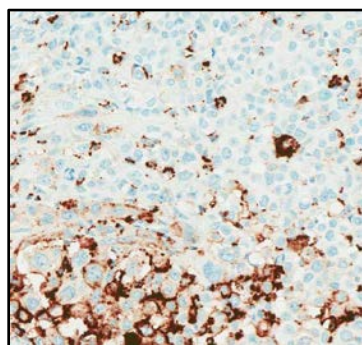


## VENTANA PD-L1 (SP142) Assay

**REF** 740-4859

07709374001

**IVD**  50



**Figure 1. PD-L1 expression in urothelial carcinoma.**

PD-L1 expression in  $\geq 5\%$  IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is associated with increased objective response rate (ORR) in a non-randomized study of TECENTRIQ™ (atezolizumab).

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

### SUMMARY AND EXPLANATION

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. and Roche/Genentech to identify patients with locally advanced or metastatic urothelial carcinoma (mUC) who are most likely to respond to treatment with TECENTRIQ.

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.<sup>1</sup>

Globally, there were an estimated 429,793 new cases of bladder cancer and 165,084 deaths in 2012.<sup>2</sup> 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.<sup>3</sup> Urothelial carcinoma presents as non-muscle-invasive, muscle-invasive, or metastatic disease. The overall 5-year survival rate for mUC is approximately 5.4%.<sup>4</sup>

PD-L1 is a transmembrane protein that downregulates immune responses through binding to its two receptors programmed death-1 (PD-1) and B7.1. 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.<sup>5</sup> 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.<sup>6</sup> PD-L1 expression has been observed in immune cells and tumor cells.<sup>7,8</sup> Aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion.<sup>5</sup> 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.

Bellmunt et al. (2015) reported that PD-L1 is widely expressed in tumor cells (TC) and tumor-infiltrating mononuclear cells (TIMCs) and showed PD-L1 expression in TIMCs to be associated with longer survival in patients who developed metastases.<sup>9</sup> The association between PD-L1 expression in 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.<sup>8,10,11</sup> 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.<sup>12</sup>

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.

### PRINCIPLE OF THE PROCEDURE

VENTANA PD-L1 (SP142) Assay utilizes a rabbit monoclonal primary antibody that binds to PD-L1 in paraffin-embedded tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) followed by the OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test)). Refer to the appropriate OptiView DAB IHC Detection Kit and OptiView Amplification Kit package inserts for further information.

### REAGENT PROVIDED

VENTANA PD-L1 (SP142) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA PD-L1 (SP142) Assay contains approximately 36  $\mu$ g of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 7  $\mu$ g/mL.

VENTANA PD-L1 (SP142) Assay contains a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

### MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

The following reagents and materials may be required for staining:

- Human benign tonsil tissues for use as control tissue
- Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
- Microscope slides, positively charged
- Bar code labels
- Xylene (Histological grade)
- Ethanol or reagent alcohol (Histological grade)
  - 100% solution: Undiluted ethanol or reagent alcohol
  - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
  - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
- Deionized or distilled water
- OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
- OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))
- EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
- Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
- ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
- ULTRA Cell Conditioning Solution(ULTRA CC1) (Cat. No. 950-224 / 05424569001)
- Hematoxylin II counterstain (Cat. No. 790-2208 / 05277965001)
- Bleuing Reagent (Cat. No. 760-2037 / 05266769001)
- Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)

17. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
18. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
19. Light microscope
20. Absorbent wipes

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

## STORAGE

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

## SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. Ventana recommends tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours. Fixation times of less than 6 hours may result in a loss of staining for PD-L1. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).<sup>13-15</sup>

Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative and other alcohol-containing fixatives have demonstrated a loss of specific staining for PD-L1 at all fixation times tested (1-72 hours); they are not recommended for use with this assay. See the interpretation guide (P/N 1014987EN) for further discussion of the impact of specimen preparation on PD-L1 staining intensity.

