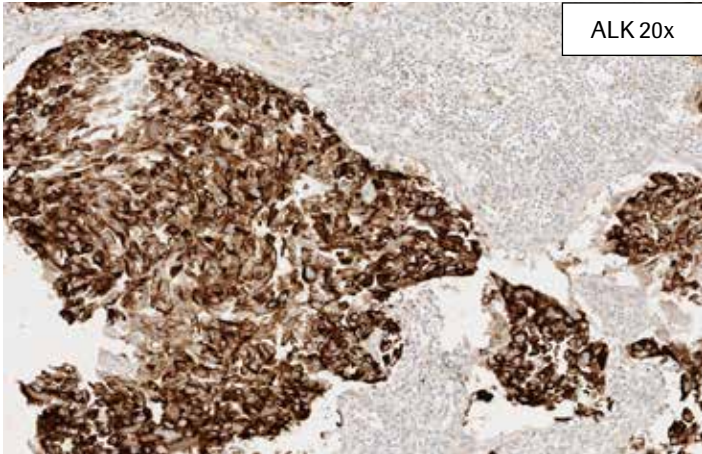
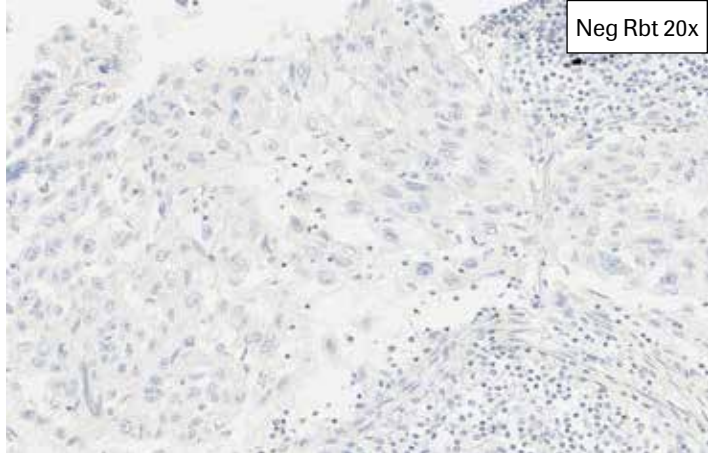
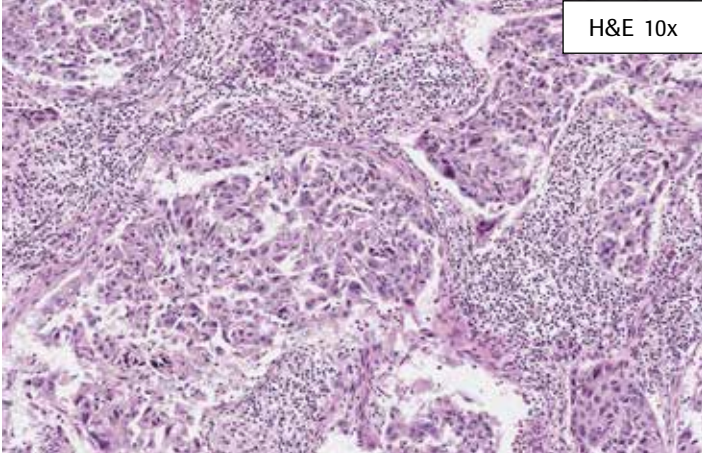
Some examples of challenging cases are shown on the following pages.

## Challenging Cases: Non-Specific Background Staining



**Challenging Case 1: Negative** This case displays a non-specific diffuse granular cytoplasmic pattern throughout the tissue that is detected above background staining observed on the associated Rabbit Monoclonal Negative Control Ig- stained slide. This case is assigned a Negative Clinical Diagnosis for ALK status.

