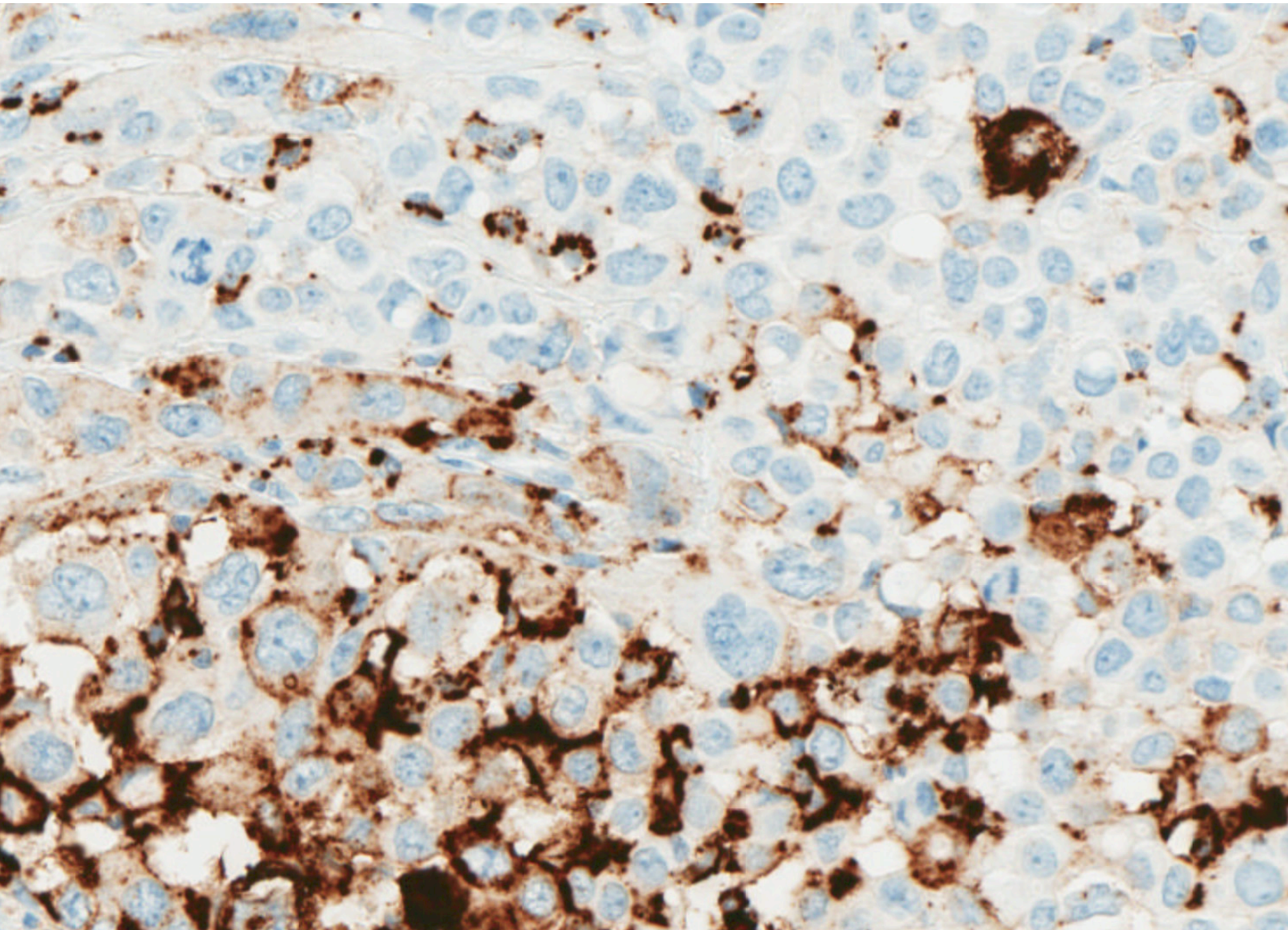
VENTANA PD-L1 (SP142) Assay

Interpretation Guide for Urothelial Carcinoma



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Introduction

VENTANA PD-L1 (SP142) Assay is an immunohistochemical assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody to recognize the programmed death-ligand 1 (PD-L1) protein. This assay was co-developed by Roche/Ventana Medical Systems, Inc. (Ventana) and Roche/Genentech to identify patients with locally advanced or metastatic urothelial carcinoma (mUC) who are most likely to respond to treatment with TECENTRIQ® (atezolizumab).

Urothelial carcinoma (also known as urothelial cell carcinoma, transitional cell carcinoma of the urinary tract, or urothelial bladder cancer) is the most common cancer of the urinary system worldwide. The majority of urothelial tumors arise in the bladder with the remainder originating in the renal pelvis, urethra, or ureter. Transitional cell carcinoma (TCC) is the most common histologic subtype associated with bladder cancer and accounts for greater than 90% of all urothelial carcinoma cases in the industrialized world; non-urothelial subtypes (e.g., squamous cell, adenocarcinoma, small cell carcinoma) are more frequent in other areas of the world.[1]

Globally, there were an estimated 429,793 new cases of bladder cancer and 165,084 deaths in 2012.[2] In Europe alone, for 2012, there were an estimated 151,297 new cases of bladder cancer and 52,411 deaths. In 2015, it was estimated that there would be 74,000 new cases of bladder cancer and 16,000 deaths in the United States.[3] Urothelial carcinoma presents as non-muscle-invasive, muscle-invasive, or metastatic disease. The 5-year relative survival rate for mUC is approximately 5.4%.[4]

PD-L1 is a transmembrane protein that down regulates immune responses through binding to its two inhibitory receptors programmed death-1 (PD-1) and B7.1 (**Figure 1**).[5,6] PD-1 is an inhibitory receptor expressed on T-cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[7] Ligation of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or exhaustion of T cells. B7.1 is a molecule expressed on antigen presenting cells and activated T-cells. PD-L1 binding to B7.1 on T-cells and antigen presenting cells can mediate downregulation of immune responses, including inhibition of T-cell activation and cytokine production.[8] PD-L1 expression has been observed in immune cells and tumor cells.[5,9] Aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion.[7] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment.

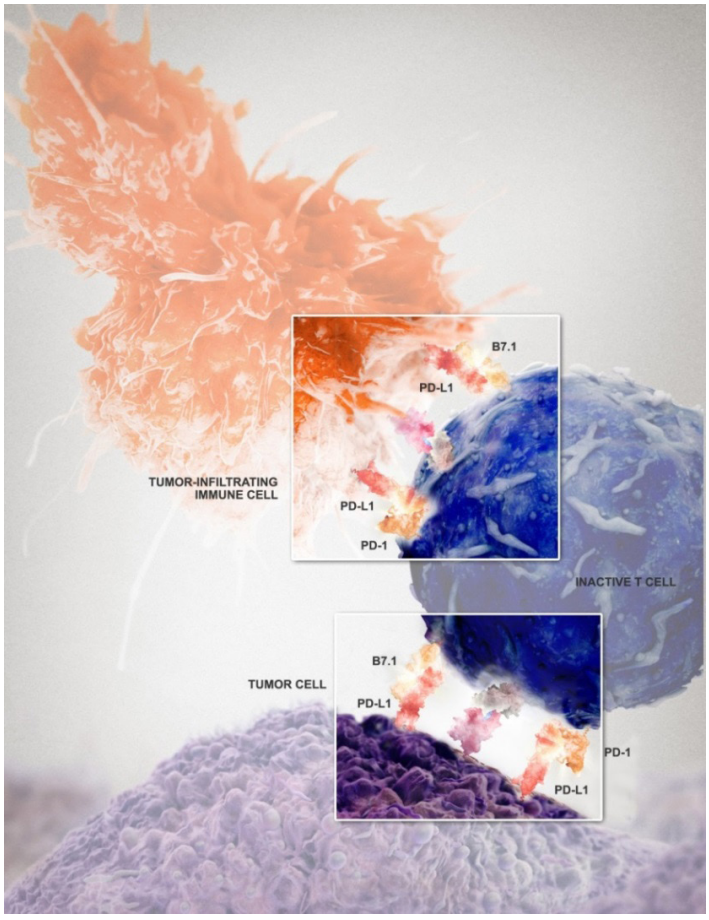


Figure 1: PD-1, PD-L1 pathway

Bellmunt et al. (2015) reported that PD-L1 is widely expressed in tumor cells and tumor-infiltrating mononuclear cells (TIMCs) and showed PD-L1 expression in TIMCs to be associated with longer survival in patients who developed metastases.[10] The association between PD-L1 expression in tumor cells (TC) or tumor-infiltrating immune cells (IC) and clinical benefit with PD-L1/PD-1 pathway inhibitors has been reported in Phase I clinical trials.[9,10,11] Furthermore, targeting the PD-L1 pathway, based on IC expression, has demonstrated activity in patients with advanced urothelial carcinoma who have failed or refused standard-of-care therapies.[13]

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1 and blocks interactions with the PD-1 and B7.1 receptors. Atezolizumab is a non-glycosylated IgG1 kappa immunoglobulin that has a calculated molecular mass of 145 kDa.

Intended Use

Intended Use of Product

VENTANA PD-L1 (SP142) Assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 clone SP142 intended for use in the assessment of the PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) urothelial carcinoma and non-small cell lung cancer (NSCLC) tissue stained with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a VENTANA BenchMark ULTRA instrument. Determination of PD-L1 status is indication-specific, and evaluation is based on either the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity.

PD-L1 expression in ≥ 5% IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is indicated as an aid in identifying urothelial carcinoma patients for treatment with TECENTRIQ (atezolizumab).

See the TECENTRIQ product label for PD-L1 expression cutoff values guiding therapy in specific clinical circumstances.

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

Refer to the VENTANA PD-L1 (SP142) Assay package insert for information on other approved indications.

Purpose of Interpretation Guide

The VENTANA PD-L1 (SP142) Assay interpretation guide is designed to assist pathologists in interpreting and scoring urothelial carcinoma tissues stained with VENTANA PD-L1 (SP142) Assay.

- The photomicrographs included as part of this training guide illustrate the staining patterns, as well as the range of PD-L1 scores, which may be present in urothelial carcinoma tissues stained with VENTANA PD-L1 (SP142) Assay.
- The use of tonsil as a tissue control in the context of PD-L1 evaluation, and the associated staining characteristics and performance are addressed.
- Challenging cases, staining artifacts, and the impact of pre-analytic conditions on the assay are also addressed.

Clinical Evaluation

Staining Overview

Immunohistochemical (IHC) staining with VENTANA PD-L1 (SP142) Assay demonstrates staining in IC (**Figure 2**) as well as TC (**Figure 3**). Detailed staining characteristics are described in the Staining Characteristics - Urothelial Carcinoma section.

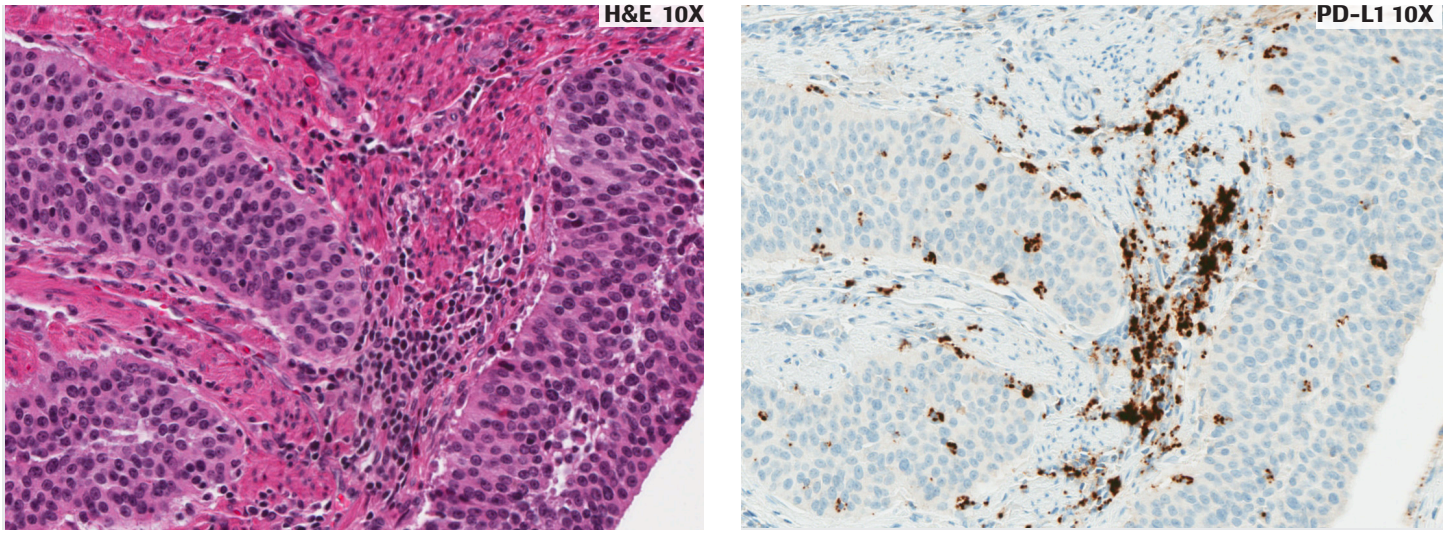


Figure 2: Urothelial carcinoma tissue showing dark brown punctate and linear IC staining

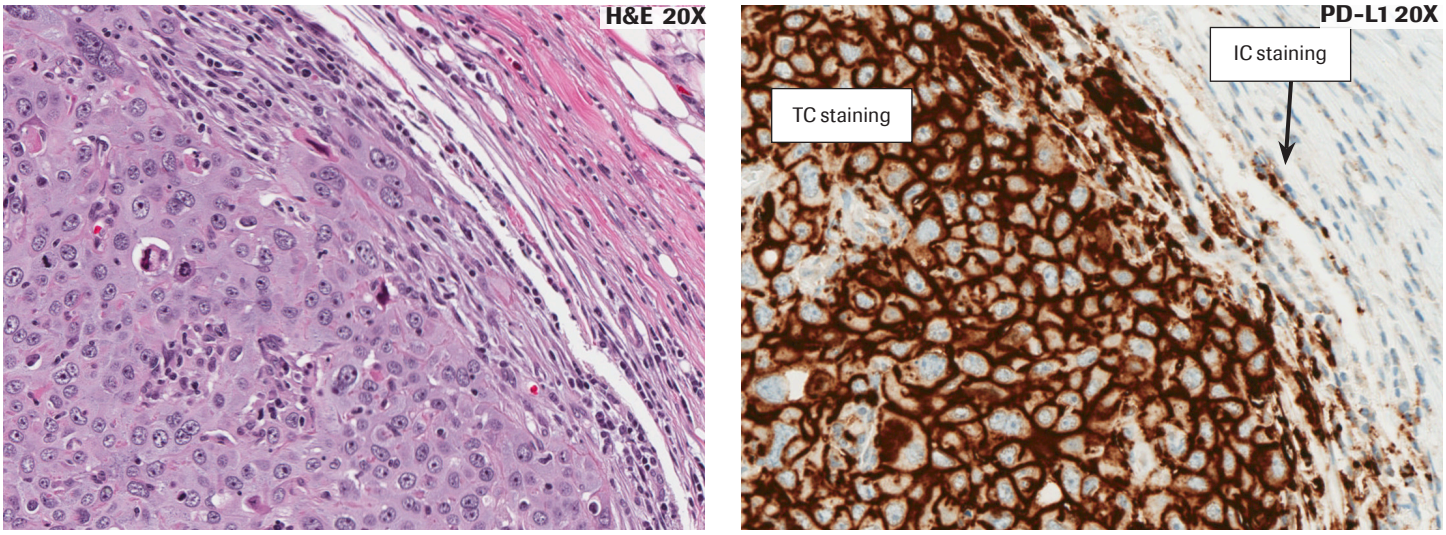


Figure 3: Urothelial carcinoma tissue showing strong circumferential TC membrane staining, as well as IC staining.

VENTANA PD-L1 (SP142) Assay Scoring Algorithm – Urothelial Carcinoma

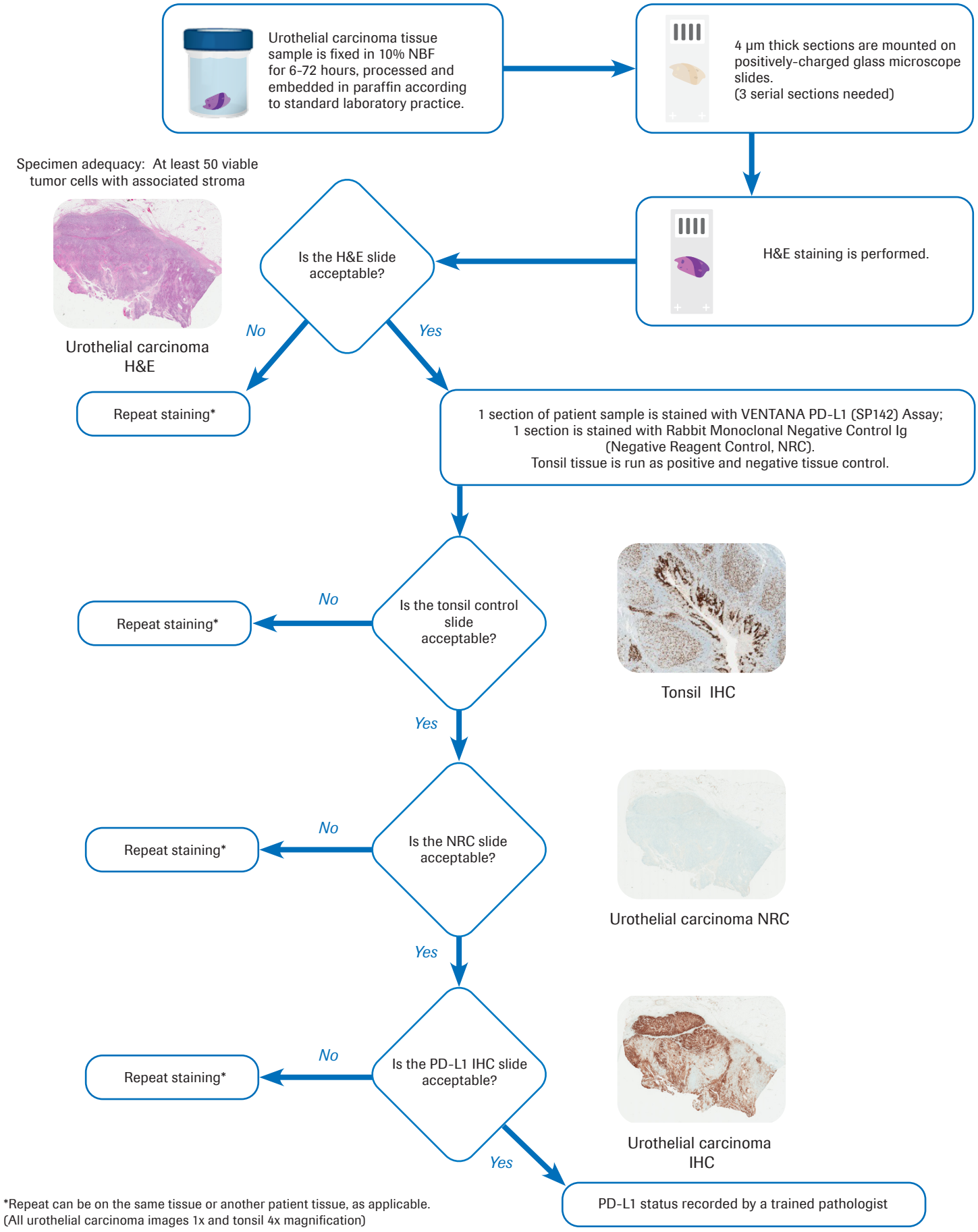
Urothelial carcinoma tissue stained with VENTANA PD-L1 (SP142) Assay will be scored according to the criteria in **Table 1**. IC are scored as the proportion of tumor area that is occupied by PD-L1 staining IC of any intensity. If the specimen contains PD-L1 staining of any intensity in tumor-infiltrating immune cells occupying ≥ 5% of tumor area, the case will be assigned a PD-L1 expression level of ≥ 5% IC. If the specimen contains PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 5% of tumor area, the case will be assigned a PD-L1 expression level of < 5% IC.