Sections should be cut approximately 4 µm thick and mounted on positively-charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised 3 months after cutting from the paraffin block for urothelial carcinoma specimens and 2 months for tonsil specimens (see the interpretation guide (P/N 1014987EN) and the Performance Characteristics section below).

## WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at [www.ventana.com](http://www.ventana.com).

## STAINING PROCEDURE

VENTANA PD-L1 (SP142) Assay has been developed for use on a BenchMark ULTRA instrument in combination with Rabbit Monoclonal Negative Control Ig, OptiView DAB IHC Detection Kit, OptiView Amplification Kit, and ancillary reagents. An assay-specific staining procedure must be used with VENTANA PD-L1 (SP142) Assay. Refer to Table 1 for the recommended staining protocol and required staining procedure. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

**Table 1.** Recommended staining protocol for VENTANA PD-L1 (SP142) Assay and Rabbit Monoclonal Negative Control Ig with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a BenchMark ULTRA instrument.

Staining Procedure: U OptiV DAB VENTANA PD-L1 (SP142)	
Protocol Parameter	Selection
Deparaffinization	Selected
Cell Conditioning	CC1 Cell Conditioning 48 minutes
Pre-primary antibody peroxidase	Selected
Primary Antibody	VENTANA PD-L1 (SP142) Selected or Negative Control Selected 16 minutes, 36°C
OptiView HQ Linker	8 minutes (default)
OptiView Amplification	8 minutes (default)
Amplifier and Amplification	Selected
Amplifier and Amplification H2O2	8 minutes
Amplification Multimer	8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

## QUALITY CONTROL PROCEDURES

### Rabbit Monoclonal Negative Control Ig

A matched negative reagent control slide must be run for every specimen to aid in the interpretation of results. Rabbit Monoclonal Negative Control Ig a negative reagent control antibody, is specifically matched for this assay and is used in place of the primary antibody to evaluate nonspecific staining. The staining procedure for the negative reagent control should equal the primary antibody incubation period. Use of a different negative control reagent, or failure to use the recommended negative control reagent, may cause false results.

### Tonsil Tissue Control

A tissue control must be included with each staining run. Qualified benign human tonsil tissue is to be used as the control. Control tissue should be fixed as soon as possible and processed in a manner identical to patient tissues. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Tonsil tissue contains positive and negative staining elements for the PD-L1 protein and is therefore suitable for use as a tissue control. The positive and negative staining tissue components are used to confirm that the assay functioned properly.

Appropriate staining of tonsil tissue components is described in Table 2 and in the interpretation guide (P/N 1014987EN).

### Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing it on a series of tissues with known IHC performance characteristics representing PD-L1 positive and negative tissues (refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist<sup>13</sup> or the CLSI Approved Guideline<sup>14</sup>). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Urothelial carcinoma tissues with known PD-L1 status, and benign human tonsil samples, are suitable for assay verification.

## INTERPRETATION OF RESULTS

The VENTANA automated immunostaining procedure causes a brown colored DAB reaction product to precipitate at the antigen sites localized by the VENTANA PD-L1 (SP142) Assay antibody. The stained slide(s) are interpreted by a qualified pathologist using light microscopy. A qualified pathologist experienced in IHC procedures must evaluate tissue controls and qualify the stained product before interpreting results.

## Tonsil Tissue Control Interpretation

The stained tonsil tissue control should be examined for appropriate staining. The presence of PD-L1 staining within the macrophages and lymphocytes in germinal centers and reticulated crypt epithelium of tonsil serve as positive tissue elements. Absence of staining in superficial squamous epithelium and negative immune cells in interfollicular regions of tonsil serve as negative tissue elements. Acceptability criteria are listed in Table 2. (Refer to the interpretation guide (P/N 1014987EN)).

If the tissue control fails to demonstrate appropriate staining, any results with the patient specimens should be considered unevaluable and repeat staining should be performed.