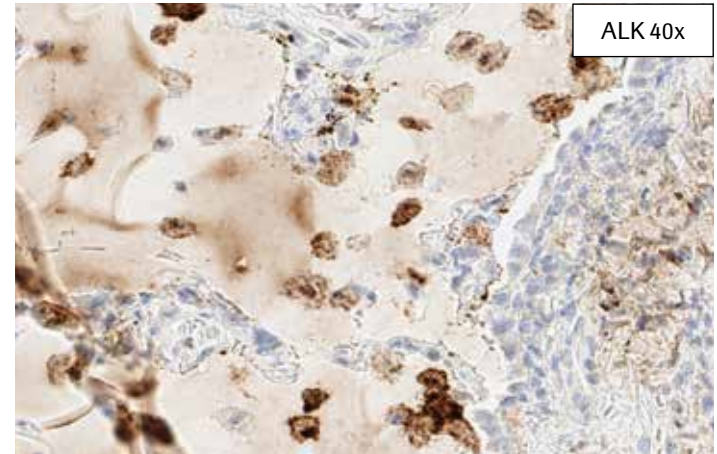
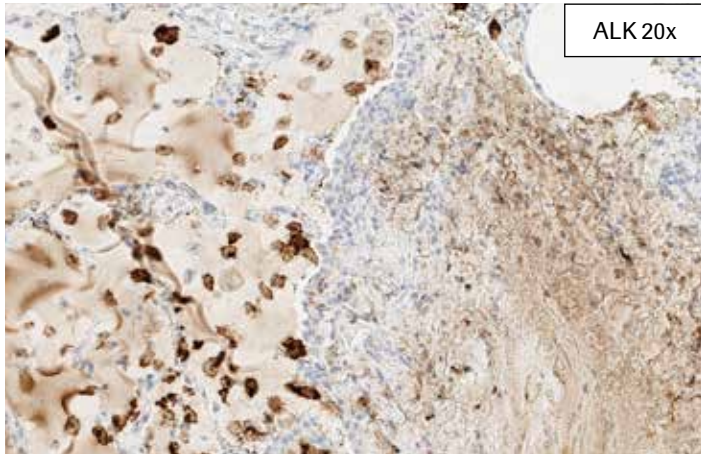
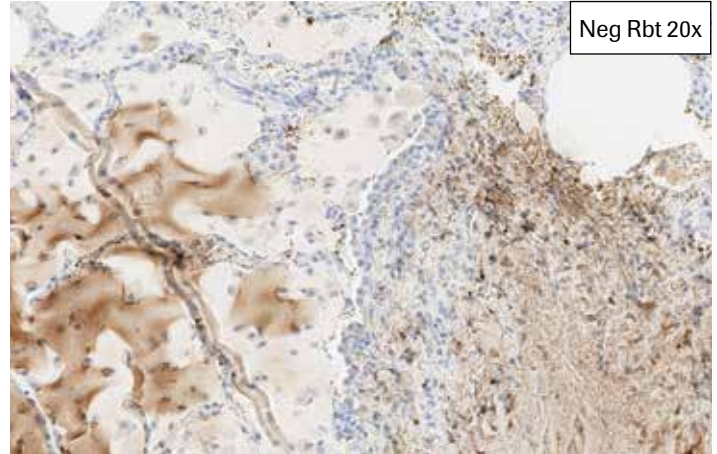
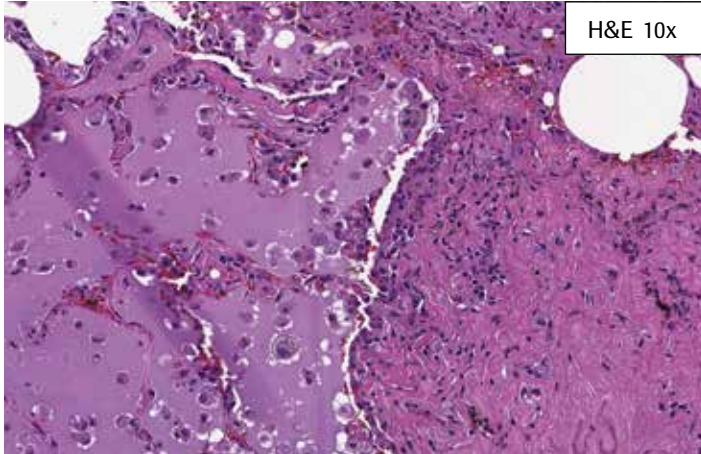




**Challenging Case 2: Positive** This case displays a non-specific diffuse granular cytoplasmic staining in the stroma that is detected above background staining observed on the associated Rabbit Monoclonal Negative Control Ig- stained slide. This case is assigned a Positive Clinical Diagnosis for ALK status, and background is considered acceptable as it does not interfere with interpretation of the specific stain.



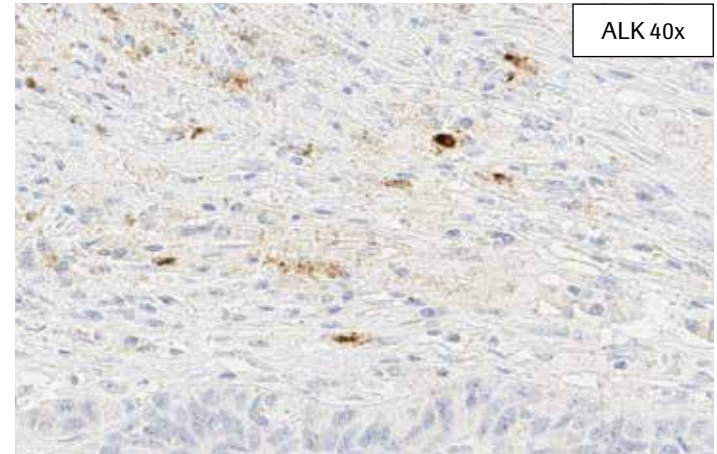
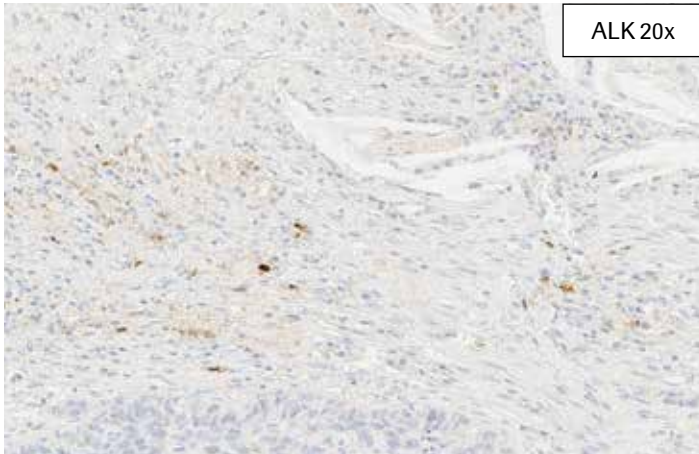
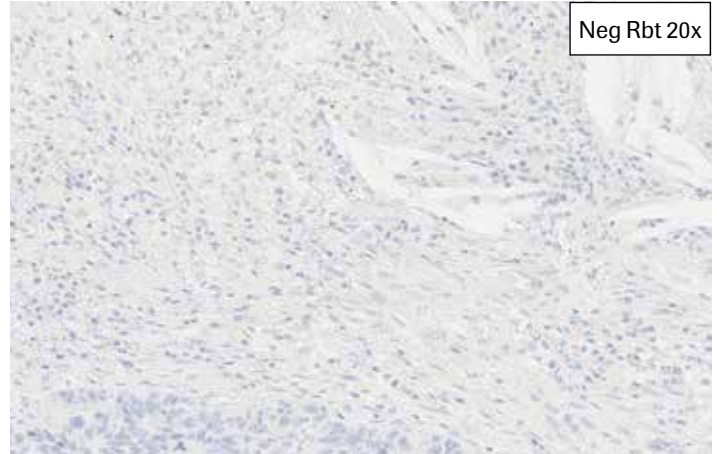
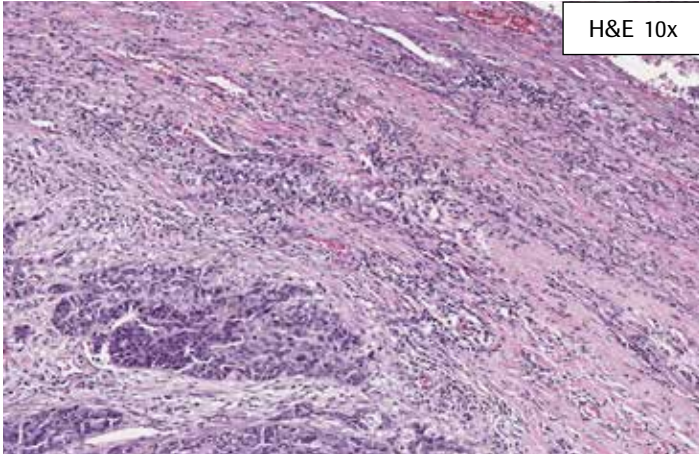
## Challenging Cases: Granular Cytoplasmic Staining in Alveolar Macrophages