Urothelial carcinoma tissue samples obtained from resections, transurethral resection of bladder tumor (TURBT), and core needle biopsies from both primary and metastatic sites are acceptable. This assay has not been validated for use with cytology samples or decalcified bone specimens. Urothelial carcinoma tissue is considered adequate for VENTANA PD-L1 (SP142) Assay evaluation if it contains at least 50 viable tumor cells with associated stroma. Staining requires three sections from each case: one serial tissue section for hematoxylin and eosin (H&E) staining, a second for negative reagent control staining, and a third for VENTANA PD-L1 (SP142) Assay staining. Pre-qualified benign tonsil tissue should be used as positive and negative tissue control for each staining run. Detailed instructions for control tissue qualification and acceptability are outlined in **Table 2**. Matched patient's tissue should be stained with negative reagent control to assess non-specific background staining.

Table 1: VENTANA PD-L1 (SP142) Assay Scoring Algorithm for Urothelial Carcinoma

Tumor-Infiltrating Immune Cell (IC) Staining	PD-L1 Expression
Absence of any discernible PD-L1 staining (OR) Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 5% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 5% IC

Specimen Flow



*Repeat can be on the same tissue or another patient tissue, as applicable.
(All urothelial carcinoma images 1x and tonsil 4x magnification)

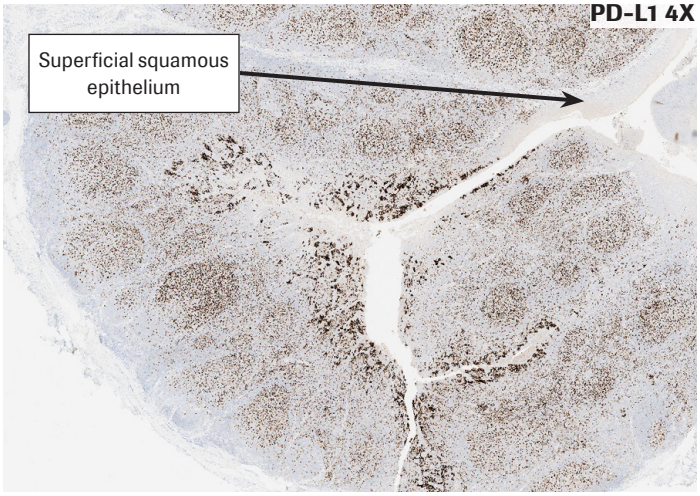
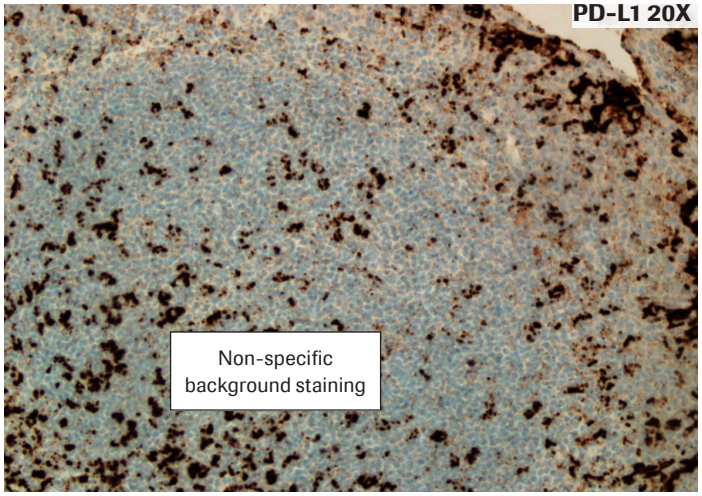
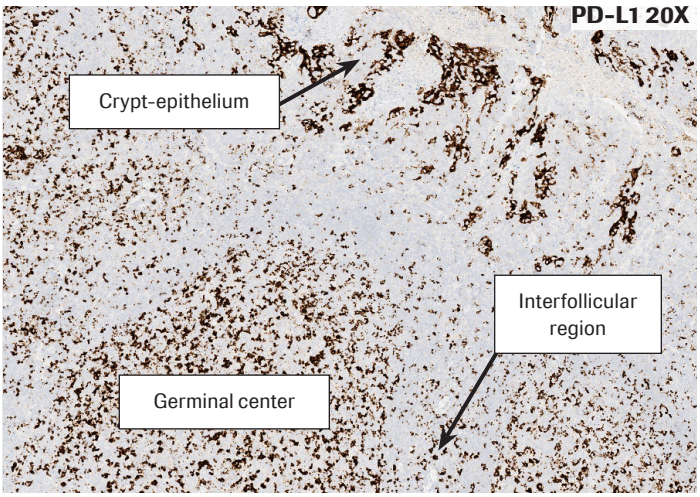
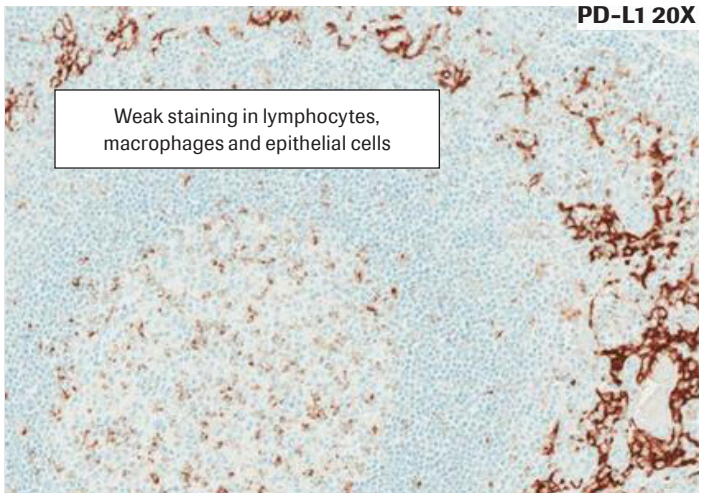
Controls

Tissue controls will be used only for monitoring the correct performance of processed tissues, test reagents and instruments, not as an aid in formulating a specific score for patient samples. One tissue control is recommended in each staining run (on-slide controls are acceptable).

Benign human tonsil is an ideal tissue control as it contains both positive and negative staining epithelial and immune cells and can serve as both a positive and negative tissue control for VENTANA PD-L1 (SP142) Assay staining. Tonsil tissue stained with VENTANA PD-L1 (SP142) Assay demonstrates staining of lymphocytes and macrophages in germinal centers, with scattered PD-L1 staining immune cells among PD-L1-negative cells in interfollicular regions. Also, diffuse membrane staining is observed in the reticulated crypt epithelial cells with absence of staining of superficial squamous epithelial cells.

Tonsil tissue fixed in 10% NBF and processed similar to patient tissues should be qualified and used as a tissue control. The tonsil tissue control should show acceptable staining for an assay run to pass. If tonsil tissue shows unacceptable staining, the run is considered invalid and a repeat run, including patient samples, should be performed. Qualification and acceptability criteria for tonsil tissue controls are listed in **Table 2**.

Table 2: Tonsil Qualification and Acceptability Criteria

Acceptable	Unacceptable
Positive tissue elements: Moderate to strong PD-L1 staining noted in lymphocytes and macrophages in germinal centers, with diffuse staining in reticulated crypt epithelial cells.	Excessive non-specific background staining obscuring the identification of PD-L1 positive cells.
Negative tissue elements: PD-L1 negative immune cells in the interfollicular regions with negative superficial squamous epithelium.	Weak to no PD-L1 staining noted in lymphocytes and macrophages in germinal centers, and reticulated crypt epithelial cells.
	
	

Staining Characteristics – Urothelial Carcinoma

PD-L1 staining with VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissues demonstrates staining in IC (**Figure 4–Figure 7**), as well as TC. The images in this interpretation guide are snapshots from scanned slides; magnification is noted for each image.

IC Staining:

IC are immune cells present in the intratumoral and contiguous peritumoral stroma. The VENTANA PD-L1 (SP142) Assay stain highlights a heterogeneous population of immune cells; the majority of which is morphologically consistent with lymphocytes, macrophages, dendritic cells, and granulocytes.

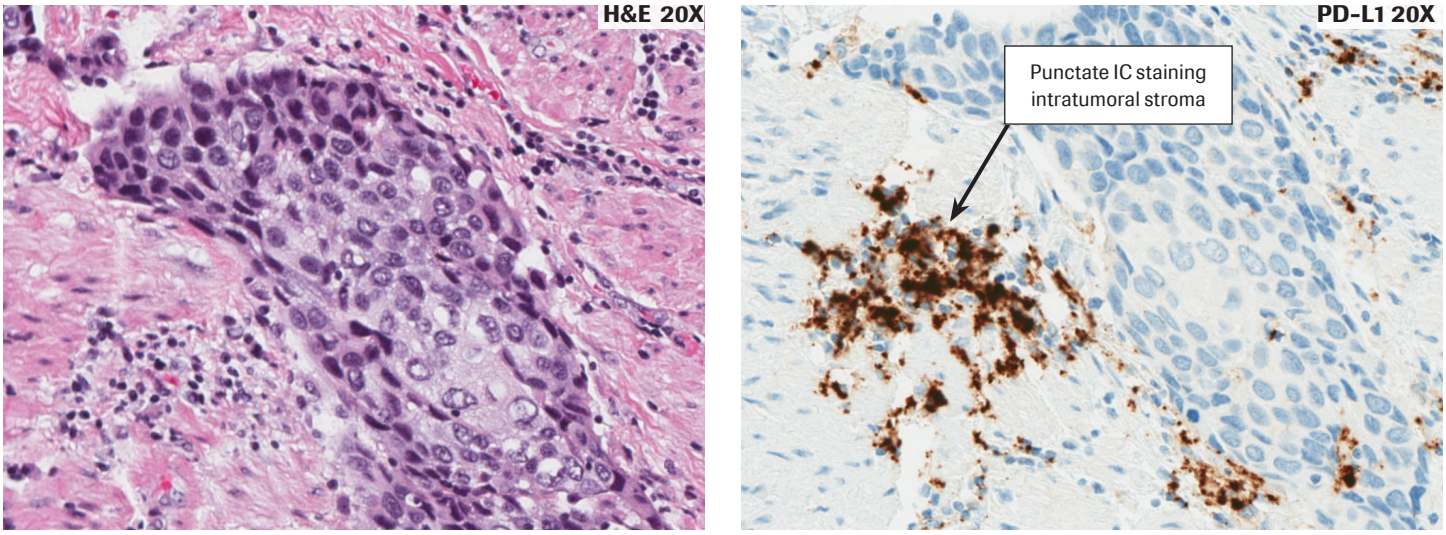


Figure 4: IC often show dark brown punctate or linear staining, which is the predominant IC staining pattern observed in the majority of tissues. IC staining is often seen as aggregates either in intratumoural or peritumoral stroma (invasive margin) or in both locations.

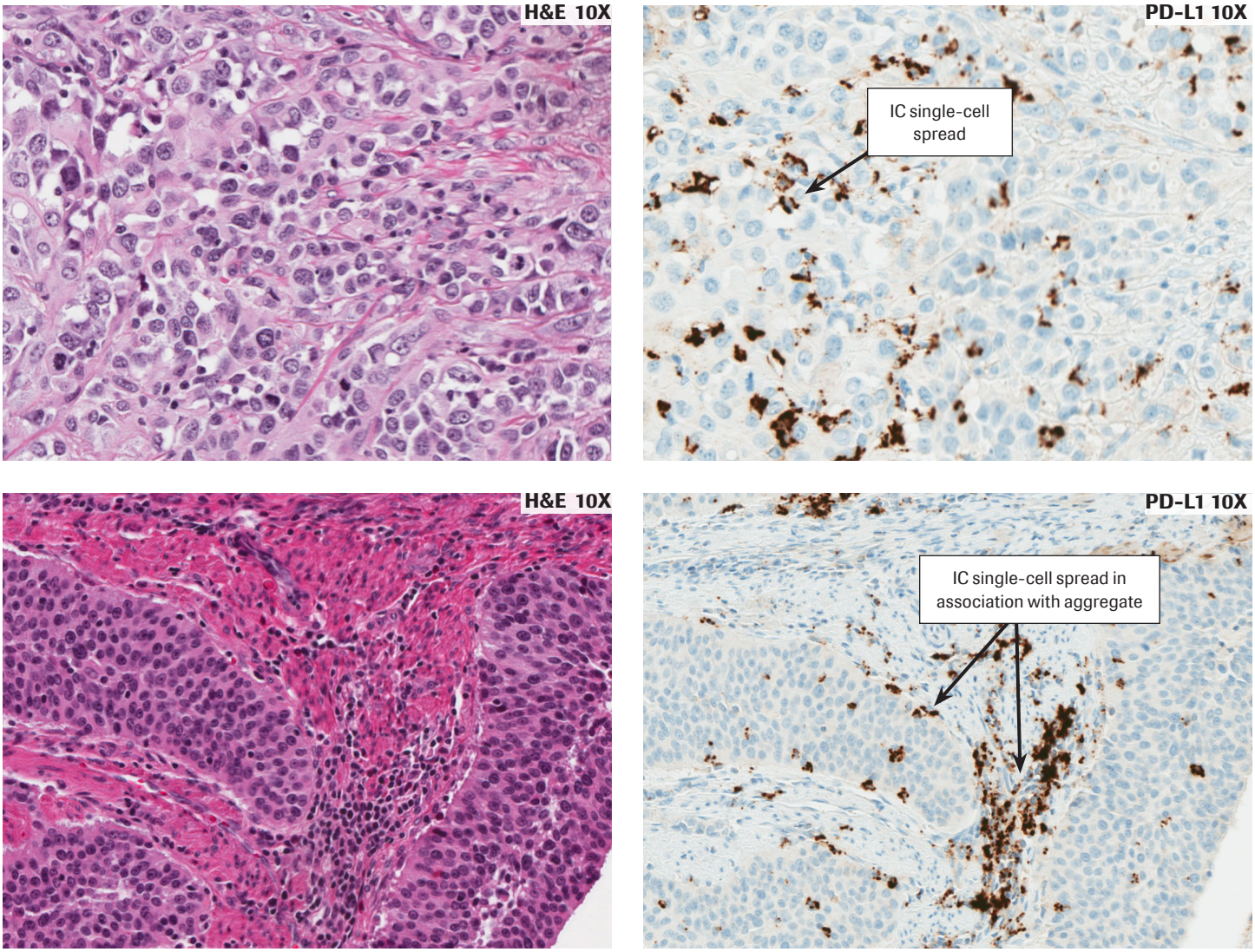


Figure 5: Occasionally, IC staining can also be observed in the form of focal or diffuse scattered single cells or small aggregates (single-cell spread) dispersed among tumor cells. This pattern may be seen in association with aggregates in tumor stroma. IC staining corresponds to the immune cells among tumor cells in the H&E image.

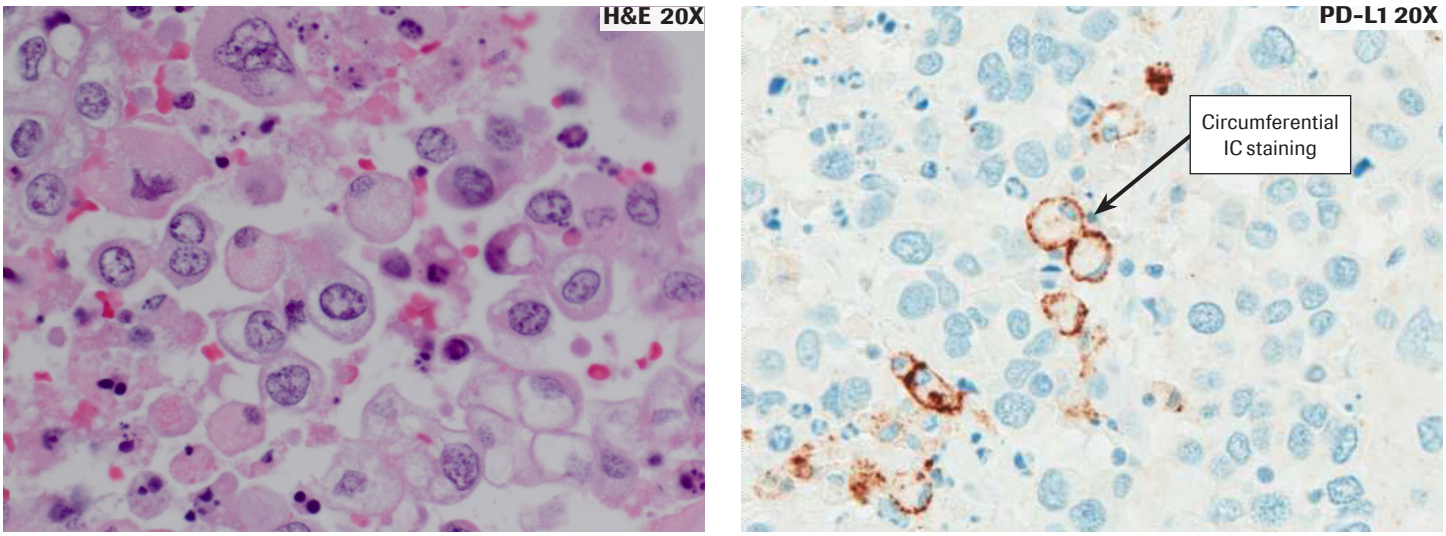
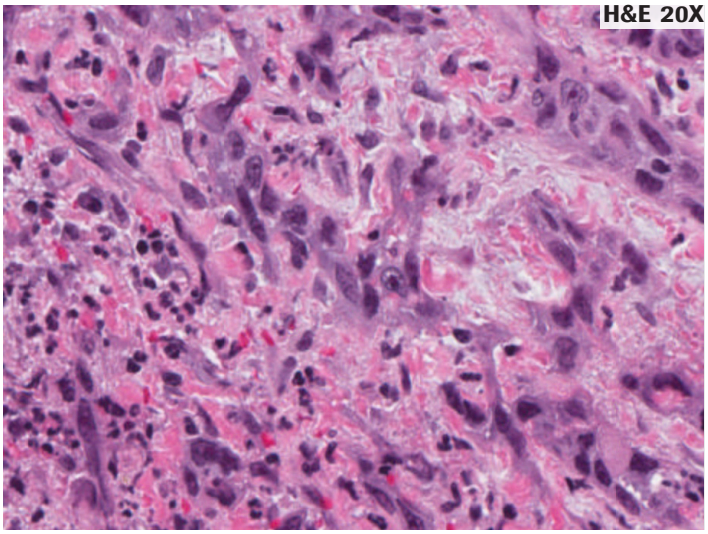


Figure 6: Occasionally circumferential immune cell membrane staining is also observed, especially in cells that are morphologically consistent with macrophages and/or dendritic cells.