**Table 2.** Tonsil tissue control evaluation 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.

## Negative Reagent Control

Non-specific staining, if present, will have a diffuse appearance and can be evaluated using the negative reagent control slide stained with Rabbit Monoclonal Negative Control Ig. Intact cells should be used for interpretation of staining results, as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid. Examples of background staining for this assay can be found in the interpretation guide (P/N 1014987EN).

## Patient Tissue

Patient tissue must be evaluated according to the VENTANA PD-L1 (SP142) Assay scoring algorithm provided in Table 3. Refer to the interpretation guide (P/N 1014987EN) for additional instructions and representative images.

**Table 3.** VENTANA PD-L1 (SP142) Assay Scoring Algorithm for Urothelial Carcinoma

Immune Cell (IC) Staining Assessment	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%
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%

PD-L1 staining in tumor cells should not be included in the scoring determination of patient tissue.

## GENERAL LIMITATIONS

- IHC is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
- Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning,

or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.

- Excessive or incomplete counterstaining may compromise proper interpretation of results.
- The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and system-level controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
- This product is not intended for use in flow cytometry, performance characteristics have not been determined.
- Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.<sup>16,17</sup>
- Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.<sup>18</sup>
- False positive results may be seen because of non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) depending on the type of immunostain used.<sup>19</sup>
- As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

## SPECIFIC LIMITATIONS

- VENTANA PD-L1 (SP142) Assay has been solely approved on the BenchMark ULTRA instrument with the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit and is not approved with any other detection or instruments.
- A patient specimen slide should be stained with Rabbit Monoclonal Negative Control Ig. Other negative control reagents are not suitable for this assay.
- VENTANA PD-L1 (SP142) Assay antibody is stable for up to eight days at 30° C. Assay performance beyond these limits has not been established.
- This assay has not been validated for use with cytology samples or decalcified bone specimens.
- Patient tissue should be stained within 3 months of sectioning from the tissue block. Loss of staining performance has been observed with VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma tissue sections that have been stored at room temperature for longer than 3 months.
- Ventana recommends that samples be fixed between 6 and 72 hours in 10% NBF. Use of fixation times or fixative types other than those recommended can lead to false negative results. Fixatives such as AFA, PREFER fixative, and other alcohol-containing fixatives have demonstrated a loss of specific PD-L1 protein staining. Refer to the interpretation guide (P/N 1014987EN) for further discussion.
- Artifacts such as DAB spots, Blank spots, DAB dots, Speckling, and/or may require repeat staining if they interfere with the interpretation of VENTANA PD-L1 (SP142) Assay. Always compare the PD-L1 stained slide to the negative reagent control to ensure that background is acceptable. Refer to the interpretation guide (P/N 1014987EN) for further discussion.
- Occasional DAB dots have been observed in benign human tonsil control, cerebellum and testicular tissues and focal nuclear staining has been observed in normal pancreatic (acinar cells) and hypophyseal tissue (Table 4), however nuclear staining is not included in scoring of VENTANA PD-L1 (SP142) Assay staining.

## PERFORMANCE CHARACTERISTICS

Tests for staining specificity, sensitivity, impact of tissue thickness, repeatability, and intermediate precision, as well as tests for reader precision, inter-laboratory reproducibility, and clinical outcome were conducted and the results are listed in the following section.

### Specificity

Arrays containing a variety of normal tissues were stained with VENTANA PD-L1 (SP142) Assay and evaluated for the presence of immune cell staining (any immune cell staining, of any intensity) as described in Table 4.