**Challenging Case 3: Negative** Strong granular cytoplasmic staining may be present in alveolar macrophages and should not be interpreted as an ALK-positive diagnosis. When evaluating a NSCLC for ALK staining it is imperative to closely examine the cell type staining to ensure that it is tumor. These staining artifacts should be excluded in slide interpretation as it is present in normal tissue elements. This case is assigned a Negative Clinical Diagnosis for ALK status.

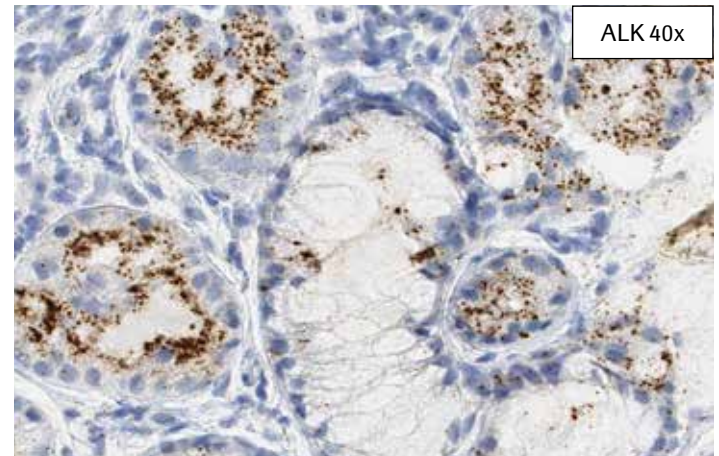
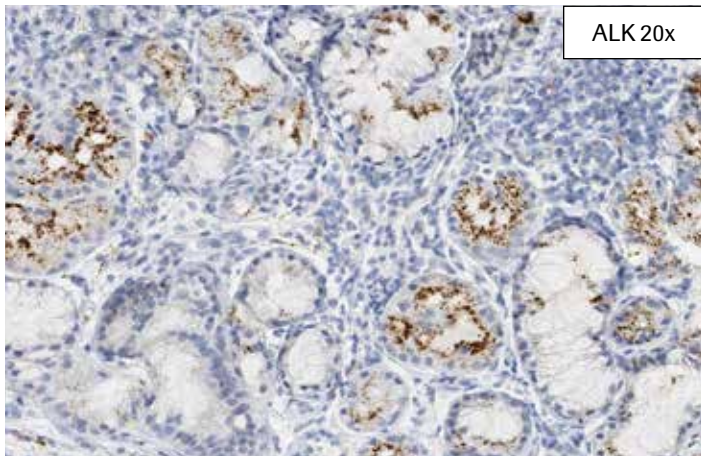
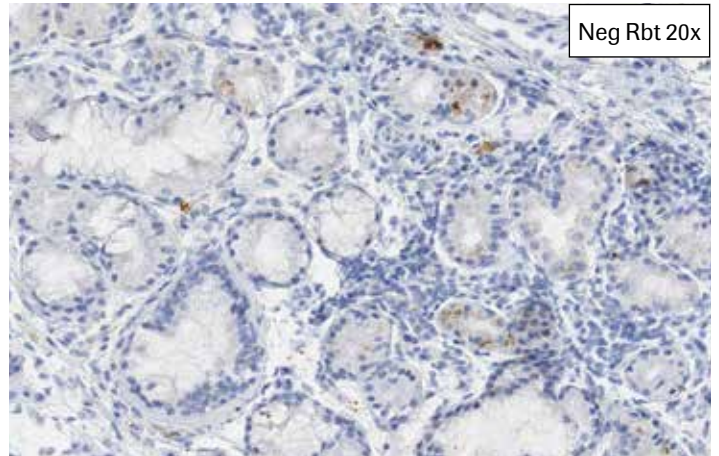
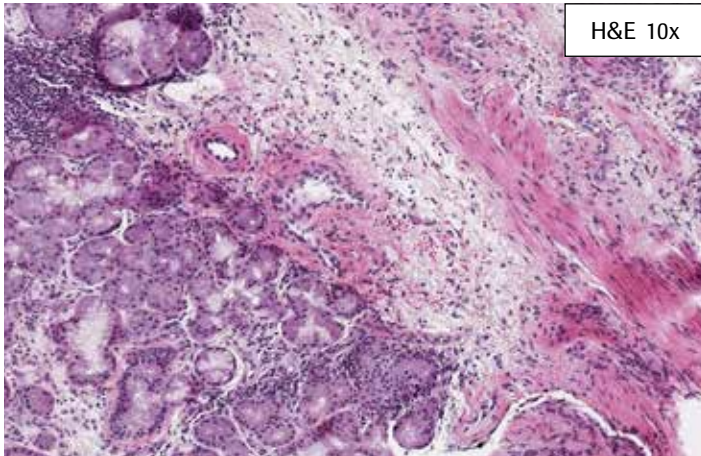