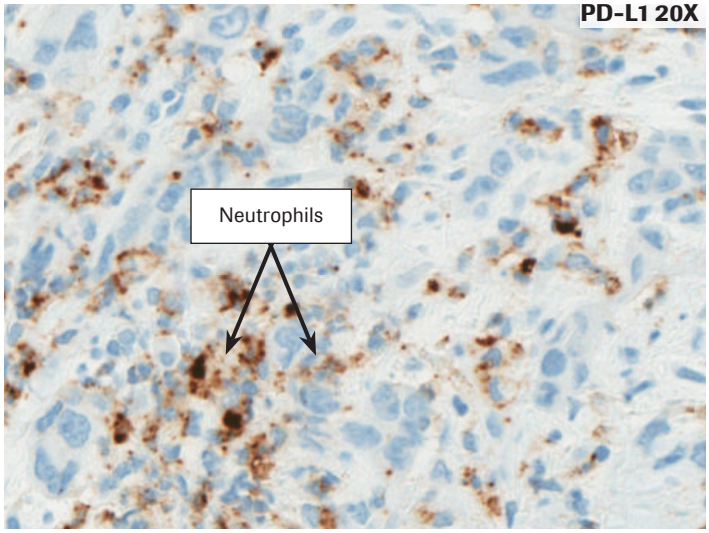
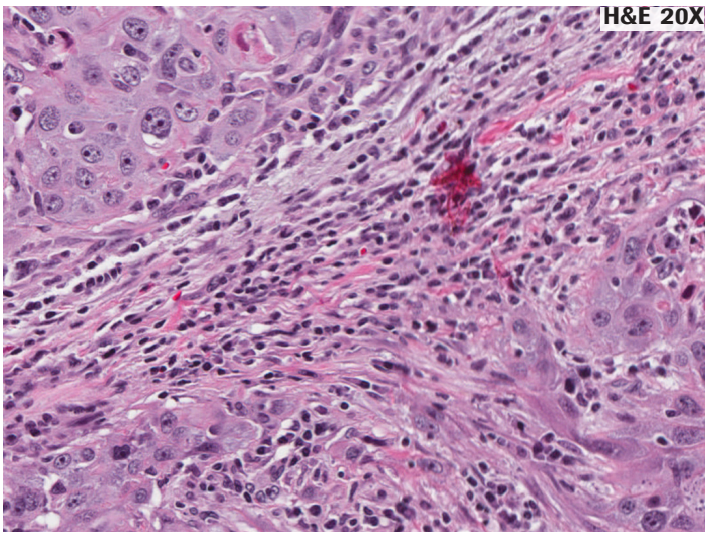


Figure 7: Rarely, IC staining can be observed in neutrophils, as fine punctate staining along with diffuse granular staining. Neutrophil staining can be seen dispersed among tumor cells, in the intratumoral or peritumoral stroma or as aggregates.

Differentiation of IC from TC Staining

IC staining can be observed in association with TC staining. The PD-L1 stained slide will only be evaluated for IC staining; hence, differentiation of IC from TC staining is essential. Review of the corresponding H&E slide will help in identifying IC among TC. This, along with a high magnification review of the PD-L1 stained slide, may aid in differentiating between IC and TC staining. The following images demonstrate different commonly observed patterns encountered in clinical practice, when IC and TC staining is observed together (**Figure 8–Figure 10**).



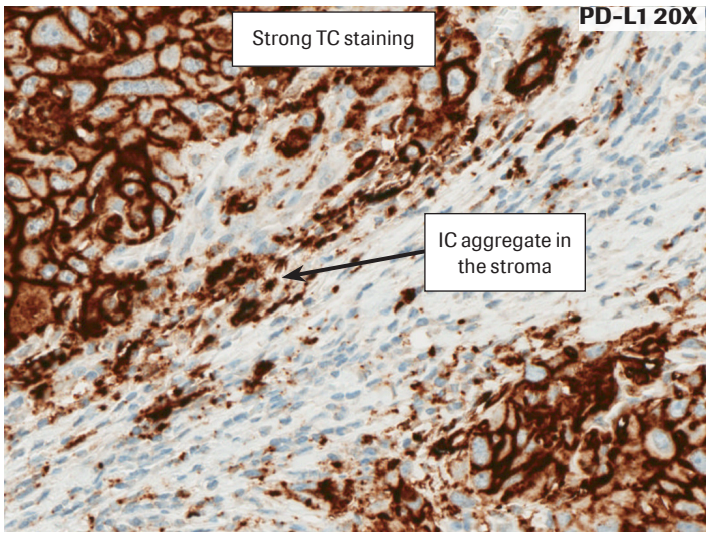


Figure 8: TC show strong membrane staining, with rare IC among the TC identified on the H&E slide; focus on evaluating IC staining in intratumoral and contiguous peritumoral stroma.

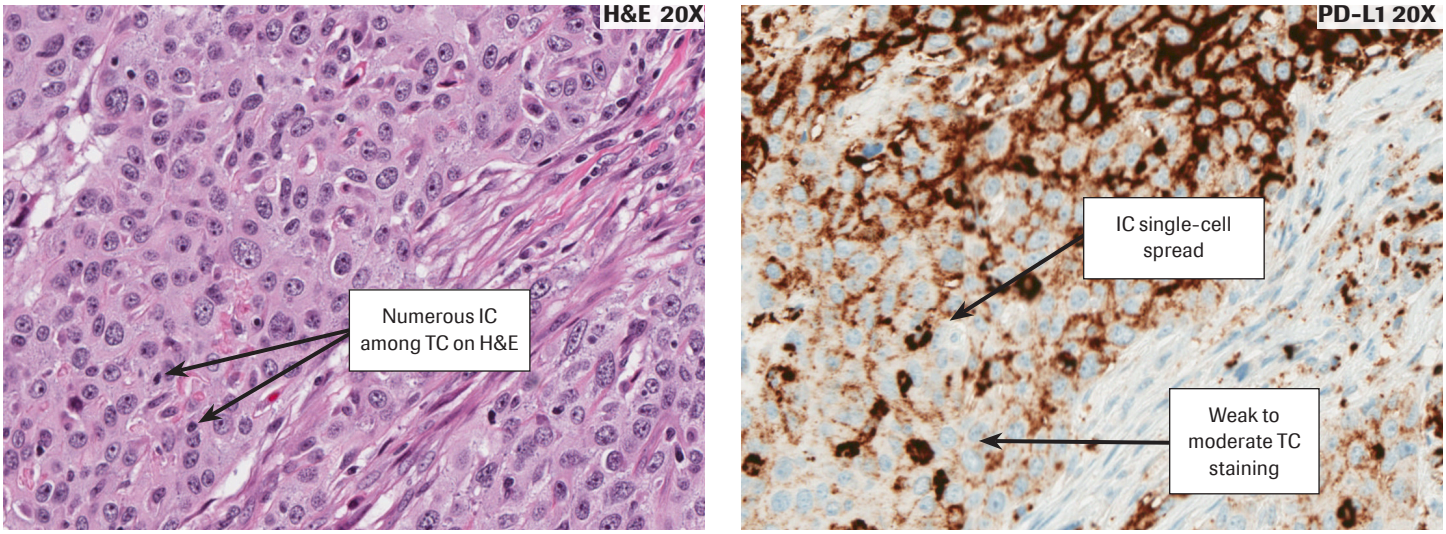


Figure 9: TC show weak to moderate membrane staining with many IC among TC identified on the H&E slide; focus on evaluating IC both among tumor cells and in the stroma.

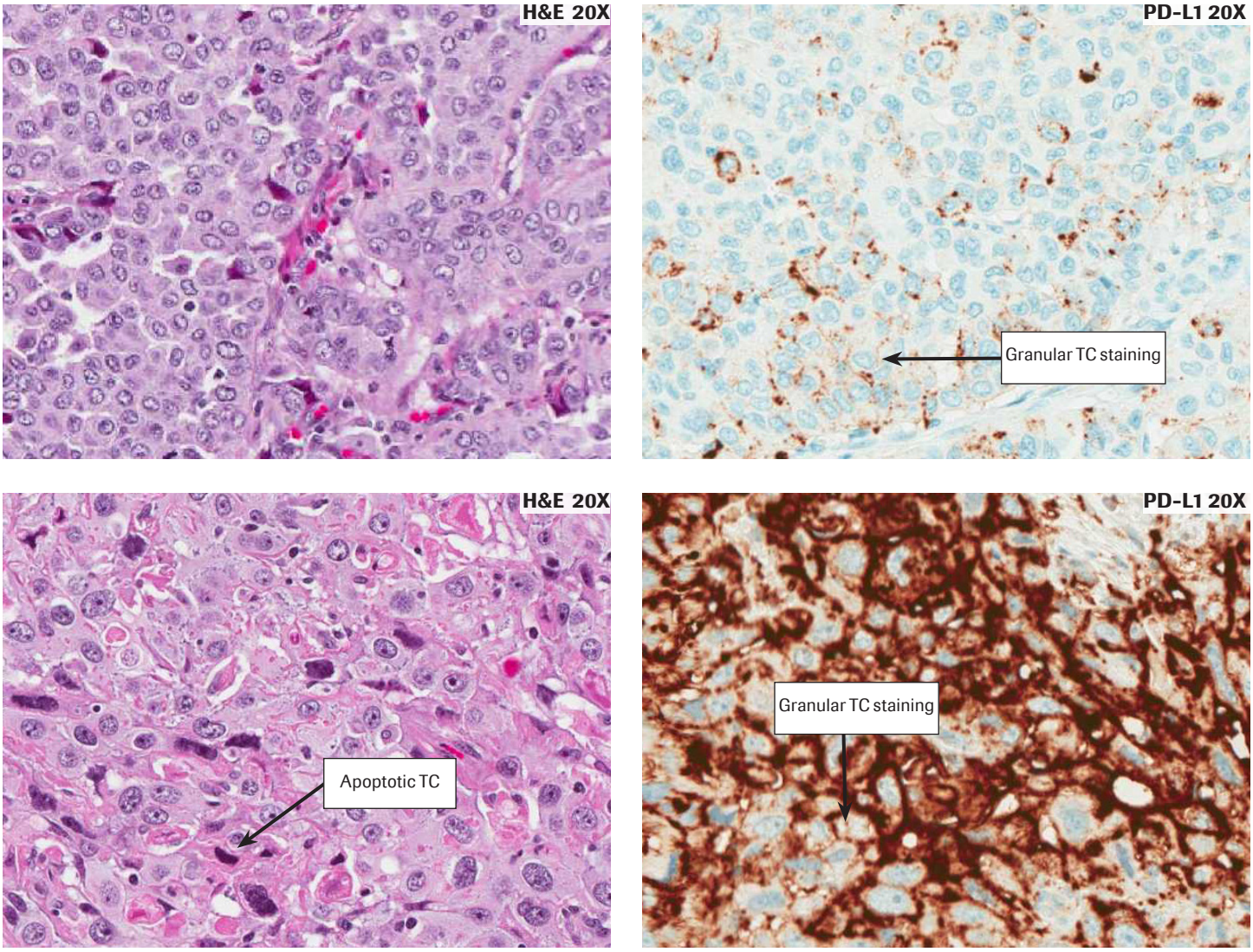


Figure 10: If the H&E slide does not show many identifiable IC, and granular or beaded staining pattern is observed among TC, then this staining should be attributed to TC rather than IC.

Scoring Method

VENTANA PD-L1 (SP142) Assay stained urothelial carcinoma tissue will only be evaluated for IC staining.

IC scoring: IC are scored as the **proportion of tumor area that is occupied by PD-L1 staining immune cells of any intensity**. Any IC staining irrespective of type of cells or localization is included.

- Tumor Area: Tumor area for PD-L1 (SP142) interpretation is defined as area occupied by viable tumor cells, and their associated intra- and contiguous peritumoral stroma (**Figure 11A**, Resection specimen: **Figure 11B&C**, TURBT: **Figure 11D&E**). Necrotic tumor is excluded from this definition of tumor area (**Figure 11F**).
- In fragmented tissue samples, including TURBT and biopsies, where distinction of intra versus peritumoral stroma is difficult, only stroma that is contiguous to individual tumor nests is included in the tumor area definition; stroma that is part of the tissue fragment, but not contiguous to viable tumor, is excluded (**Figure 11B&C**).

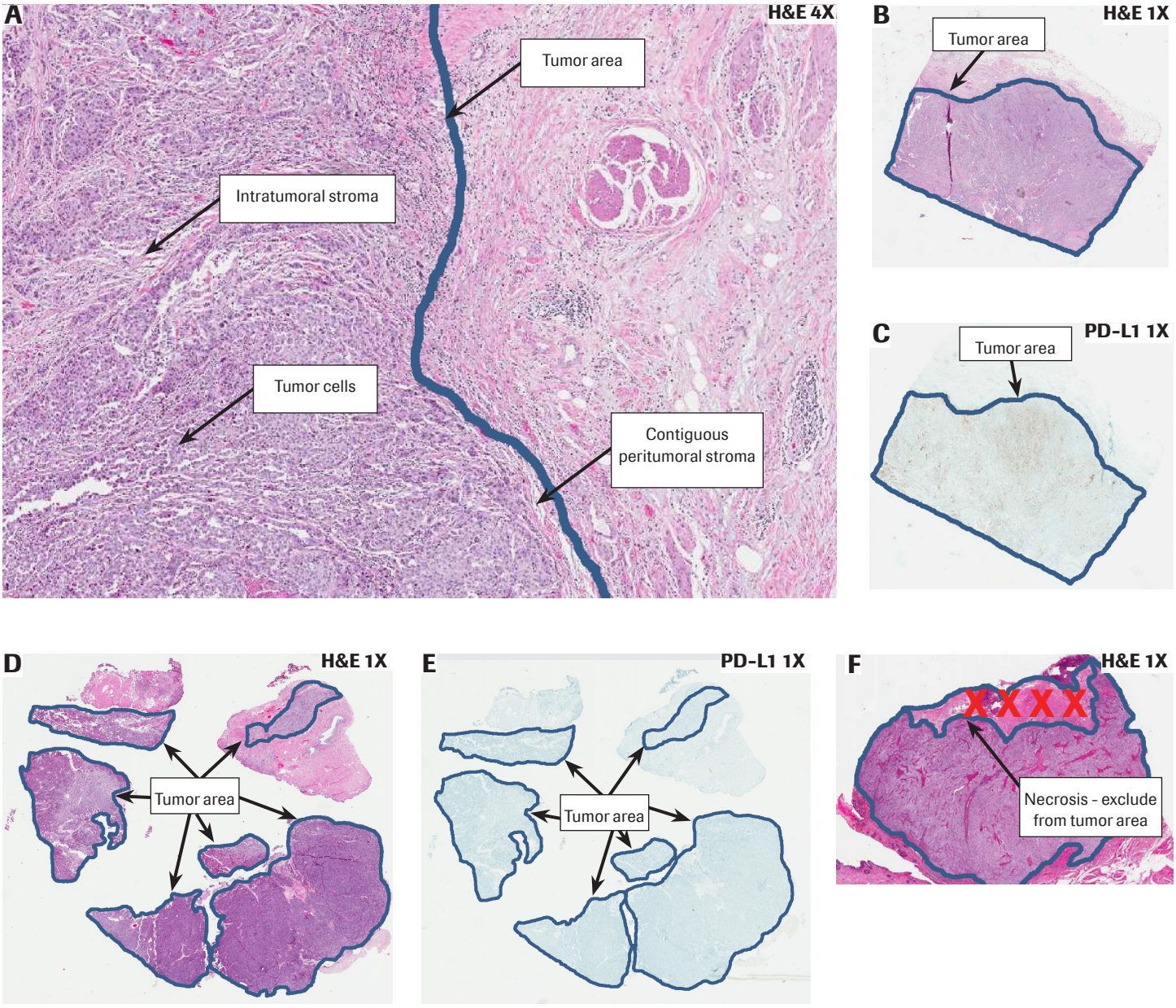
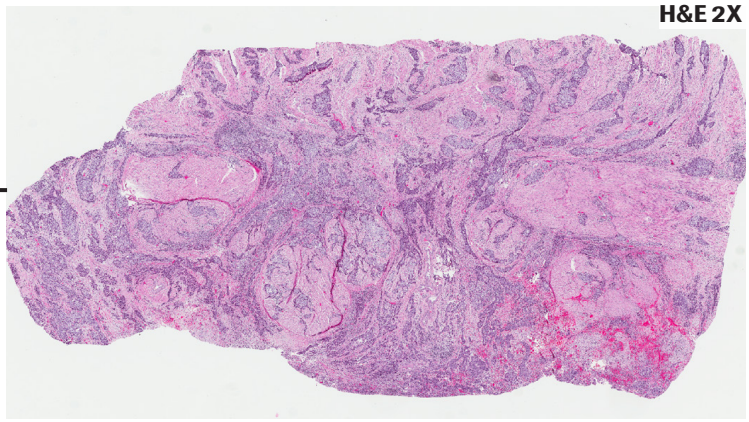


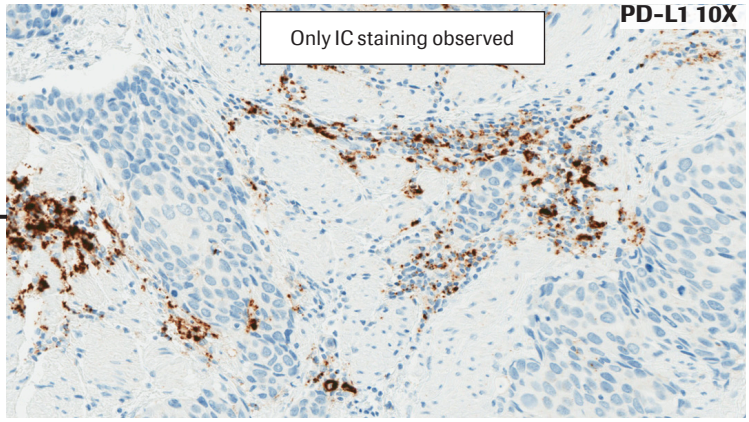
Figure 11: **A** Tumor Area; **B&C** Tumor area in a resection specimen; **D&E** Tumor area in a transurethral resection of bladder tumor (TURBT) specimen; **F** Tumor area in a specimen containing necrosis.