**Table 4.** Specificity of VENTANA PD-L1 (SP142) Assay staining was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive <sup>*</sup> /total cases	Tissue	# positive <sup>*</sup> /total cases
Adrenal gland	1/3	Muscle, cardiac	0/3
Bladder	3/36**	Muscle, skeletal	0/2
Breast	1/3	Myeloid	0/2
Cerebellum	0/3***	Nerve, peripheral	0/3
Cerebrum	0/3	Ovary	0/3
Cervix	2/2	Pancreas	0/3****
Colon	2/3	Parathyroid	0/2
Endometrium	1/3	Prostate	0/3
Esophagus	0/3	Salivary gland	2/3
Hypophysis	0/3****	Skin	0/3
Intestine, small	1/3	Spleen	3/3
Kidney	2/3	Stomach	0/3
Lingual gland	0/1	Testis	0/3***
Liver	0/3	Thymus gland	3/3
Lung	1/3	Thyroid gland	1/3
Lymph node	3/3	Tonsil	3/3
Mesothelium	0/3		

\* Immune cell staining of any intensity \*\* Focal immune cell staining

\*\*\* Focal DAB dots were observed in 1/3 cerebellum and 1/3 testis tissues

\*\*\*\* Nuclear staining was observed in 1/3 pancreas and 1/3 hypophysis tissues

### Sensitivity

3750 urothelial carcinoma specimens (including 55 metastases) were evaluated with VENTANA PD-L1 (SP142) Assay. Of these, 2545 (67.9%) showed immune cell staining of any percentage; 466 (12.4%) showed  $\geq 5\%$  immune cell staining; 512 (13.7%) showed tumor cell staining of any percentage. In addition, an array of neoplastic tissues was evaluated for immune cell and tumor cell staining with VENTANA PD-L1 (SP142) Assay as described in Table 5.

**Table 5.** Sensitivity of VENTANA PD-L1 (SP142) Assay staining was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Origin	Pathology	# positive <sup>*</sup> /total cases	
		Immune cells	Tumor cells
Abdomen	Malignant mesothelioma	1/1	0/1
Back	Neurofibroma	1/1	0/1
Bladder	Low grade malignant leiomyosarcoma	0/1	0/1
Bladder	Transitional cell carcinoma	1/1	0/1
Bone	Osteosarcoma	0/1	0/1
Breast	Invasive ductal carcinoma	1/1	0/1
Breast	Intraductal carcinoma with early infiltrate	1/1	0/1
Cerebrum	Glioblastoma	0/1	0/1
Cerebrum	Atypical meningioma	0/1	0/1
Cerebrum	Malignant ependymoma	0/1	0/1
Cerebrum	Oligodendroglioma	0/1	0/1
Colon	Adenocarcinoma	1/1	0/1
Colon	Interstitialoma	0/1	0/1
Esophagus	Squamous cell carcinoma	0/1	0/1
Esophagus	Adenocarcinoma	1/1	0/1
Intestine	Adenocarcinoma	1/1	0/1
Intestine	Stromal sarcoma	1/1	0/1
Kidney	Clear cell carcinoma	1/1	0/1
Liver	Hepatocellular carcinoma	0/1	0/1
Liver	Hepatoblastoma	1/1	0/1
Lung	Adenocarcinoma	0/1	0/1
Lung	Small cell undifferentiated carcinoma	1/1	0/1
Lung	Squamous cell carcinoma	1/1	0/1
Lymph node	Diffuse B-cell lymphoma	1/1**	1/1**
Lymph node	Hodgkin's lymphoma	1/1	0/1
Mediastinum	Diffuse B-cell lymphoma	1/1**	1/1**
Muscle, smooth	Moderate malignant leiomyosarcoma	1/1	0/1
Muscle, striated	Embryonal rhabdomyosarcoma	0/1	0/1
Ovary	Serous adenocarcinoma	1/1	0/1
Ovary	Adenocarcinoma	1/1	0/1
Pancreas	Islet cell tumor	0/1	0/1