## Challenging Cases: Granular Cytoplasmic Staining in Scattered Lymphoreticular Cells within Lymphocytic Infiltrate



**Challenging Case 4: Negative** Strong granular cytoplasmic staining may be present in lymphoreticular cells within lymphocytic infiltrate and should not be interpreted as an ALK-positive diagnosis. When evaluating a NSCLC for ALK staining it is imperative to closely examine the cell type staining to ensure that it is tumor. This staining should be excluded in slide interpretation as it is present in normal tissue elements. This case is assigned a Negative Clinical Diagnosis for ALK status.

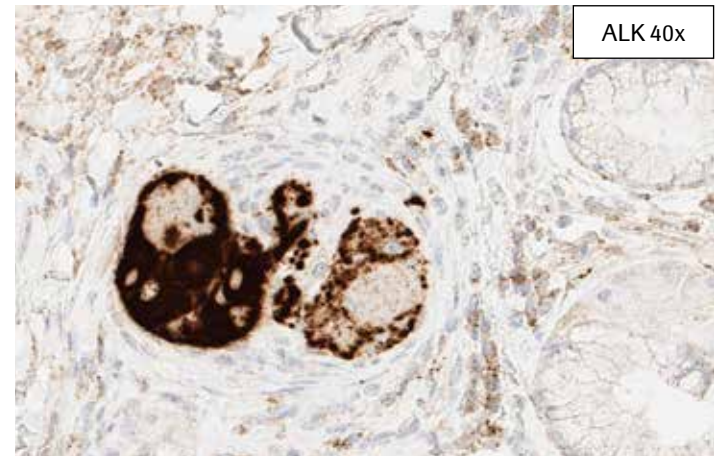
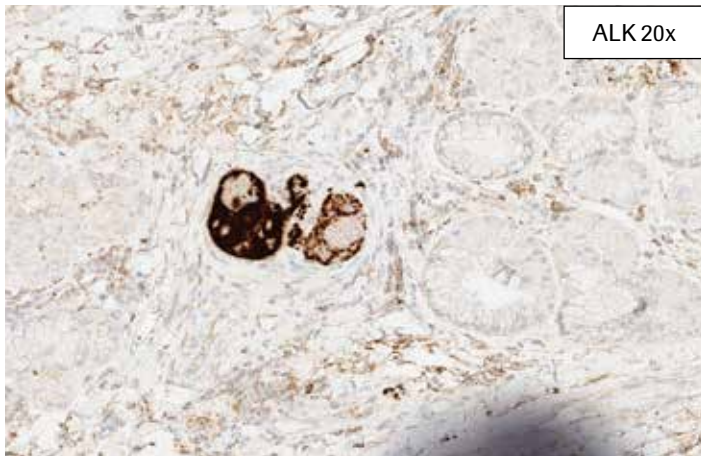
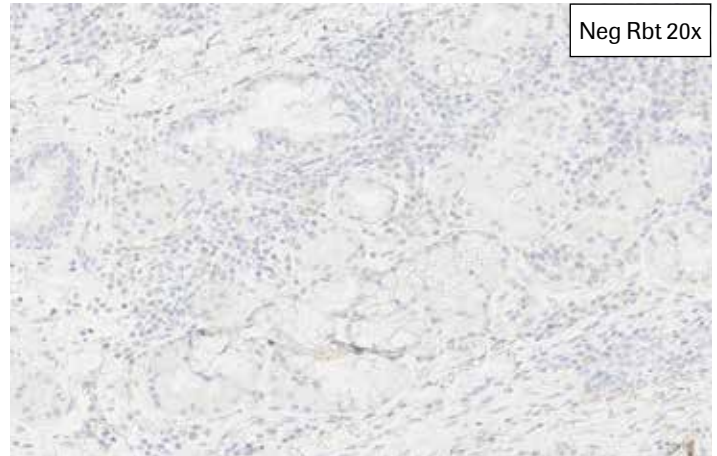
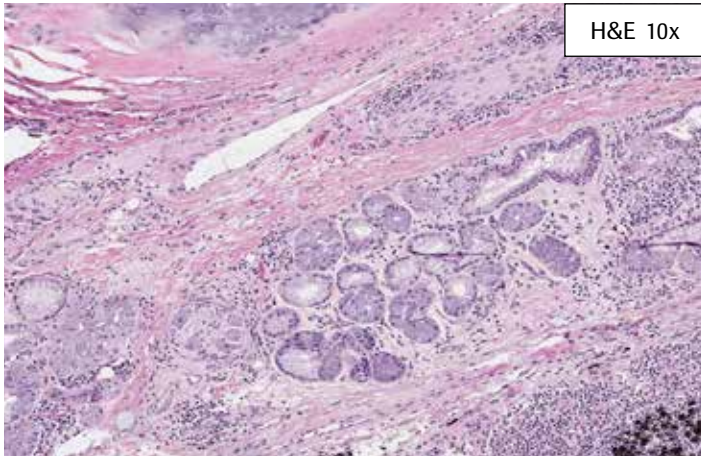
## Challenging Cases: Granular Cytoplasmic Staining in Benign Glandular Epithelium



**Challenging Case 5: Negative** Granular cytoplasmic staining in benign glandular epithelium may be present on the ALK -stained slides. These staining artifacts should be excluded in slide interpretation as it is present in normal tissue elements but not in the tumor cells. This case is assigned a Negative Clinical Diagnosis for ALK status.