Scoring of PD-L1 IC aggregate staining:

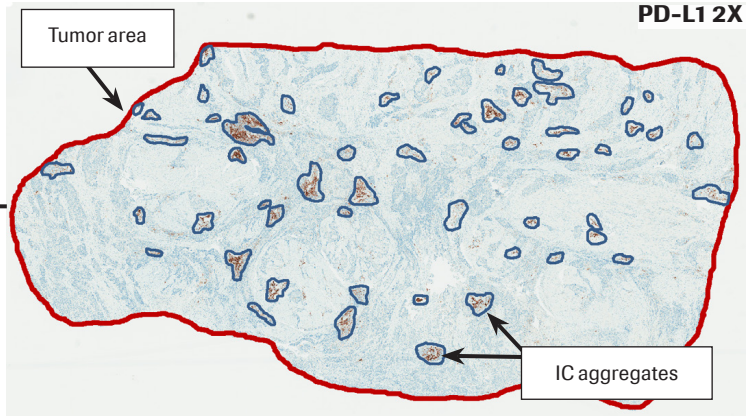
Review the H&E stained slide for the presence of tumor, necrosis, adequacy (at least 50 viable tumor cells with associated stroma), and assessment of tumor area.



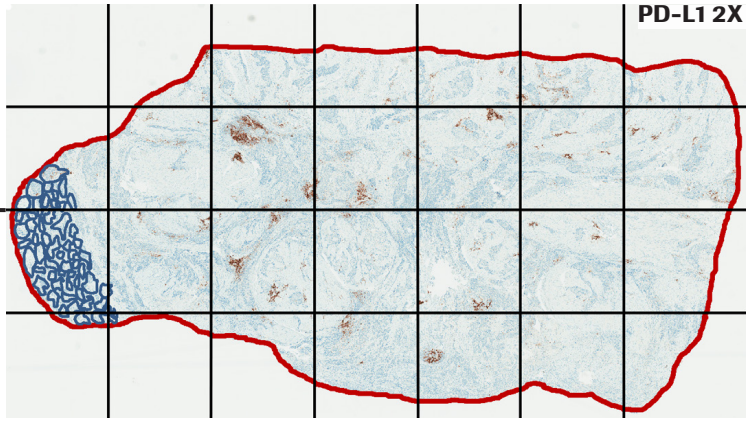
Review the PD-L1 stained slide at 10x or 20x magnification and confirm overall staining pattern as IC, TC or both.



Visually encircle IC aggregates as closely as possible using 2x or 4x magnification.

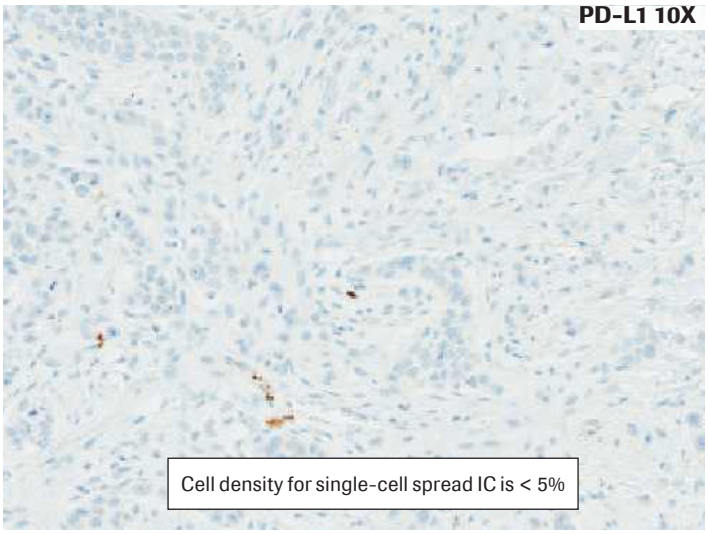


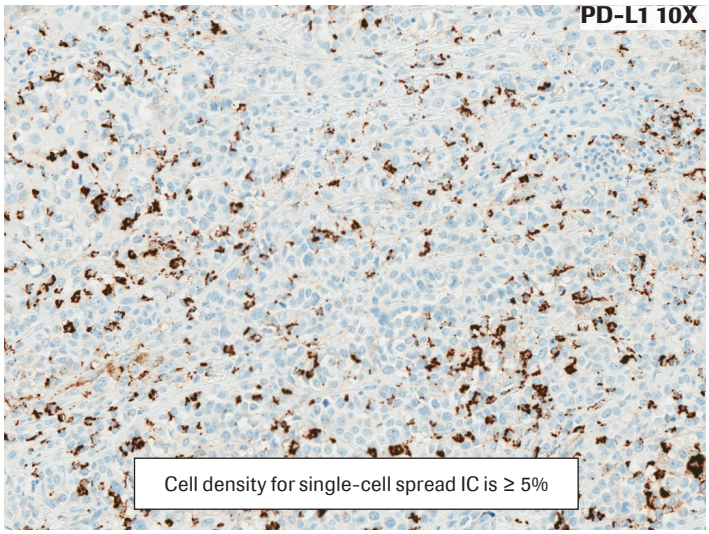
Combine the aggregate staining and estimate the proportion of tumor area occupied by IC aggregates using 2x or 4x magnification. The score for this case is ≥ 5% IC. Reference images in the Reference Images section should be used as an aid in scoring.



Scoring of PD-L1 IC single-cell spread staining:

Single-cell spread IC is scored based on the density of single cell spread, using the Reference Images section of this guide.





Scoring Methods: Challenges and Pitfalls

1. **Tumor area in papillary urothelial carcinoma:** In specimens with papillary urothelial carcinoma, the stroma in fibrovascular cores is considered intratumoral stroma. Lamina propria at the base of the papillary lesion may contain lymphoid aggregates that show staining of PD-L1. Only staining that is contiguous to the base of the tumor is considered part of tumor area (**Figure 12**).

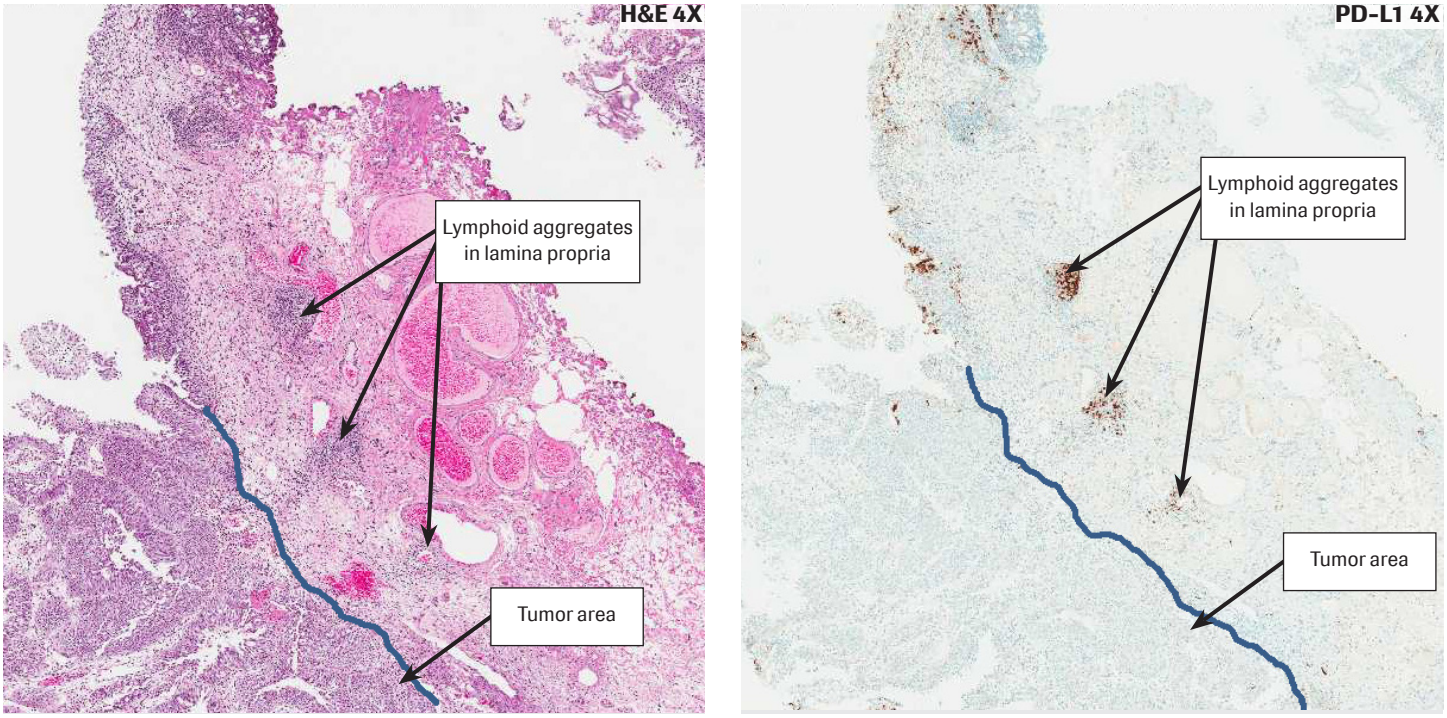


Figure 12: Lymphoid aggregates in the lamina propria are excluded from IC scoring in a papillary urothelial carcinoma.

2. **Intravascular Immune cells:** Vasculature in tumor stroma may show PD-L1 positive immune cells (**Figure 13**). This is observed most often in fibrovascular cores of papillary urothelial carcinoma. These are not considered towards IC scoring.

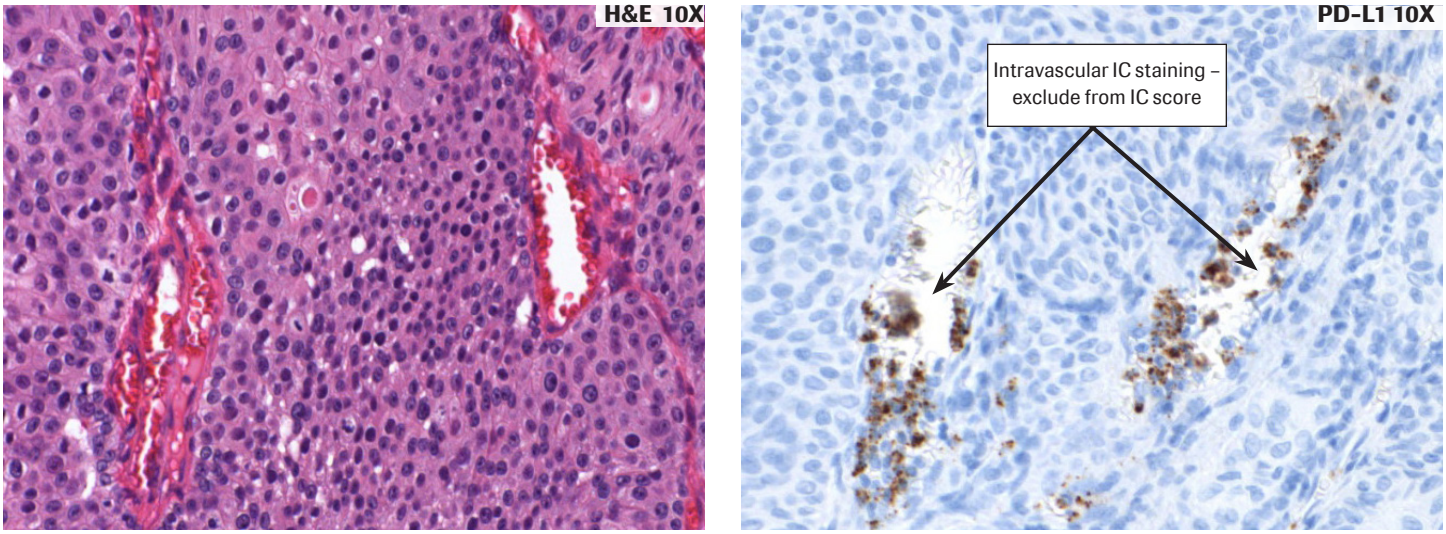


Figure 13: Intravascular immune cell staining – exclude from IC scoring.

3. **Staining in granulomas:** Some urothelial carcinoma tissues can show granulomas, possibly due to treatment effect. Staining of PD-L1 can be seen in macrophages and giant cells in these granulomas and can be mistaken for tumor cell staining (**Figure 14**). Review of the corresponding H&E slide will faciliate their identification as immune cell staining. If these granulomas are present within the tumor area, they should be included in IC scoring.

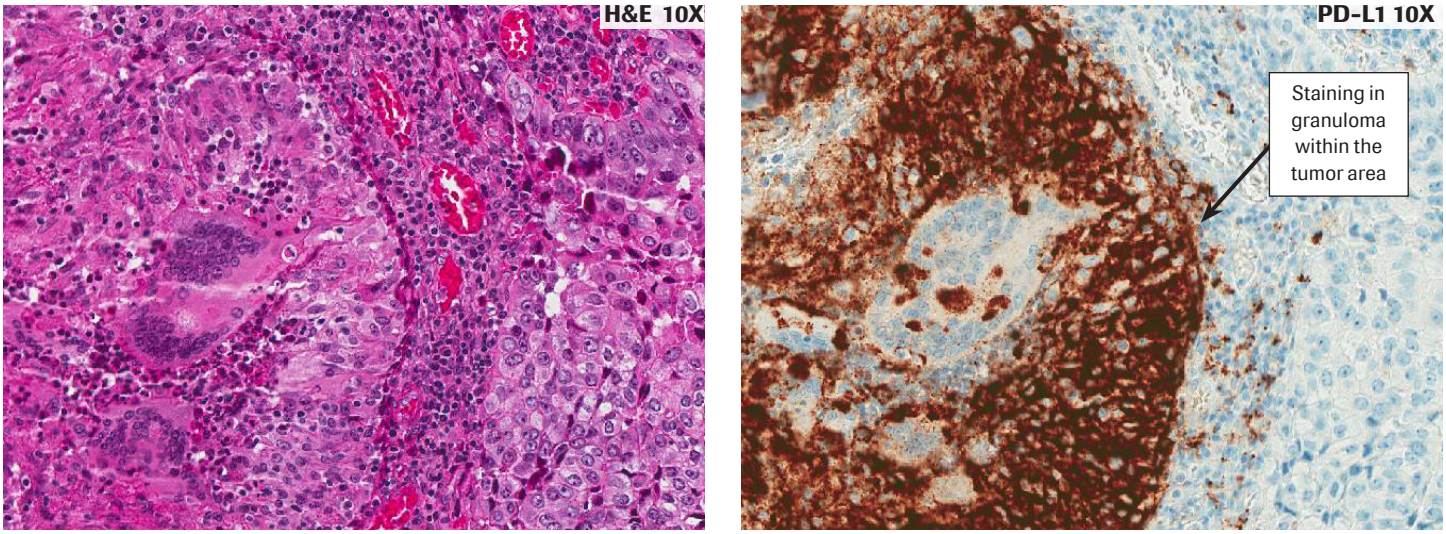


Figure 15: Staining in granuloma – consider towards IC if present in tumor area

4. **Staining in necrotic debris:** Necrotic debris or immune cells in the periphery of necrotic or apoptotic regions can show PD-L1 staining. This staining may be granular and can be mistaken for IC staining. This staining, as well as the neutrophil staining observed as aggregates, should be excluded from scoring (**Figure 15**).

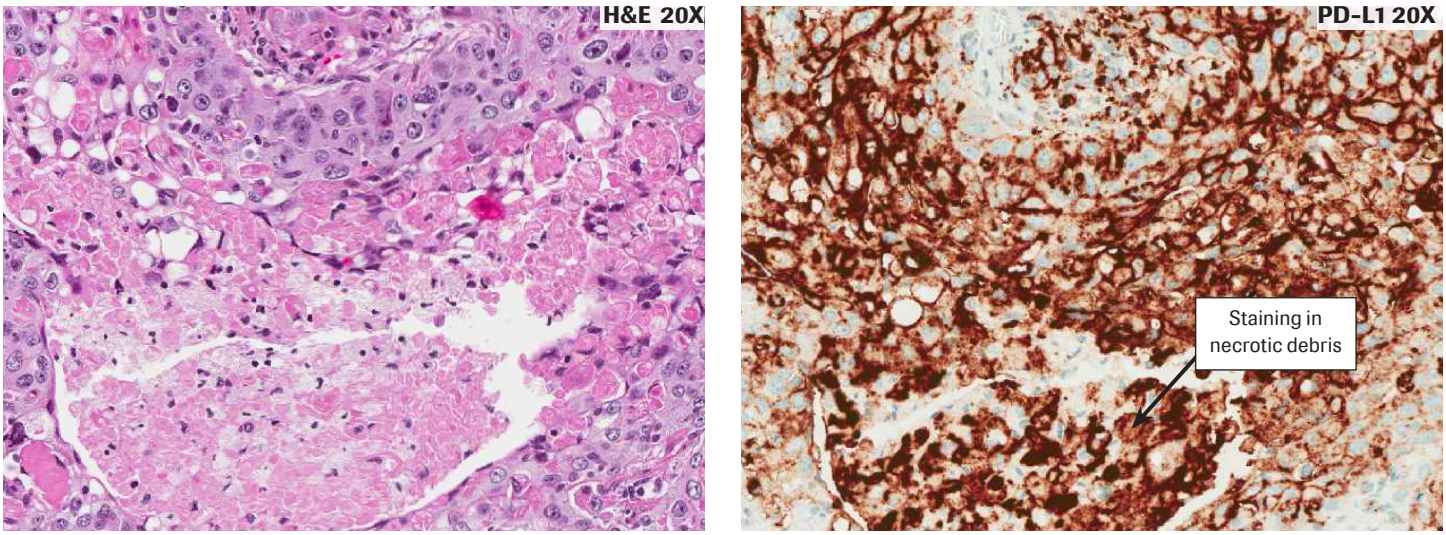


Figure 15: Necrotic debris showing PD-L1 staining. This should not be included in IC scoring. Note the presence of TC staining, which is not scored.