Origin	Pathology	# positive*/total cases	
		Immune cells	Tumor cells
Pancreas	Adenocarcinoma	1/1	0/1
Pelvic cavity	Anaplastic large cell lymphoma	1/1**	1/1**
Prostate	Adenocarcinoma	0/2	0/2
Rectum	Adenocarcinoma	1/1	1/1
Rectum	Moderate malignant interstitialoma	0/1	0/1
Rectum	Malignant melanoma	1/1	0/1
Retroperitoneum	Neuroblastoma	1/1	0/1
Retroperitoneum	Spindle cell rhabdomyosarcoma	0/1	0/1
Skin	Basal cell carcinoma	1/1	0/1
Skin	Squamous cell carcinoma	1/1	0/1
Spleen	Diffuse B-cell lymphoma	1/1*	1/1*
Stomach	Signet-ring cell carcinoma	1/1	0/1
Testis	Seminoma	1/1	0/1
Testis	Embryonal carcinoma	0/1	0/1
Thyroid	Medullary carcinoma	0/1	0/1
Thyroid	Papillary carcinoma	0/1	1/1
Uterine cervix	Squamous cell carcinoma	2/2	0/2
Uterus	Leiomyoma	0/1	0/1
Uterus	Adenocarcinoma	1/1	0/1
Uterus	Clear cell carcinoma of endometrium	1/1	1/1

\* Immune cell or tumor cell staining of any intensity

\*\* Tumor cell and immune cell staining could not be differentiated

### Tissue Thickness

Tissue thickness was evaluated using 5 unique urothelial carcinoma specimens (3 PD-L1  $\geq$  5% and 2 PD-L1 < 5%). Duplicate sections at 2, 3, 4, 5, 6, and 7 microns were tested for each case. All tissue thicknesses demonstrated appropriate specific staining for PD-L1 and acceptable background levels for VENTANA PD-L1 (SP142) Assay staining. No sections exhibited a change in PD-L1 status within the range of thickness tested. Ventana recommends that specimens be cut at 4 microns for staining with VENTANA PD-L1 (SP142) Assay.

### Repeatability and Intermediate Precision

Repeatability studies for VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens were completed to demonstrate:

- Intra-day Repeatability – Five replicate slides each from 10 unique urothelial carcinoma specimens (4 PD-L1  $\geq$  5% and 6 PD-L1 < 5%) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument within one day.

- Inter-day Precision – Two replicate slides each from 10 unique urothelial carcinoma specimens (4 PD-L1  $\geq$  5% and 6 PD-L1 < 5%) were stained with VENTANA PD-L1 (SP142) Assay on a single BenchMark ULTRA instrument across 5 non-consecutive days.
- Inter-instrument and Inter-lot Precision – 27 replicate slides each from 18 unique urothelial carcinoma specimens (9 PD-L1  $\geq$  5% and 9 PD-L1 < 5%) were stained with VENTANA PD-L1 (SP142) Assay using three lots of VENTANA PD-L1 (SP142) antibody and three paired lots of OptiView DAB IHC Detection Kit and OptiView Amplification Kit, on three BenchMark ULTRA instruments.

All slides were blinded and randomized, and then evaluated using the VENTANA PD-L1 (SP142) Assay scoring algorithm (Table 3). Results are summarized in Table 6.

**Table 6.** Repeatability and Intermediate Precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens

Repeatability/Intermediate precision parameter	Positive agreement %* (95% CI)	Negative agreement %** (95% CI)	Overall agreement % (95% CI)
Intra-day Repeatability (within a single day)	100.0% (83.9-100.0%)	100.0% (88.6-100.0%)	100.0% (92.9-100.0%)
Inter-day Precision (5 non-consecutive days)	90.0% (76.9-96.0%)	100.0% (94.0-100.0%)	96.0% (90.2-98.4%)
Inter-instrument and Inter-lot Precision (3 instruments, 3 antibody lots, and 3 detection and amplification kit lots)	99.6% (97.7-99.9%)	99.6% (97.7-99.9%)	99.6% (98.5-99.9%)