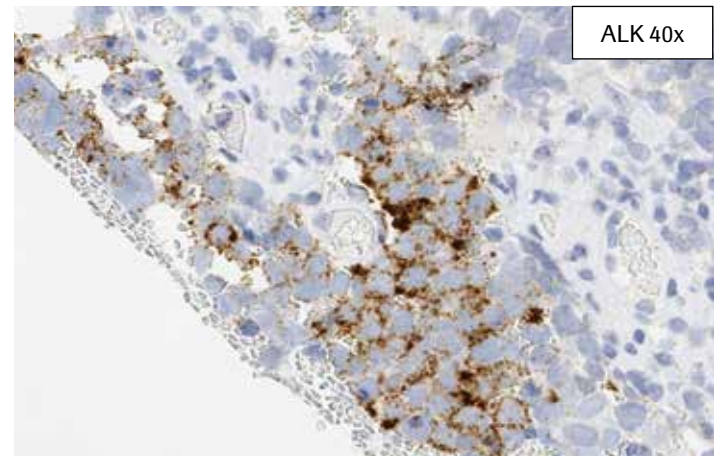
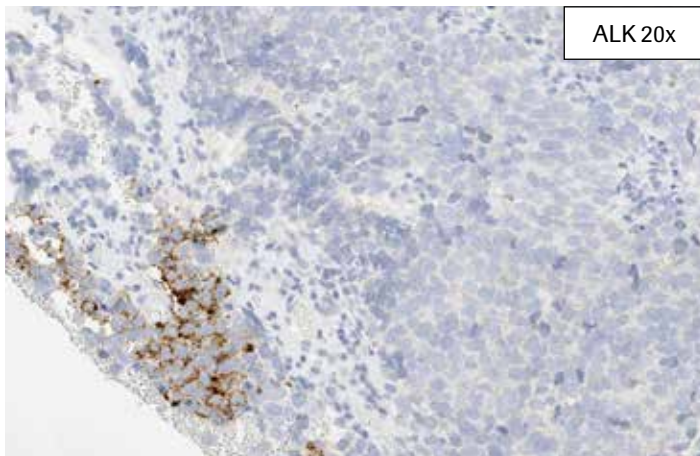
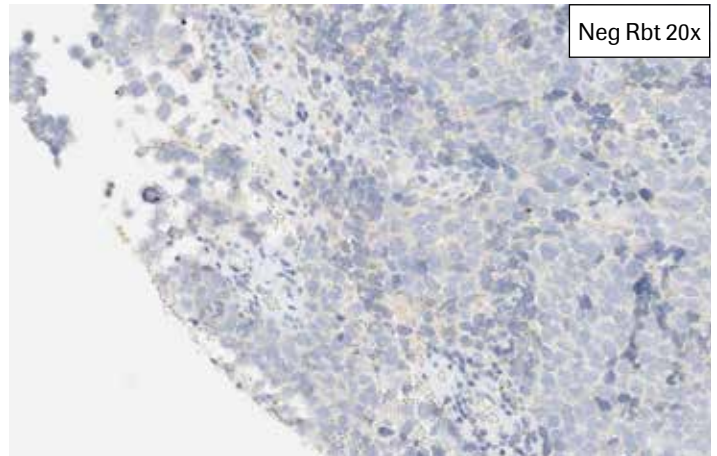
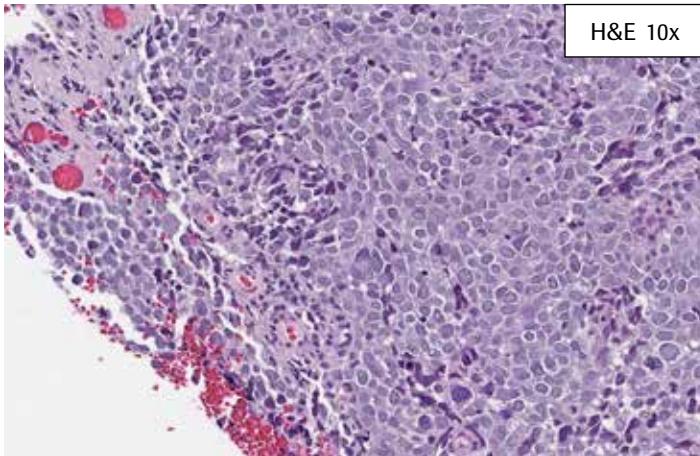


## Challenging Cases: Granular Cytoplasmic Staining in Ganglion Cells



**Challenging Case 6: Negative** Granular cytoplasmic staining in ganglion cells may be present on the VENTANA ALK (D5F3) CDx Assay-stained slides. This staining should be excluded in slide interpretation as it is present in normal tissue elements but not in the tumor cells. This case is assigned a Negative Clinical Diagnosis for ALK status.