5. **Lymph node metastasis:** VENTANA PD-L1 (SP142) Assay can be used to test both primary and metastatic samples. Metastatic samples can originate from various organs which include, but are not limited to, lymph node, liver, adrenal gland, bone, and soft tissue. Metastases from bone are not suitable for staining with VENTANA PD-L1 (SP142) Assay. Lymph node metastases deserve special attention, given the presence of native immune cells which show staining for PD-L1. In tumors metastasizing to lymph nodes only immune infiltrate staining contiguous to the tumor cells should be counted towards the PD-L1 IC percentage (**Figure 16**).

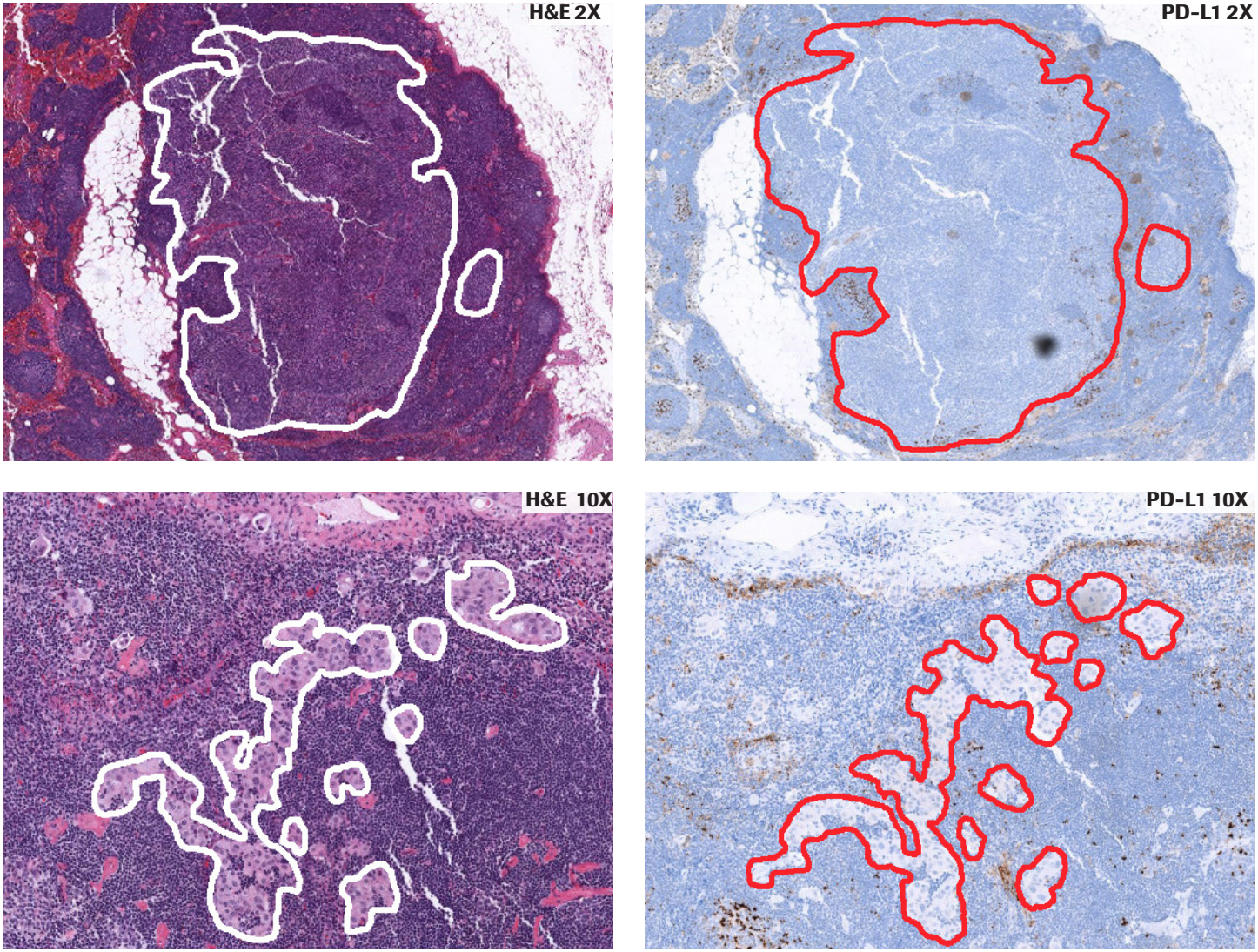


Figure 16: A & **B**: Lymph node with well circumscribed metastasis. **C** & **D**: Lymph node with multiple small tumor cell nests. Tumor area is outlined both on H&E and PD-L1 images.

Reference Images

VENTANA PD-L1 (SP142) Assay: IC Scoring

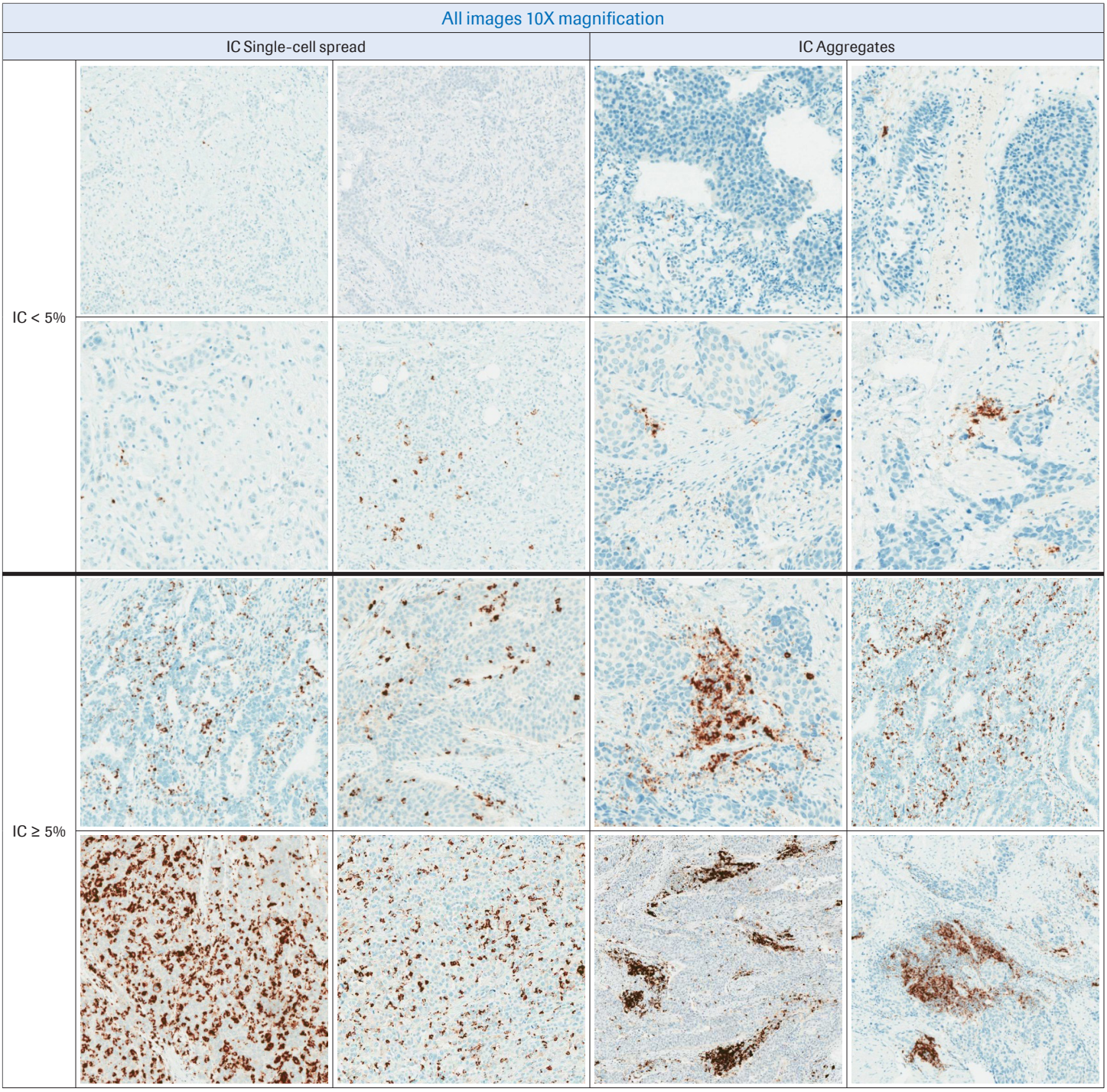
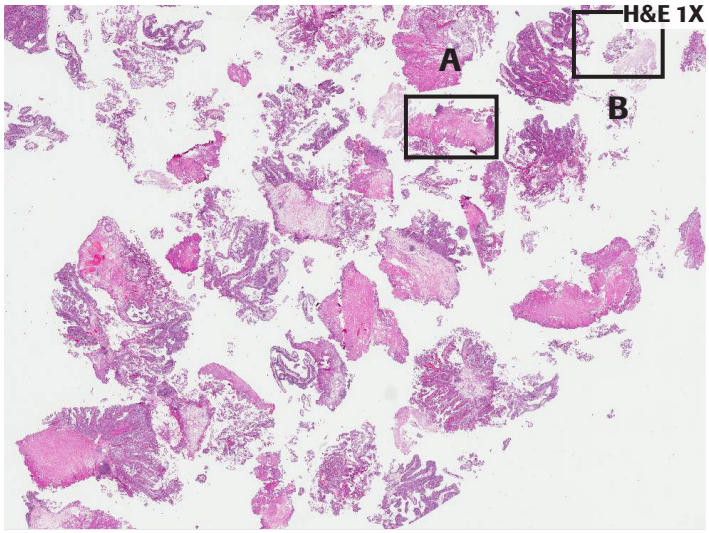
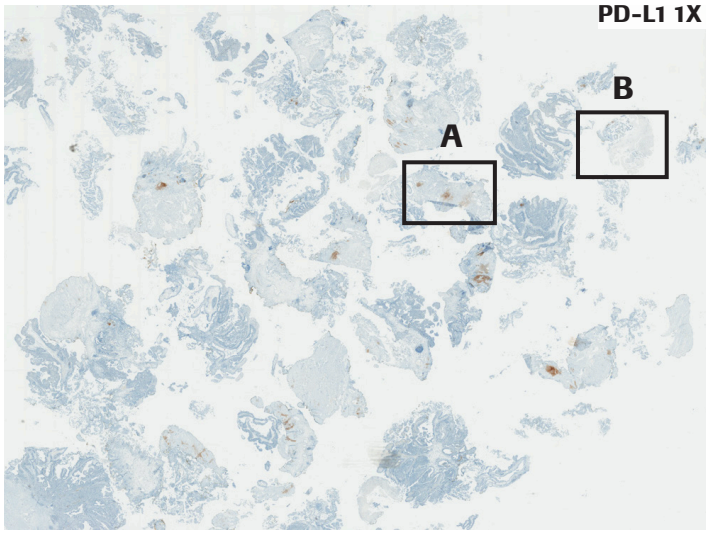
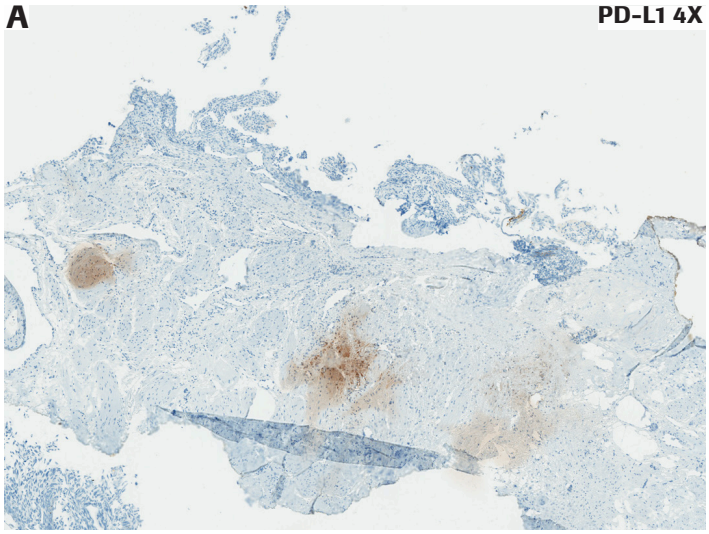


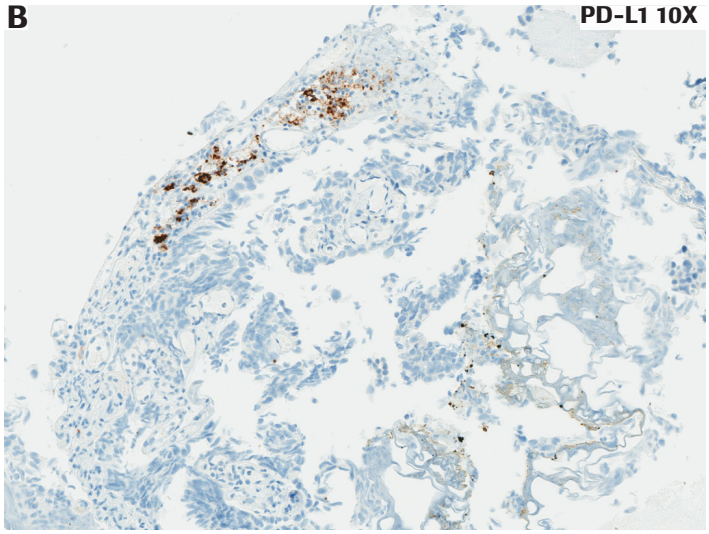
Table 3: Tumor-Infiltrating Immune Cell (IC) Characteristics	
Type of cells showing staining	Lymphocytes, macrophages, dendritic cells, and granulocytes
Type of cells included in scoring	Lymphocytes, macrophages, dendritic cells, and granulocytes
Pattern	Aggregates in stroma, single cells dispersed among tumor cells with punctate, linear or circumferential staining
Denominator for scoring	Tumor area

VENTANA PD-L1 (SP142) Assay: IC < 5%

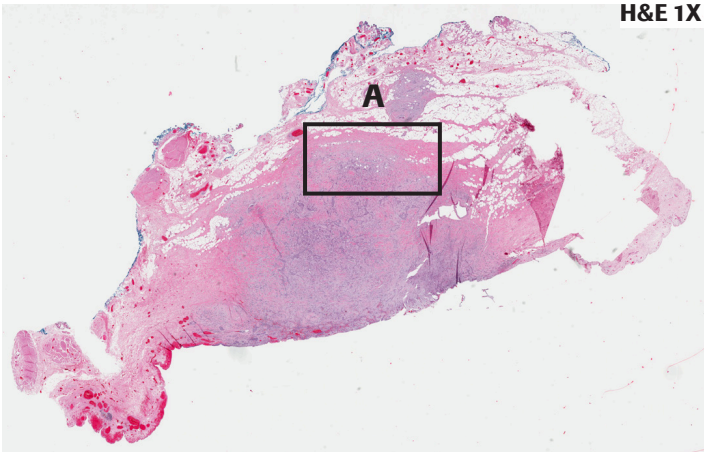


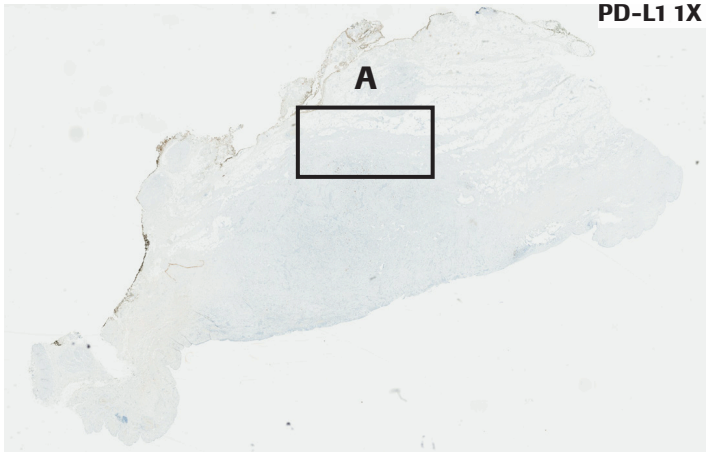


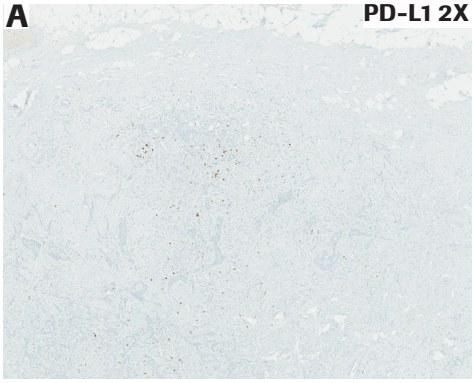


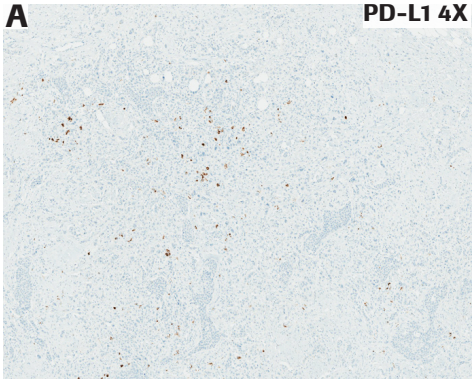


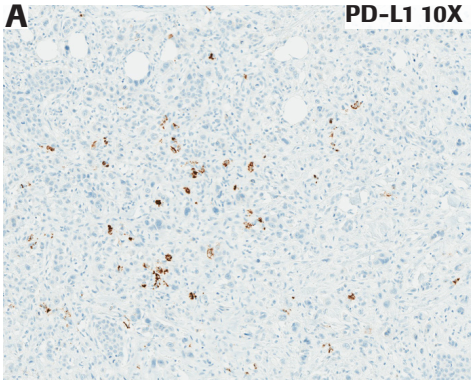
Case 1: This case is IC < 5%. Score: IC: <1%; TC 0%. At low magnification, this case exhibits several regions of brown staining. When examined at high magnification most of the brown staining appears to be due to trapping of reaction products (dye) under the folded tissue (dye trapping). It is important to distinguish between dye trapping (**Region A**) and specific IC staining (**Region B**).