\* Positive Percent Agreement

\*\* Negative Percent Agreement

### Reader Precision

To assess Inter- and Intra-reader Precision, three pathologists evaluated sixty unique urothelial carcinoma specimens (30 PD-L1  $\geq$  5% and 30 PD-L1 < 5%) that were stained with VENTANA PD-L1 (SP142) Assay. Specimens were blinded and randomized prior to evaluation for PD-L1 status using the VENTANA PD-L1 (SP142) Assay scoring algorithm (Table 3). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers compared to a consensus score across two reads and between each pathologist's reads are summarized in Table 7.

**Table 7.** Inter- and Intra-reader Precision of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens

Reader Precision	Positive agreement %* (95% CI)	Negative agreement %** (95% CI)	Overall agreement % (95% CI)
Inter-reader Precision (average of all three readers compared to a consensus score and across two reads)	92.6% (84.7-96.6%)	98.9% (92.7-99.8%)	95.8% (91.3-98.0%)
Intra-reader Precision (average of all three readers' agreement rates between first and second reads)	89.3% (77.6-95.3%)	97.8% (86.0-99.7%)	93.6% (86.8-97.0%)

\* Positive Percent Agreement

\*\* Negative Percent Agreement

### Inter-laboratory Reproducibility Study

An Inter-laboratory Reproducibility Study for VENTANA PD-L1 (SP142) Assay was conducted to demonstrate reproducibility of the assay in determining PD-L1 status in urothelial carcinoma tissue specimens. Twenty-eight unique urothelial carcinoma specimens (14 PD-L1  $\geq 5\%$  and 14 PD-L1  $< 5\%$ ) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. Prior to staining, slides were blinded and randomized. At each site, the stained slides were independently evaluated by 2 pathologists (readers). The sample set consisted of a total of 420 case slides (140 slides per site) generated from 28 unique urothelial carcinoma specimens. The final staining acceptability rate for the VENTANA PD-L1 (SP142) Assay was 99.9% in this study. Results are summarized in Table 8.

**Table 8.** Inter-laboratory Reproducibility of VENTANA PD-L1 (SP142) Assay staining of urothelial carcinoma specimens.

Inter-laboratory Reproducibility	Positive agreement % (95% CI)	Negative agreement % (95% CI)	Overall agreement % (95% CI)
Overall agreement (across sites, days and readers)	98.3% (96.6-99.2%)*	87.4% (83.8-90.2%)**	92.8% (90.9-94.4%)
Inter-site agreement (average of site-to-site pairwise comparisons)	90.7% (81.2-96.3%)†	88.3% (78.5-94.9%)††	89.6% (82.5-95.5%)
Inter-reader agreement (average of reader-to-reader pairwise comparisons within each site)	89.3% (78.1-96.0%)†	86.6% (75.1-94.6%)††	88.1% (84.6-90.8%)

n = 419 evaluable case slides

\* Positive Percent Agreement

\*\* Negative Percent Agreement

† Average Positive Agreement

†† Average Negative Agreement

### Clinical Outcome Study

The performance of VENTANA PD-L1 (SP142) Assay was investigated in Study 1, a multicenter, open-label, two-cohort trial designed to evaluate the efficacy of TECENTRIQ in patients with locally advanced or metastatic urothelial carcinoma.

Patient specimens were stained with VENTANA PD-L1 (SP142) Assay and evaluated for staining acceptability and for PD-L1 expression per the scoring algorithm specified in Table 3 on page 3. Patient specimens were FFPE urothelial carcinoma tissue from biopsies (21.2%), resections (46.6%), transurethral resection of bladder tumor (TURBT, 30.5%), or of unknown type (1.6%); 74.9% were from primary tumors and 25.1% from metastatic tumors.