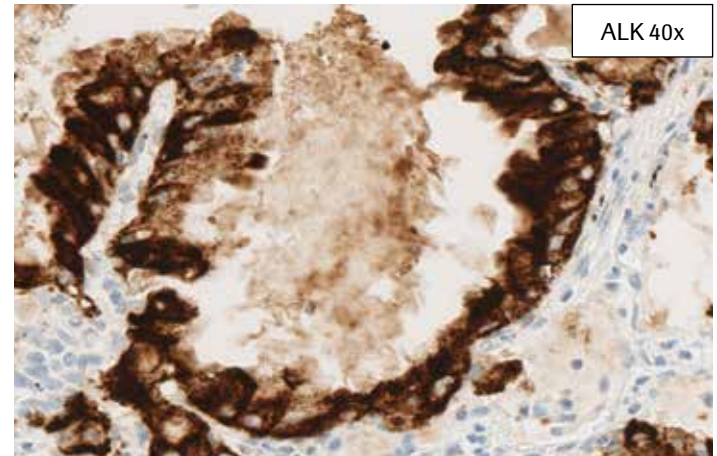
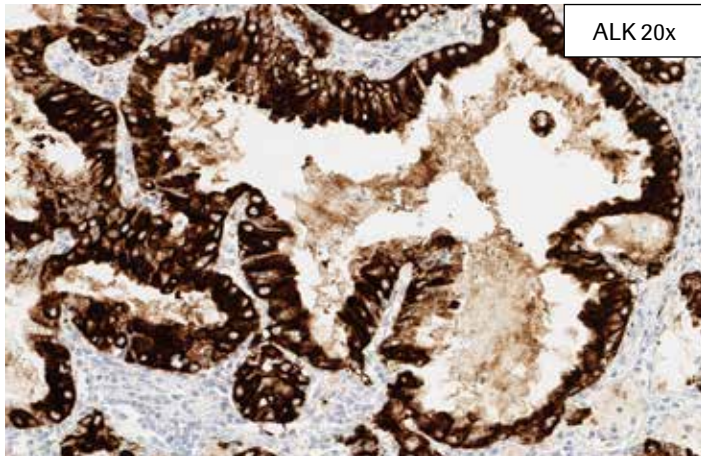
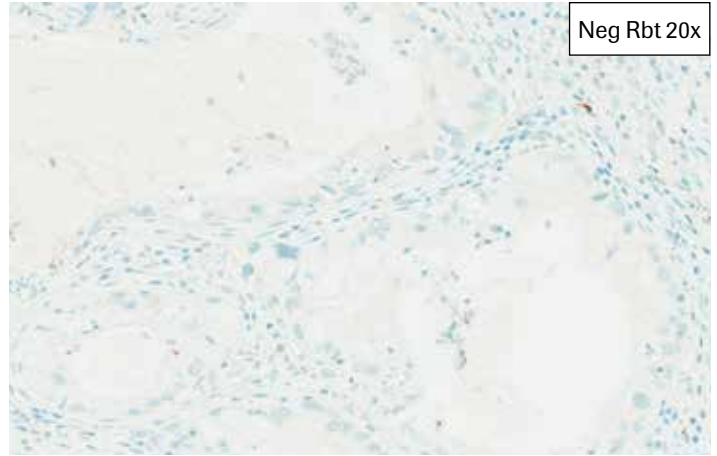
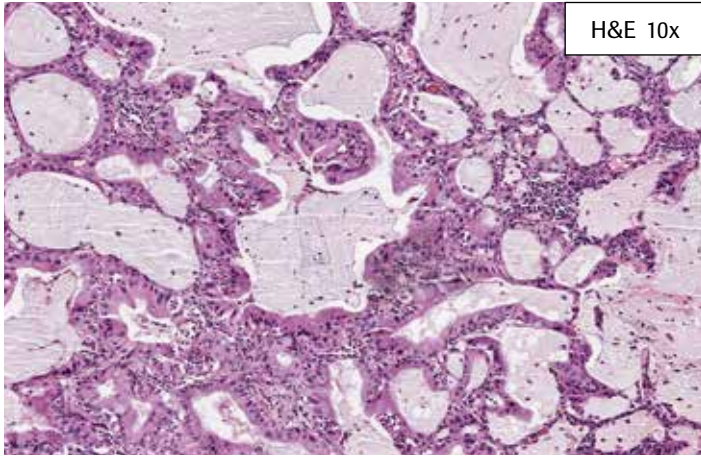
## Challenging Cases: Strong Granular Membrane Alk-Staining



**Challenging Case 7: Negative** Membrane or non-cytoplasmic staining should not be interpreted as an ALK-positive diagnosis for this case. ALK-positive specific staining must be strong granular cytoplasmic staining within viable tumor cells. This case is assigned a Negative Clinical Diagnosis for ALK status.