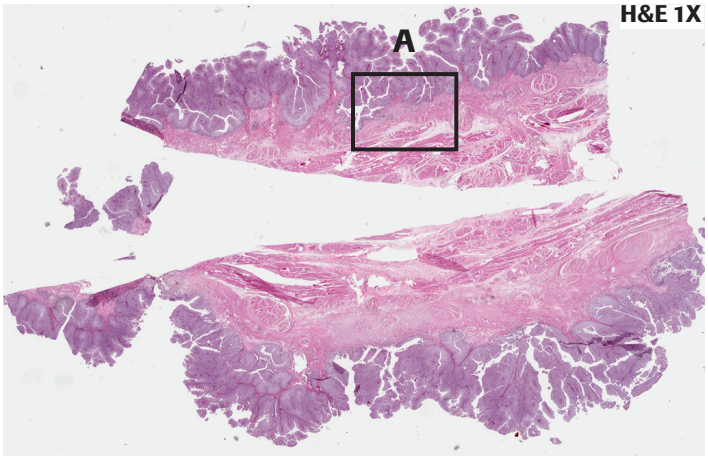


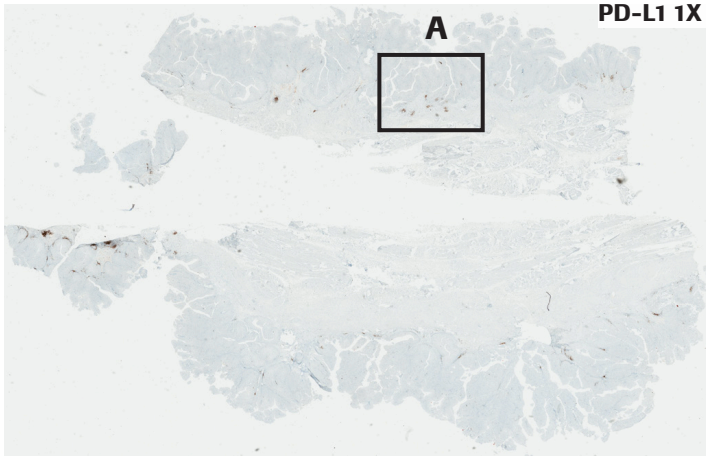


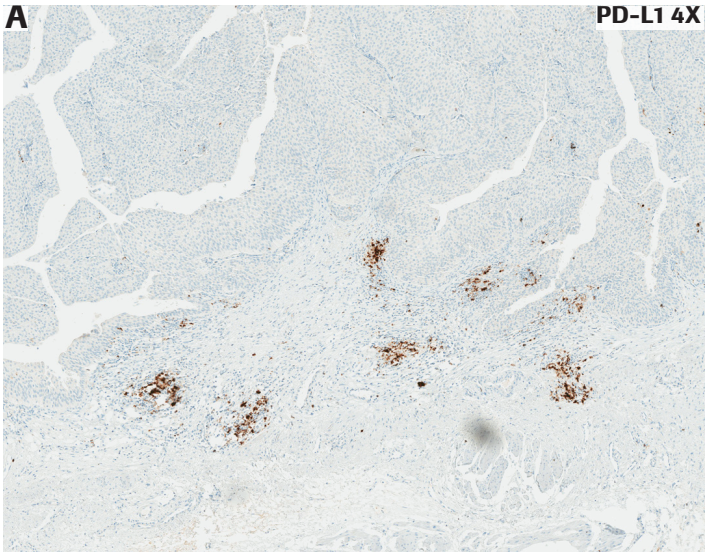


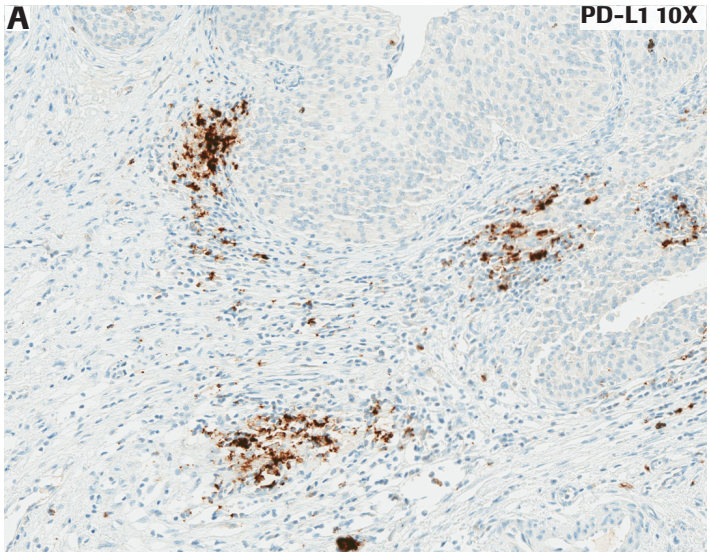


Case 2: This case is IC < 5%. Scores: IC: 1%; TC: 0%. Note the presence of focal IC single cell spread of PD-L1 staining IC, which when normalized to the entire tumor area is < 5%.



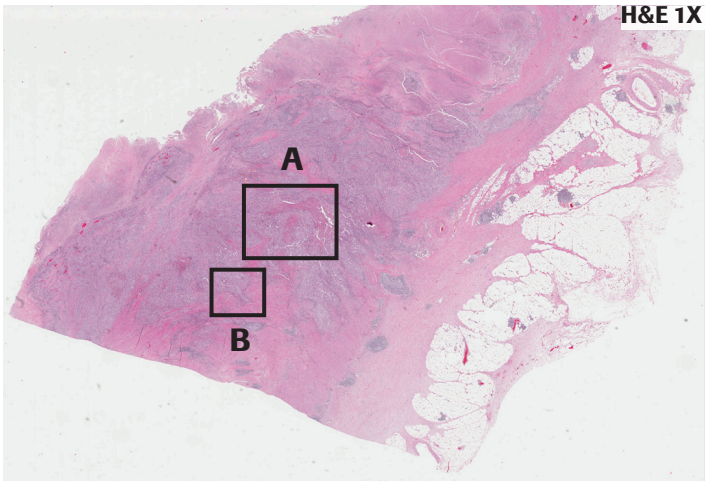


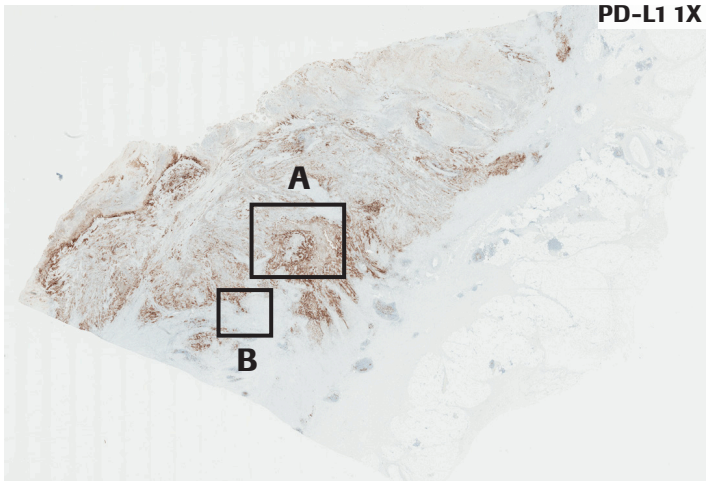


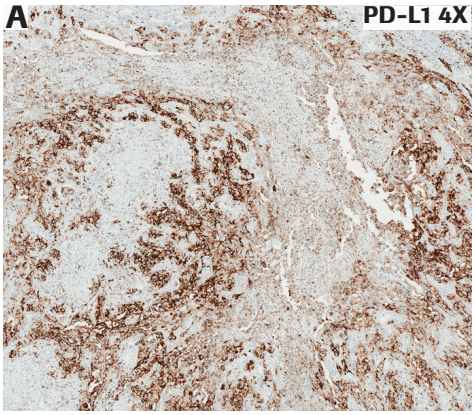


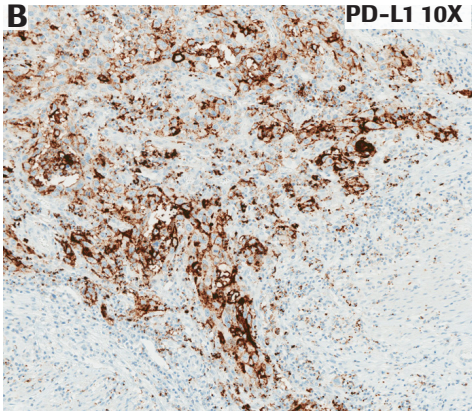
Case 3: This case is IC < 5%. Scores: IC 3%; TC: 0%. This is a papillary urothelial carcinoma with several lymphoid aggregates in the lamina propria. Only lymphoid aggregates contiguous to the tumor base are included for IC scoring.

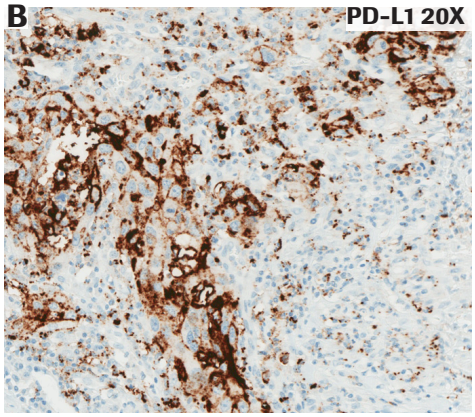
VENTANA PD-L1 (SP142) Assay: IC ≥ 5%



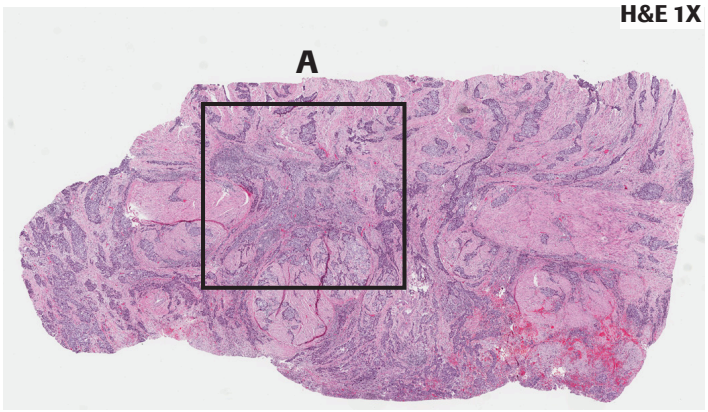


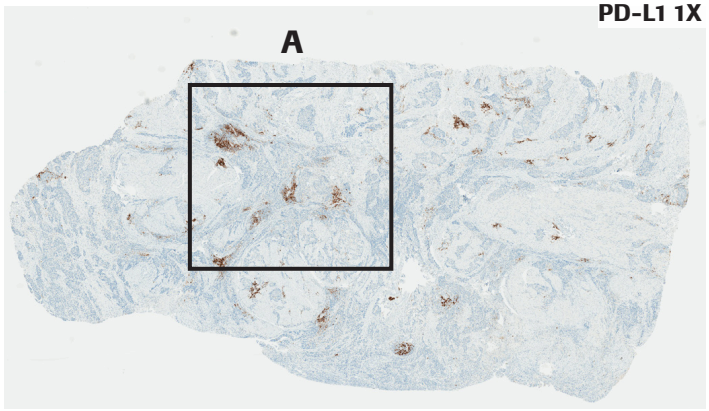


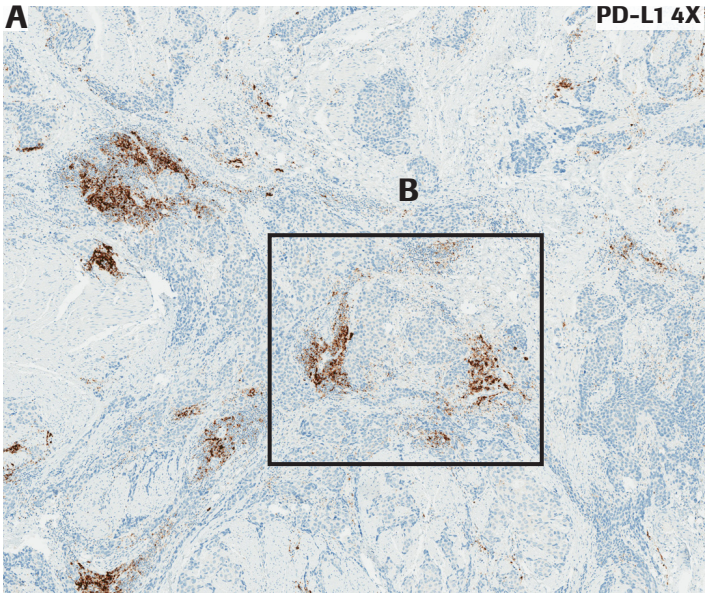


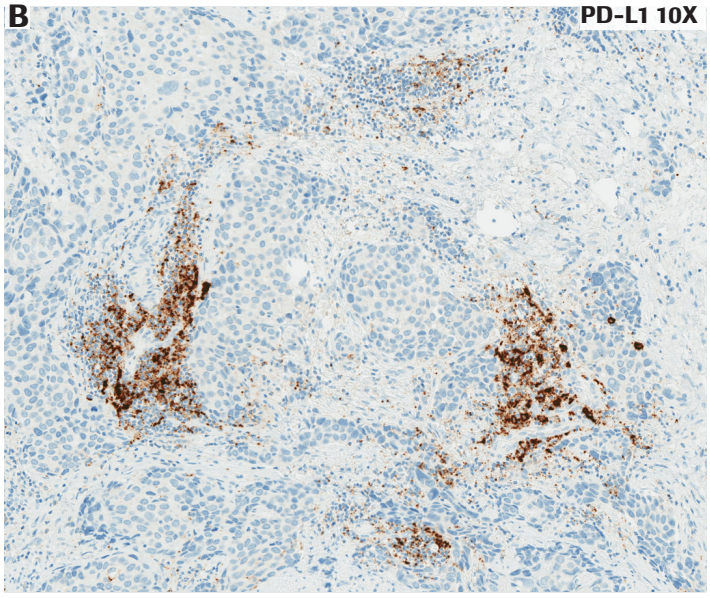


Case 4: This case is IC ≥ 5%. Scores: IC: 10%; TC: 90%. This case illustrates IC staining along with TC staining. At low magnification it is difficult to distinguish these 2 cell types, but when examined at a higher magnification (10x), punctate IC staining can be distinguished from circumferential TC staining. This case illustrates the importance of examining at high magnification to distinguish immune infiltrate staining from tumor cell staining.

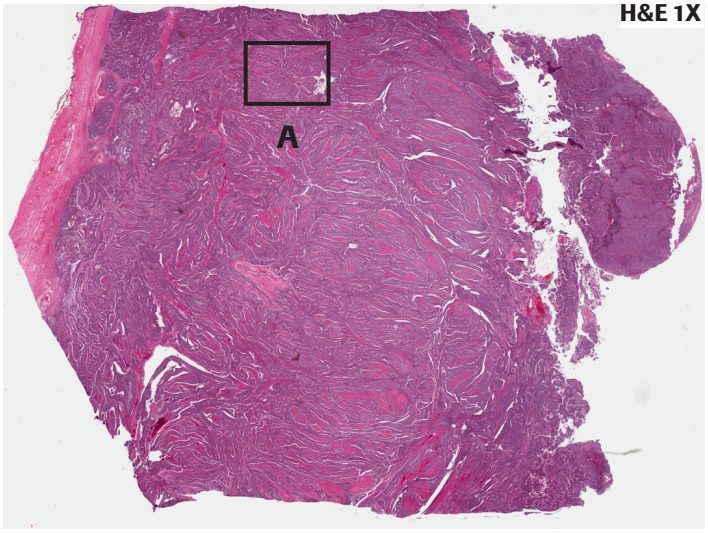


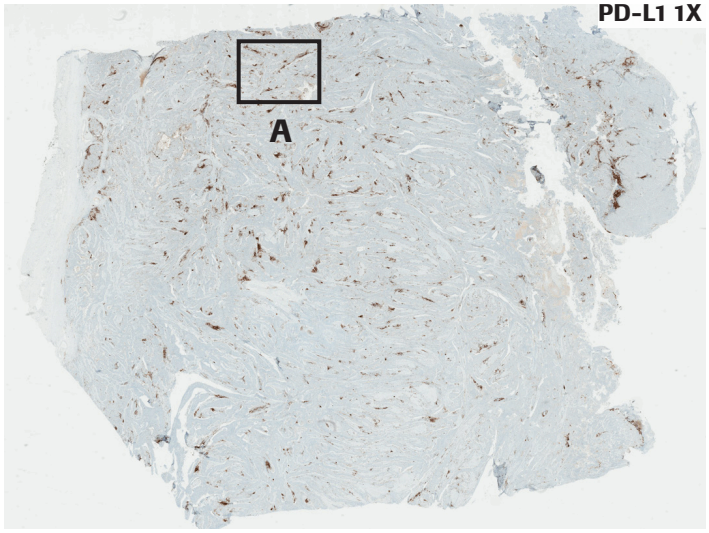


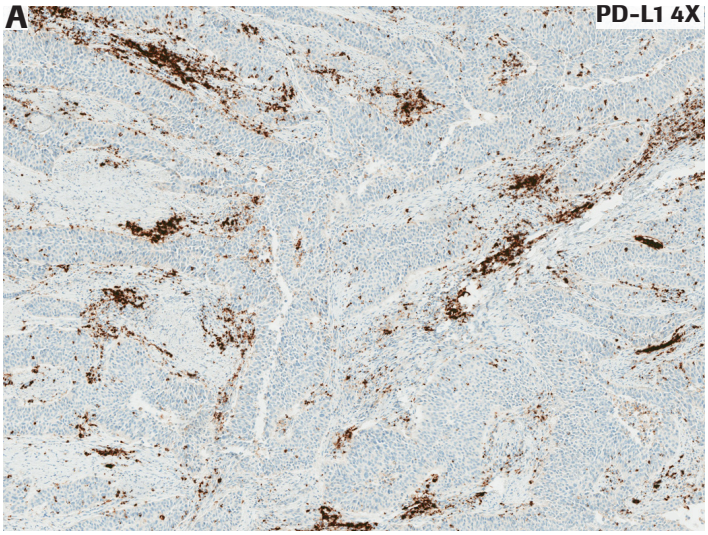


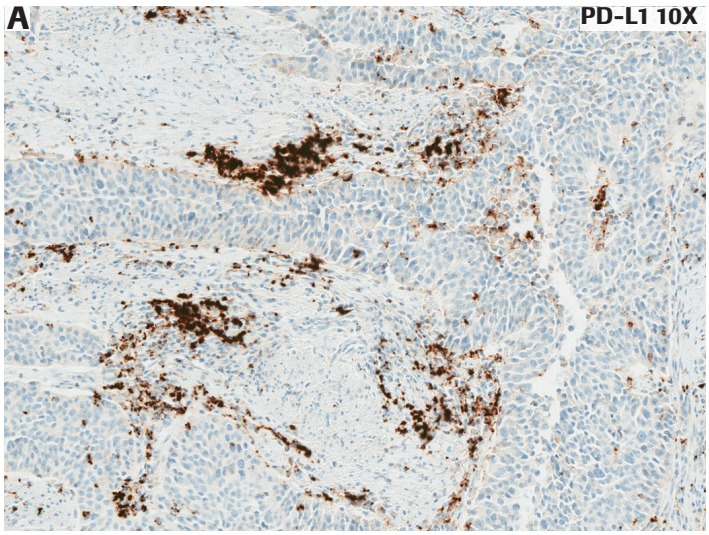


Case 5: This case is IC ≥ 5%. Scores: IC: 5%; TC: 0%. Note the presence of IC aggregate staining.



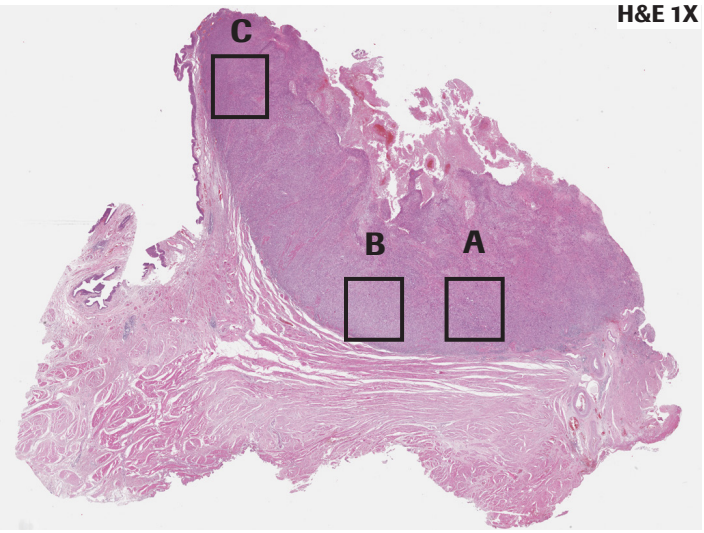


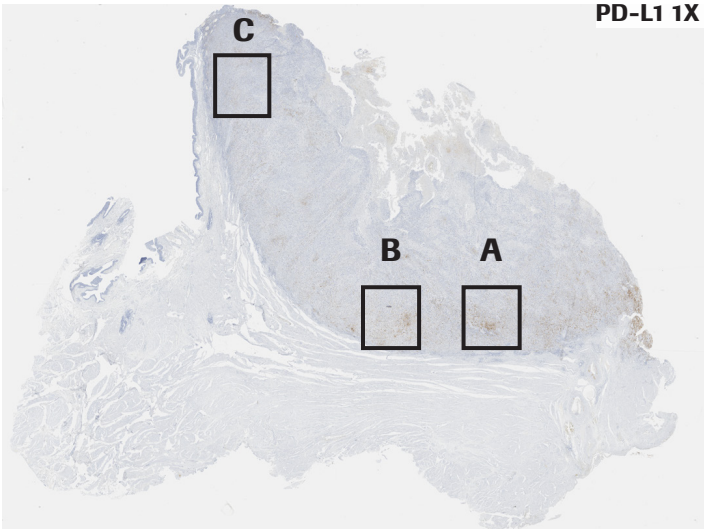


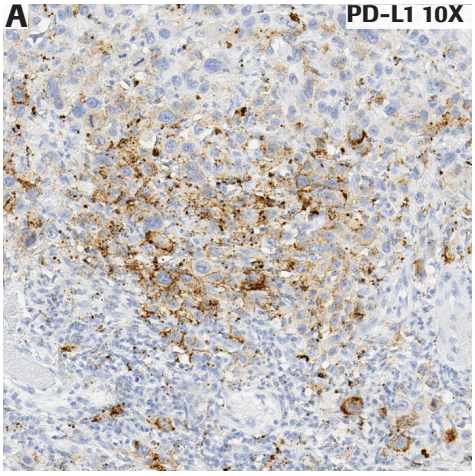


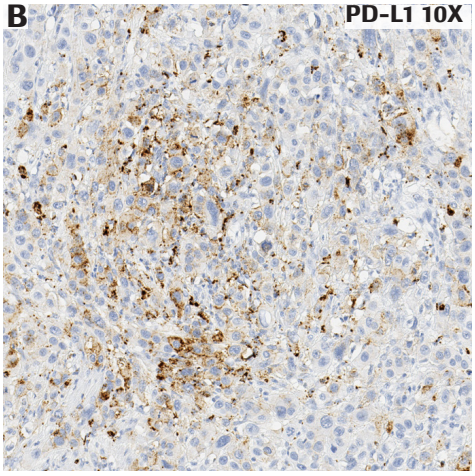
Case 6: This case is IC ≥ 5%. Scores: IC: 20%; TC 0%. Note the presence of IC aggregates along with foci of single-cell spread.

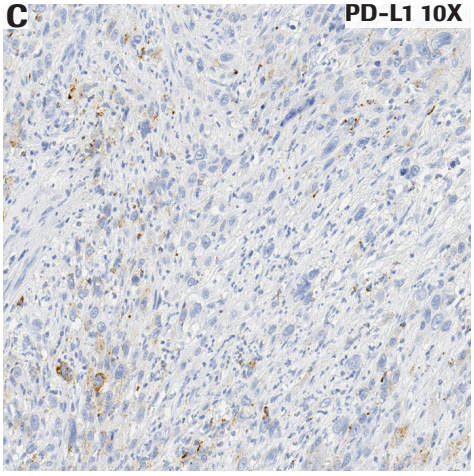
Challenging Cases



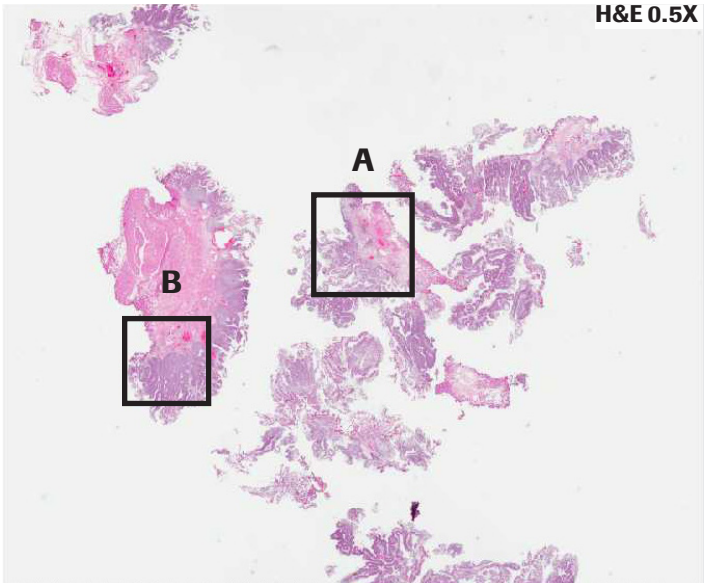


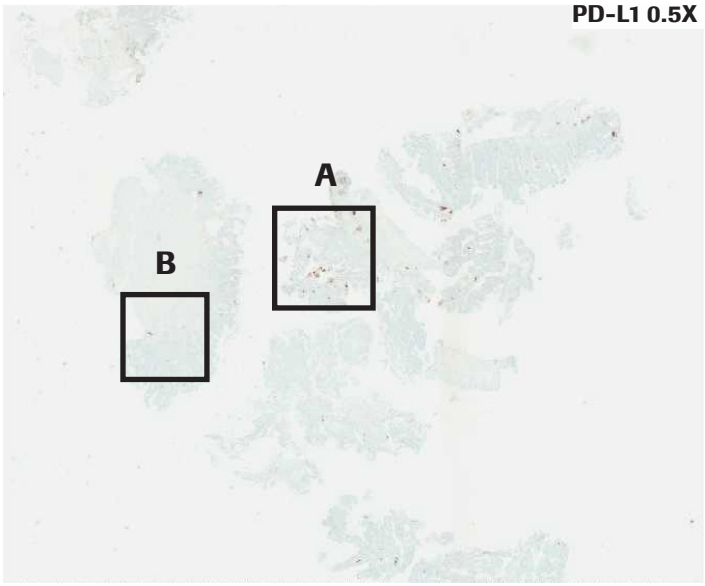


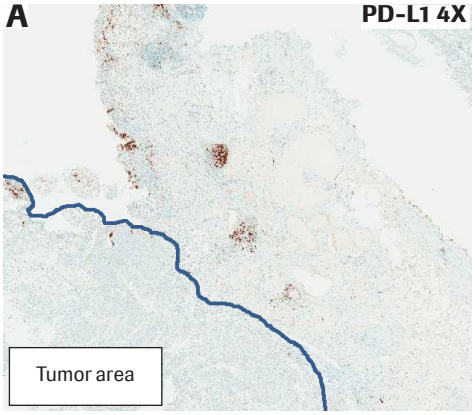


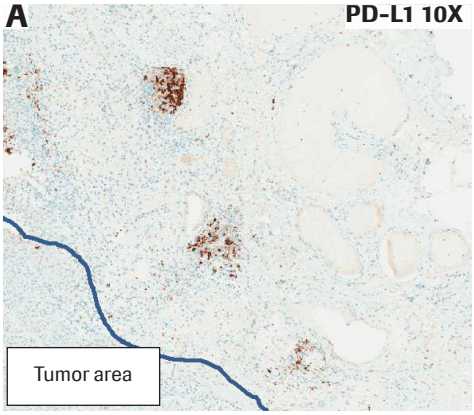


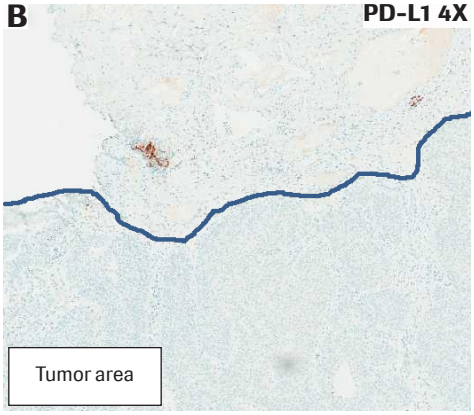
Case 7: This case is IC < 5%. Scores: IC: 3%; TC: 5%. Note the presence of granular TC staining admixed with punctate IC staining. Also note the focal distribution of TC and IC staining. At high magnification **Region A** shows predominance of TC staining, **Region B** shows equal amount of IC and TC staining and **Region C** shows paucity of either. When viewed at low magnification, IC staining is concentrated at one edge of the tumor (**Regions A & B**). This case was scored as < 5% (3% IC). This case illustrates the importance of examining at high magnification to distinguish IC and TC staining and heterogeneity of staining.







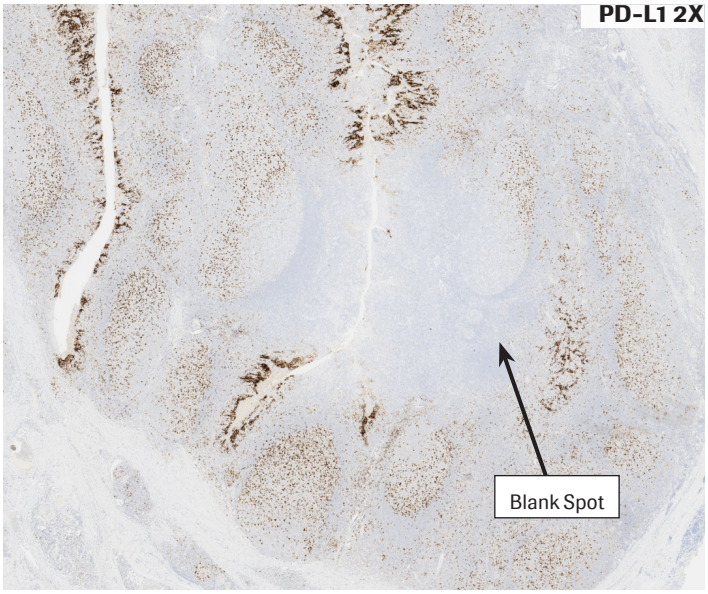


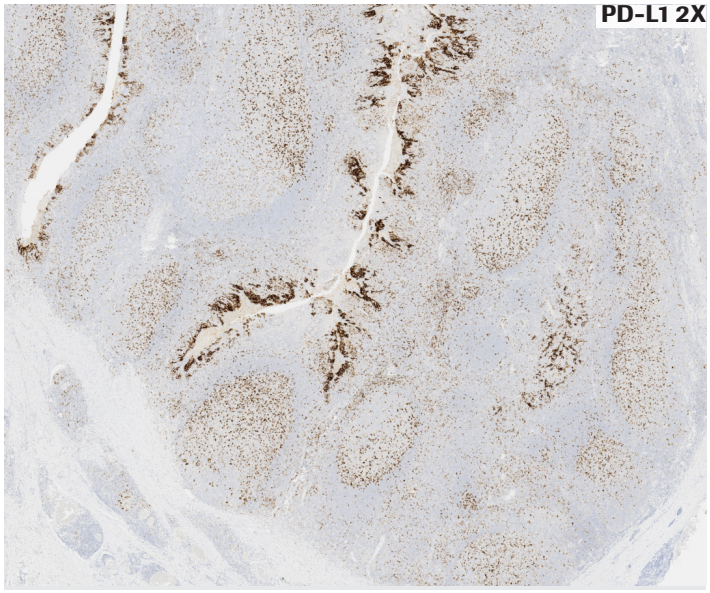


Case 8: This case is IC < 5%. Scores: IC: 2%; TC: 0%. Note the presence of papillary urothelial carcinoma in a TURBT specimen. Highlighted in **Regions A & B** are lymphoid aggregates in lamina propria that are not contiguous with the tumor and can be mistakenly included in scoring IC. The line defines the border of the tumor area.

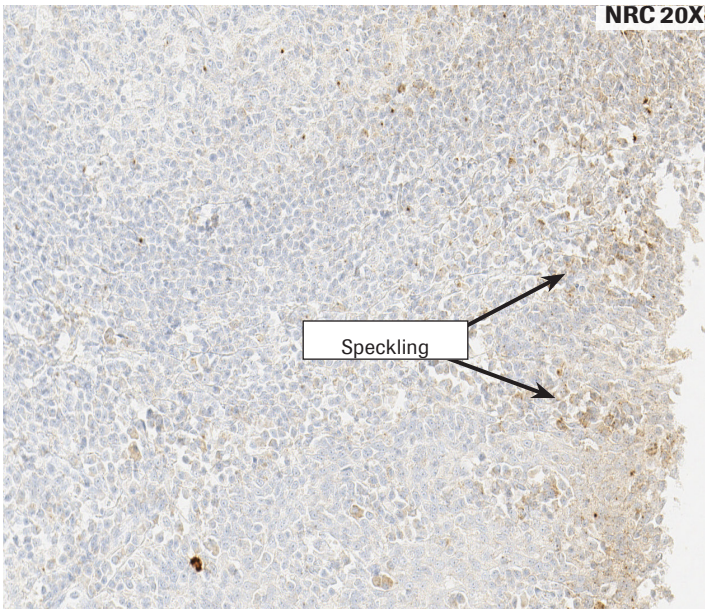
Staining Artifacts

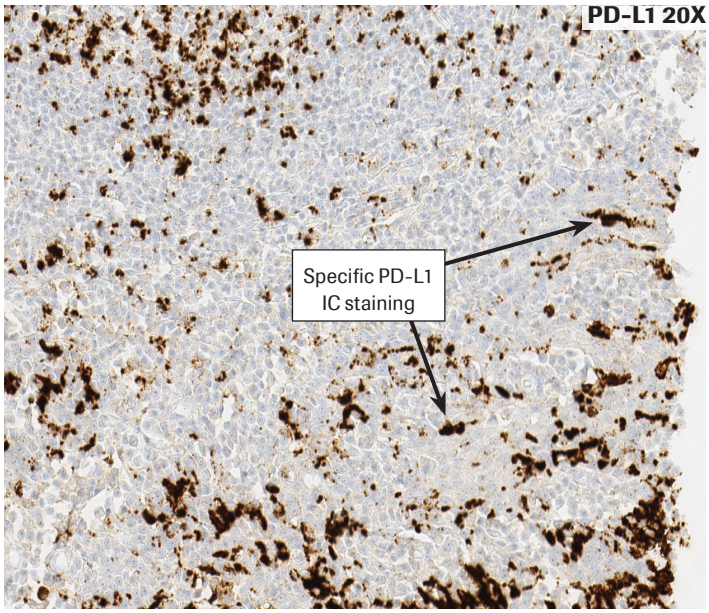
Artifacts noted in this section can be observed on Negative Reagent Control and VENTANA PD-L1 (SP142) Assay-stained slides. The presence of these artifacts may require repeat staining if they interfere with interpretation of VENTANA PD-L1 (SP142) Assay staining. Always review the corresponding Negative Reagent Control slide to ensure that non-specific background staining is within acceptable limits.



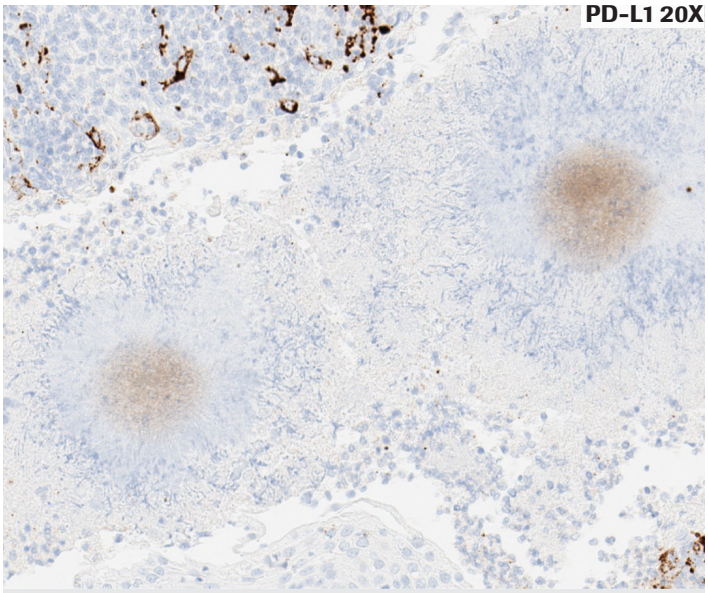


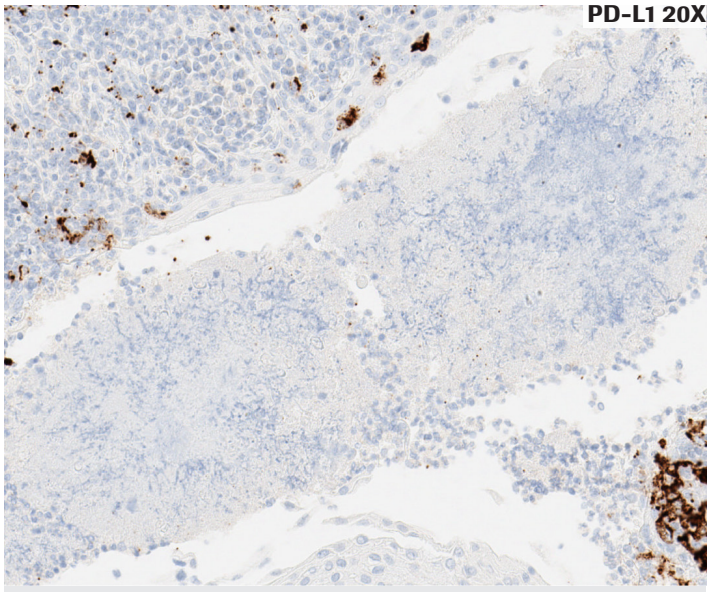
Blank Spots: Blank spots are light to non-staining areas that are typically circular and are due to a static bubble formed during staining procedure. The image on the left depicts an example of a blank spot opposed to the appropriate staining depicted in the image on the right. If the blank spot interferes with interpretation of the PD-L1-stained slide, a repeat run may be required.