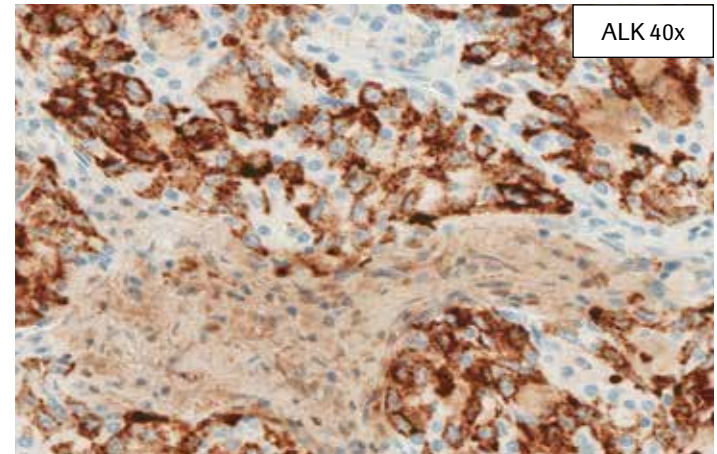
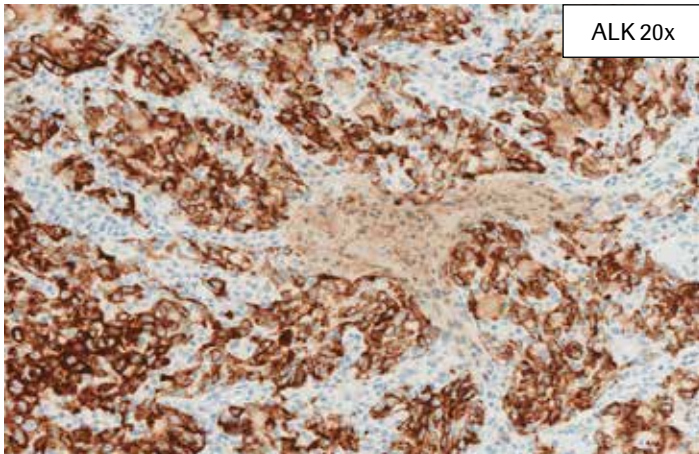
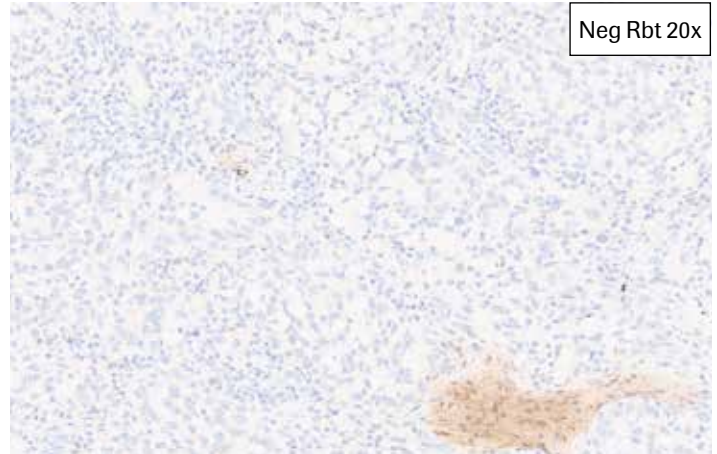
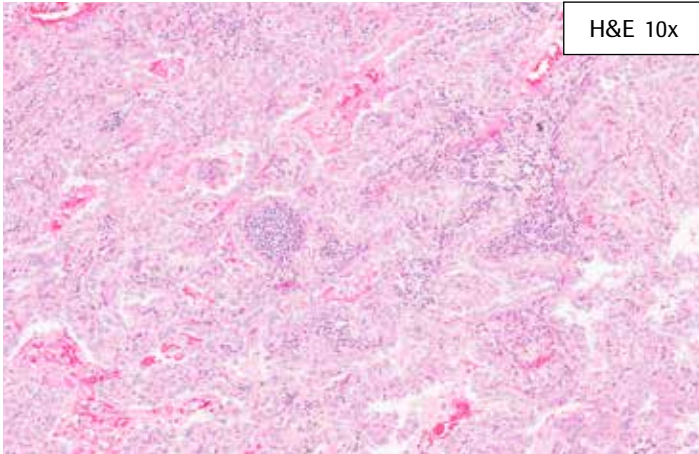


## Challenging Cases: Mucin Staining



**Challenging Case 8: Positive** Staining of mucin should be excluded from slide interpretation providing that the staining does not interfere with interpretation of the case. This case is assigned a Positive Clinical Diagnosis for ALK status.

## Challenging Cases: DAB Trapping



**Challenging Case 9: Positive** Occasionally, cases may have DAB trapping within the tissue. This artifact should be excluded from slide interpretation providing that the trapping does not interfere with interpretation of the case. Otherwise the specimen should be restained. This case is assigned a Positive Clinical Diagnosis for ALK status.

## Reproducibility of the VENTANA ALK (D5F3) CDx Assay

The advantage of the VENTANA ALK (D5F3) CDx Assay is that the use of the OptiView DAB IHC Detection Kit and OptiView Amplification Kit enables the vast majority of NSCLC cases to easily be interpreted as a “Positive” or “Negative” result. The enhanced sensitivity of the assay means that the reader does not need to provide a semi-quantitative assessment of percent tumor cell staining or staining intensities, as is the case for other biomarkers assessed by IHC assays. However, Ventana recognizes that the OptiView Amplification Kit is new technology for many pathologists. One factor that is apparent in more sensitive detection systems is that there can be more slide-to-slide variability in total staining intensity, compared with DAB only.

## **Pre-Analytical Conditions and their Impact on VENTANA ALK (D5F3) CDx Assay Staining**

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# Pre-Analytical Conditions and their Impact on VENTANA ALK (D5F3) CDx Assay Staining

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## Fixative Examples (Xenografts)

Ventana has conducted studies using the NCI-H2228 cell line (positive for ALK) generated as xenograft tumors in SCID mice as a model system for determining the impact of pre-analytical factors on the assay. The tumors were harvested and fixed with different fixatives across a range of times and stained with the VENTANA ALK (D5F3) CDx Assay.

Consistent with ASCO/CAP guidelines for HER2 testing, tissues must be fixed using 10% neutral buffered formalin (NBF) for a period of at least 6 hours, for optimal VENTANA ALK (D5F3) CDx Assay staining results.<sup>12-15</sup> Zinc formalin also yielded acceptable staining results at >6 hour time-points, and can be used with the ALK assay. However, fixation times below 6 hours (under-fixation) in NBF and in Zinc formalin resulted in a significant decrease in staining intensity for ALK.









Fixatives other than NBF and Zinc formalin, including AFA, B5, and PREFER, also were tested and should not be used with the VENTANA ALK (D5F3) CDx Assay as the staining results were severely compromised. Intensity of VENTANA ALK (D5F3) CDx Assay was dramatically decreased under all time-points tested with AFA, B5, and PREFER fixatives. In addition, fixing in 95% alcohol for as little as one hour resulted in a significant negative impact to ALK staining intensity and should not be performed with this assay.

Ventana also investigated the impact that delay to fixation has on the VENTANA ALK (D5F3) CDx Assay staining results. Xenograft samples were excised and left un-fixed for times ranging from 30 minutes to 24 hours, then fixed for 12 hours in 10% NBF. The staining results indicated that ALK intensity was compromised if the time to fixation in NBF was delayed >6 hours.