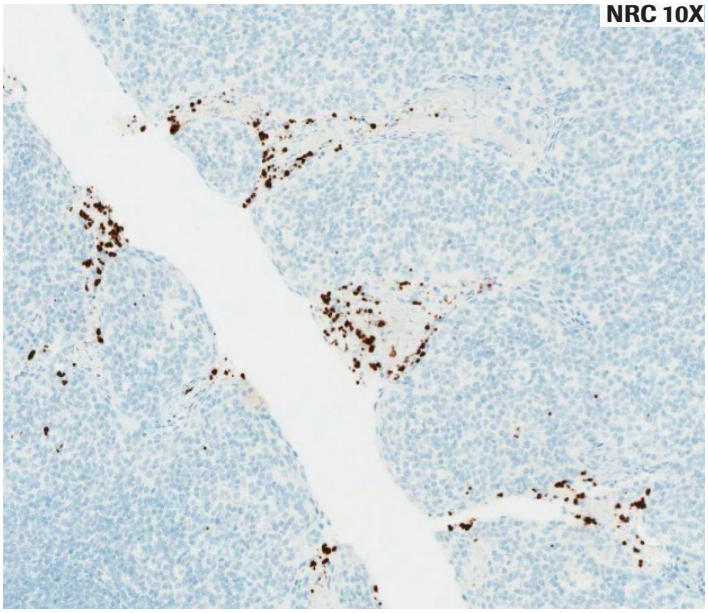


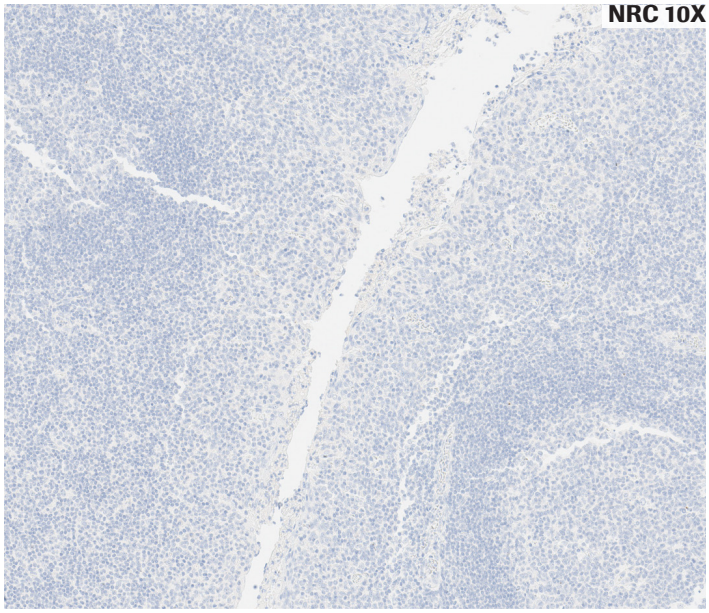
Speckling: Speckling, depicted in the image to the left, is weak to moderate non-specific staining that appears as a uniformly distributed fine granular precipate most often in the cytoplasm. Speckling does not conform to either IC or TC staining characteristics. This artifact should not be confused with specific staining such as depicted in the image to the right.



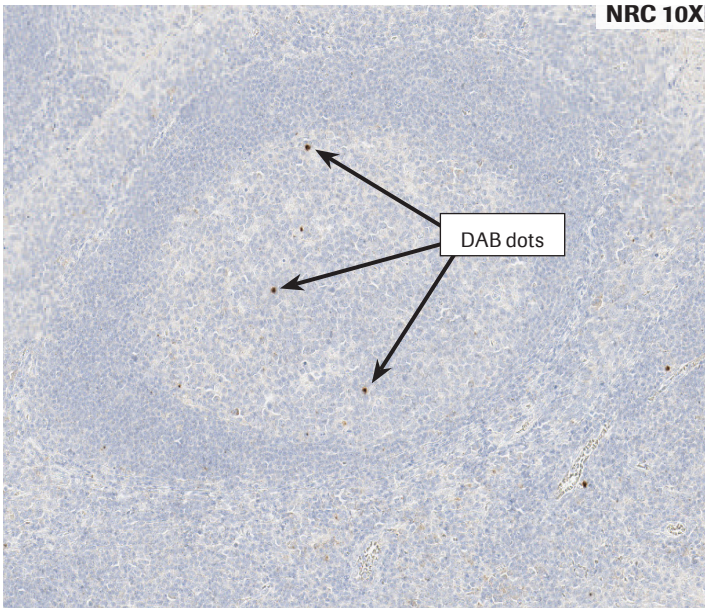


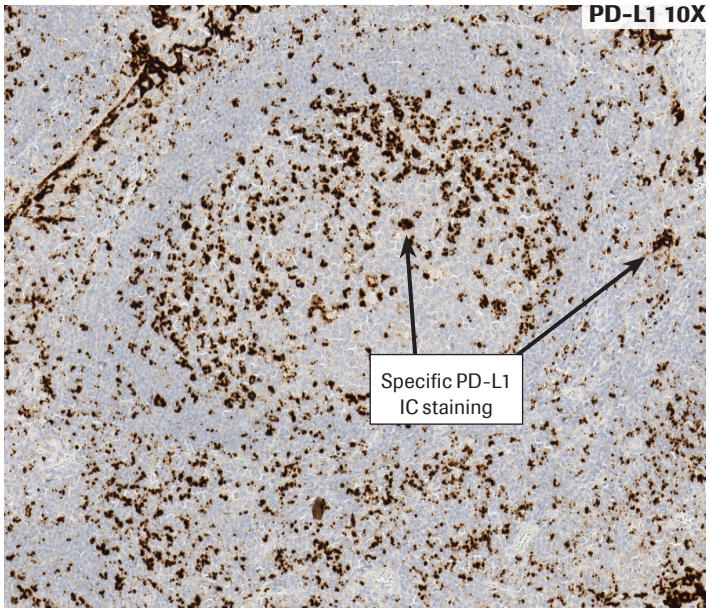
DAB Spots: DAB spots are circular spots that may form due to trapped DAB underneath the tissue section during the staining procedure. If this artifact interferes with the interpretation of the PD-L1-stained slide, repeat the stain with fresh unstained slides. In the image to the right, the DAB spot is not present with repeat staining of a serial section.



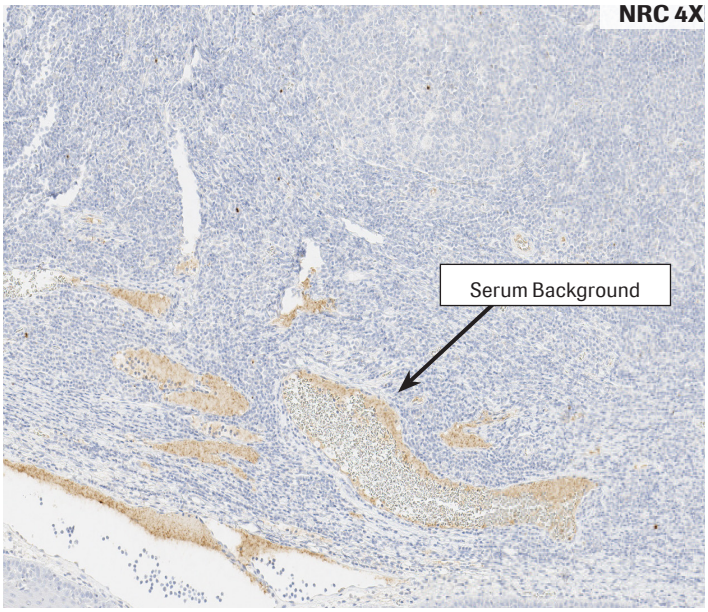


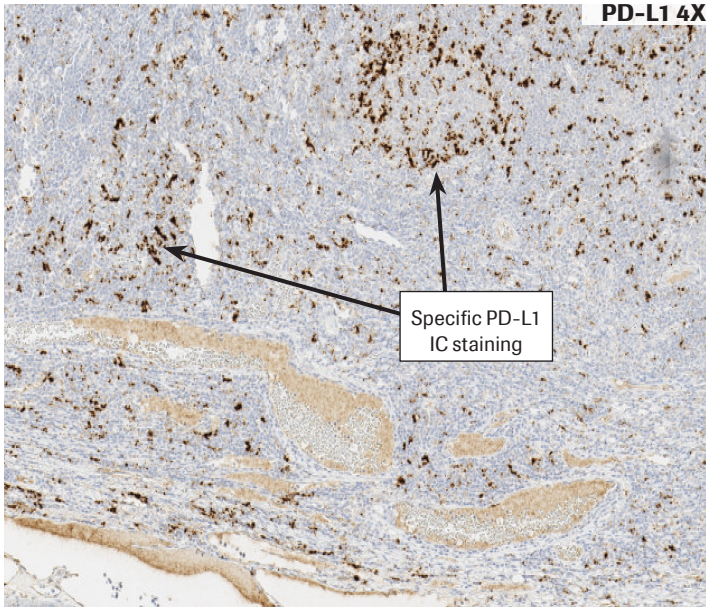
Luminal Debris: Tonsil stained with VENTANA PD-L1 (SP142) Assay can serve as both a positive and negative tissue control due to positive and negative staining elements being present. Luminal staining due to cross reactivity with an unknown antigen can be observed in the image of a tonsil stained with NRC on the left. An appropriate example of tonsil stained with NRC is depicted on the right. If you choose to run NRC on tonsil control tissue and luminal debris is observed, the sample should not be used as control.





DAB Dots: Non-specific punctuate background may be observed in tissue of any indication, and are small, indiscriminate staining artifacts from the amplification detection system. In comparison to PD-L1 staining of IC, DAB dots are smaller and exhibit a different, crisp morphology outline than the punctate IC staining. Expected immune cell staining can be seen in the image to the right.





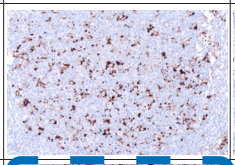
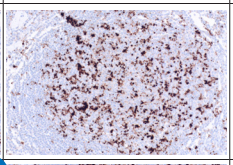
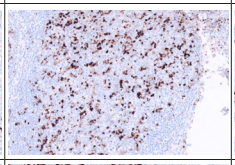
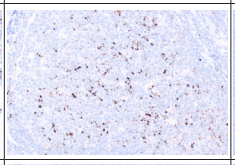
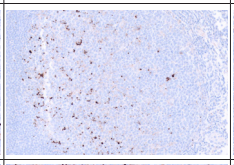
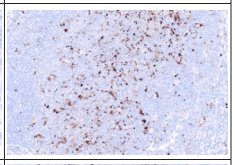
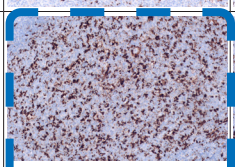
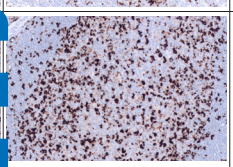
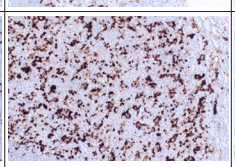
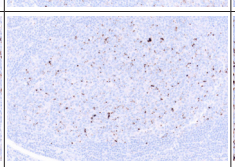
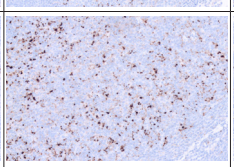
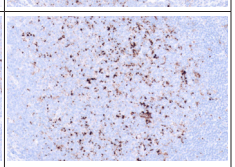
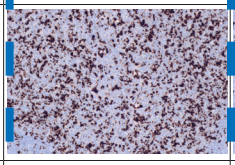
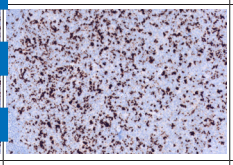
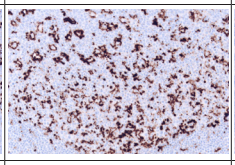
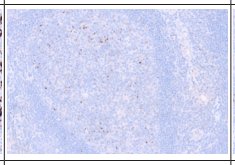
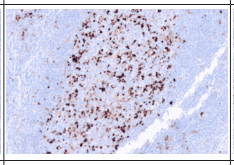
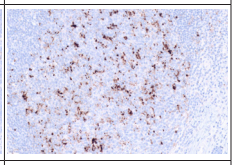
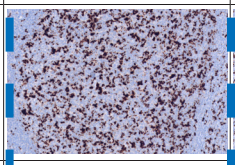
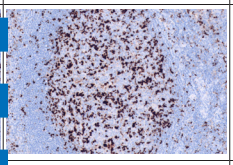
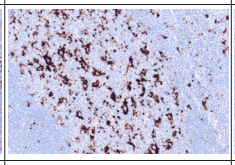
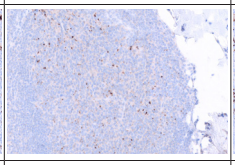
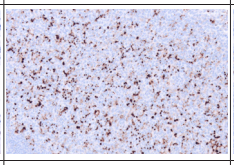
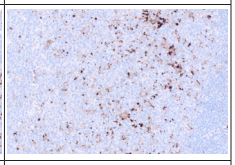
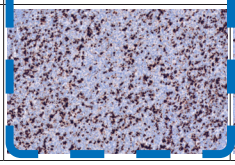
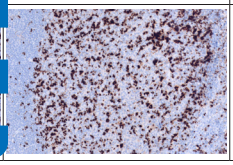
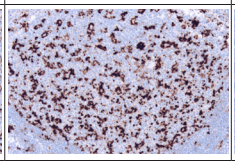
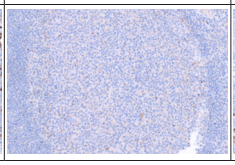
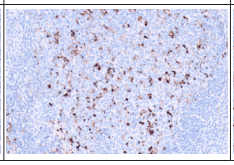
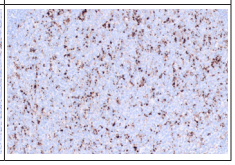
Serum Background: Serum background is non-specific staining in vascular spaces and serum extravasates. This is depicted in the bottom of each image above. It should not be confused with specific PD-L1 IC staining as depicted in the image to the right.

Impact of Pre-Analytical Conditions on VENTANA PD-L1 (SP142) Assay

Acceptable Fixation Conditions to Achieve Optimal Staining Results with VENTANA PD-L1 (SP142) Assay

- Ventana recommends fixation in 10% NBF for 6-72 hours.
- Zinc Formalin demonstrates comparable staining to 10% NBF.
- Less than 6 hour fixation is not recommended.
- Samples fixed with Z-5 demonstrate inconsistent staining with those fixed in 10% NBF; Z-5 fixation is not recommended.
- PREFER (Anatech, Ltd.) and alcohol fixatives including AFA (weaker staining) are not recommended.

Table 4: VENTANA PD-L1 (SP142) Assay Staining of Tonsil Tissue Across Fixatives and Fixation Time

Time point (Hrs)	Fixative					
	10% NBF	Zinc Formalin	Z-5*	PREFER* (Anatech, Ltd.)	AFA*	95% Alcohol*
1*						
6						
12						
24						
72						

Recommended

*Not recommended

(all images 20X magnification)

Antigen Stability on Cut Tissue Sections

Cut sections (unstained slides) of urothelial carcinoma should be stained within 3 months of sectioning and human tonsil tissues should be stained within 2 months of sectioning. Tissue cut sections (unstained slides) stored at ambient temperature show a significant loss of staining after this time (**Figure 18**).

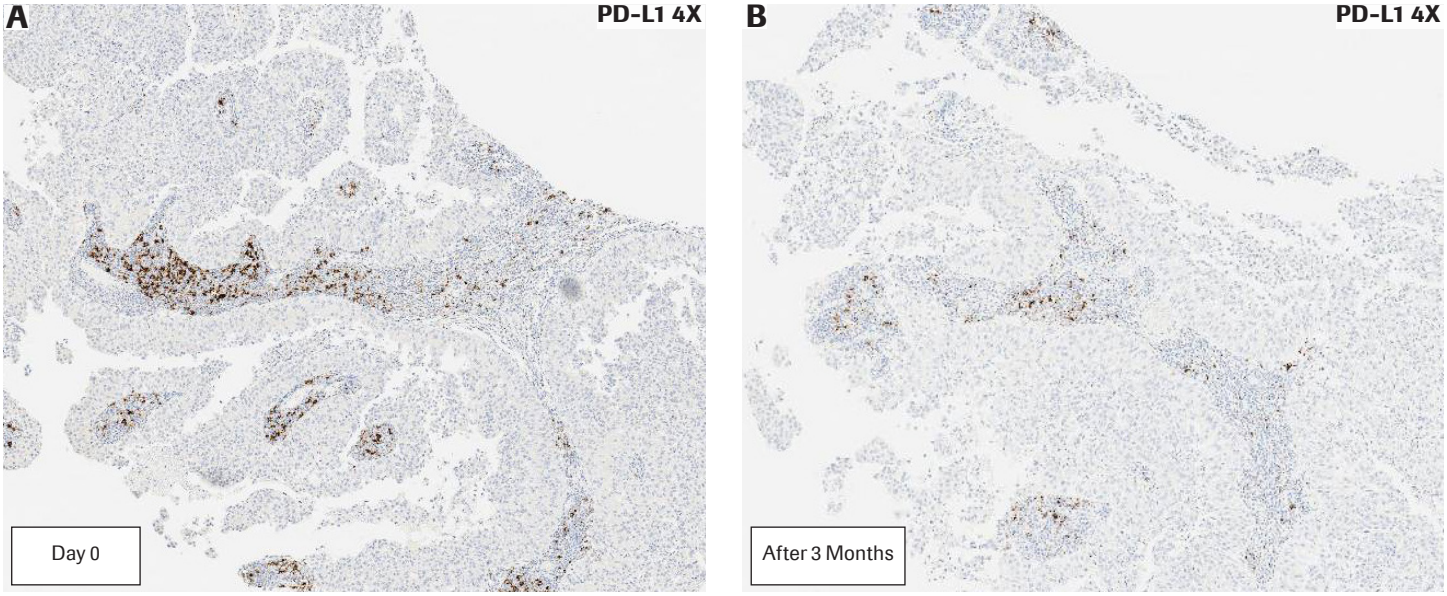


Figure 18: Serial sections of urothelial carcinoma tissue stained at Day 0 (**A**) and after three months storage at ambient temperature (**B**).

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Refer to the corresponding VENTANA PD-L1 (SP142) Assay package insert for manufacturer contact information.

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