Finally, it is important to emphasize that the ALK protein appears to be more sensitive to pre-analytical factors when compared with other lung markers detected by IHC (such as TTF1 and EGFR) using the xenograft models. Representative data are shown below. Ventana emphasizes that fixation conditions for human lung specimens be carefully monitored and controlled to ensure optimal staining results with VENTANA ALK (D5F3) CDx Assay.




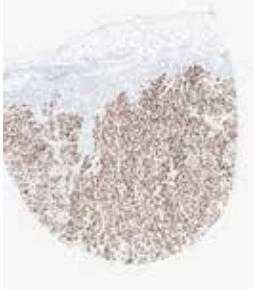
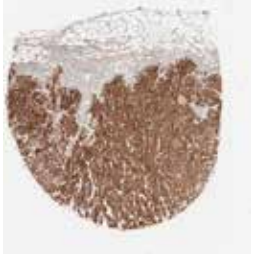
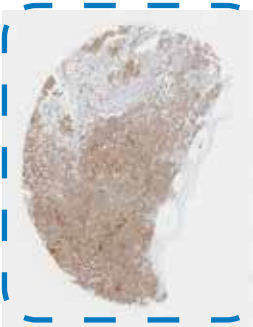


## Examples of the Impact of Fixation Conditions with VENTANA ALK (D5F3) CDx Assay

Fixation Time (Hours)	Fixative			
	10% NBF	Zinc Formalin	AFA	95% Ethanol
1				
12				

Ventana recommends fixation in 10% NBF for 6–48 hours. ALK staining results within the dotted line are acceptable. Fixation for less than 6 hours is not recommended. The use of PREFER, Bouin's (not pictured), and alcohol fixatives, such as AFA and 95% Ethanol, is not recommended due to weaker staining.

It is important to emphasize that the ALK protein (as detected by the VENTANA ALK (D5F3) CDx Assay and the anti-ALK 5A4 clone) appears to be more sensitive to pre-analytical factors when compared with other lung markers detected by IHC (TTF1 and EGFR) using the xenograft models. Representative images are shown below. Ventana emphasizes that fixation conditions for human lung specimens be carefully monitored and controlled to ensure optimal staining results with the VENTANA ALK (D5F3) CDx Assay.

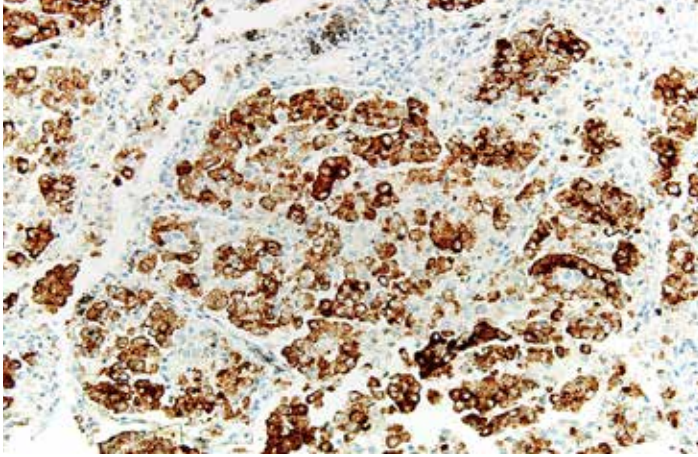
## Comparison of IHC Lung Markers Fixed with 10% NBF

10% NBF Fixation Time (Hours)	IHC Lung Marker		
	ALK	TTF-1	EGFR
1			
12			

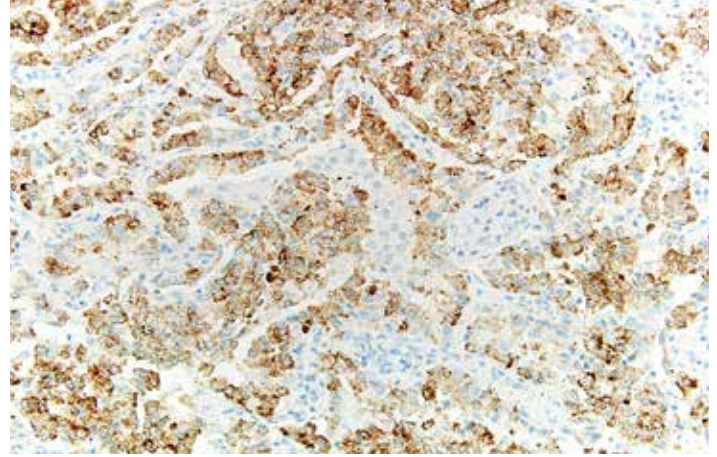
Note that when comparing fixation times between 1 and 12 hours, TTF-1 and EGFR do not exhibit the same loss of staining intensity as ALK.. ALK staining result within the dotted line is clinically positive; whereas, 1 hour fixation (pictured above) represents clinically negative staining. Therefore, Ventana recommends 10% NBF fixation times not less than 6 hours.

## Cut Slide Stability

Ventana has determined that VENTANA -ALK (D5F3) CDx Assay should not be performed on cut slides that have been stored longer than 3 months. The intensity of the staining decreased when slides were stored at room temperature (although none of the ALK positive cases tested at that time point changed its status from ALK positive to ALK negative). Examples are shown below. Ventana has not tested the impact of cut slide stability combined with different fixatives, and 3 months may not be the optimal stability for fixatives other than NBF.



Case 1 freshly sectioned and stained.



Case 1 slides stored ambient (room temperature) for 4 months.

Although both slides are positive for ALK, note diminished staining on the slide stored ambient (room temperature) for 4 months (right panel) compared to the freshly sectioned stained slide (left panel).





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## References

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Ventana Medical Systems, Inc.  
1910 E. Innovation Park Drive  
Tucson, Arizona 85755  
USA  
+1 520 887 2155  
+1 800 227 2155 (USA)



[www.ventana.com](http://www.ventana.com)

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