Table 9 describes the overall staining acceptability rate for the VENTANA PD-L1 (SP142) Assay among all urothelial carcinoma specimens screened for Study 1. The rates of acceptable morphology and acceptable background for PD-L1 stained slides are also reported. Out of a total of 650 specimens, 25 failed the initial staining attempt and staining was repeated. On the final staining attempt, 13 of the 25 samples remained unacceptable (7 due to unacceptable negative reagent control, 5 due to unacceptable morphology, and 1 for both unacceptable background and morphology). VENTANA PD-L1 (SP142) Assay demonstrated high initial and final overall staining acceptability rates; 96.2% and 98.0%, respectively. Final morphology and background acceptability rates were greater than 99%.

**Table 9.** VENTANA PD-L1 (SP142) Assay staining performance characteristics for clinical study specimens.

Attribute	Acceptability rate % (n/N) (95% CI)	
	Initial*	Final**
Overall staining acceptability rate	96.2% (625/650) (94.4-97.4%)	98.0% (637/650) (96.6-98.8%)
Morphology	98.1% (628/640) (96.8-98.9%)	99.1% (637/643) (98.0-99.6%)
Background	98.9% (625/632) (97.7-99.5%)	99.8% (637/638) (99.1-100.0%)

\* Initial staining attempt \*\* Final staining attempt

In Cohort 2 of Study 1, 310 patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following a platinum-containing chemotherapy regimen or who had disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen were treated with TECENTRIQ. This study excluded patients who had: a history of autoimmune disease, active or corticosteroid-dependent brain metastases, administration of a live, attenuated vaccine within 28 days prior to enrollment, or administration of systemic immunostimulatory agents or systemic immunosuppressive medications. Patients received an intravenous infusion of 1200 mg of TECENTRIQ every 3 weeks until unacceptable toxicity or either radiographic or clinical progression. Tumor response assessments were conducted every 9 weeks for the first 54 weeks and every 12 weeks thereafter. Major efficacy outcome measures included confirmed objective response rate (ORR) as assessed by independent review facility (IRF) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1) and duration of response (DOR).

In this cohort, the median age was 66 years, 78% were male, 91% patients were Caucasian. Twenty-six percent had non-bladder urothelial carcinoma and 78% of patients had visceral metastases. Sixty-two percent of patients had an ECOG score of 1 and 35% of patients had a baseline creatinine clearance of  $< 60$  mL/min. Nineteen percent of patients had disease progression following prior platinum-containing neoadjuvant or adjuvant chemotherapy. Forty-one percent of patients had received  $\geq 2$  prior systemic regimens in the metastatic setting. Seventy-three percent of patients received prior cisplatin, 26% had prior carboplatin, and 1% were treated with other platinum-based regimens.

Tumor specimens were evaluated prospectively using VENTANA PD-L1 (SP142) Assay at a central laboratory and the results were used to define subgroups for pre-specified analyses. Of the 310 patients, 32% were classified as having PD-L1 expression of  $\geq 5\%$ ; the remaining 68% of patients were classified as having PD-L1 expression of  $< 5\%$ . Confirmed ORR in all patients and the two PD-L1 subgroups are summarized in Table 10. The median follow-up time for this cohort was 14.4 months. In 59 patients with disease progression following neoadjuvant or adjuvant therapy, the ORR was 22.0% (95% CI: 12.3%, 34.7%).

**Table 10.** Summary of Efficacy from Cohort 2 of Study 1

	All Patients (N = 310)	PD-L1 Expression Subgroups	
		$< 5\%$ <sup>1</sup> (N = 210)	$\geq 5\%$ <sup>1</sup> (N = 100)
Number of IRF-assessed Confirmed Responders	46	20	26
ORR % (95% CI)	14.8% (11.1, 19.3)	9.5% (5.9, 14.3)	26.0% (17.7, 35.7)
Complete Response (CR) (%)	5.5%	2.4%	12.0%
Partial Response (PR) (%)	9.4%	7.1%	14.0%
Median DOR, months (range)	NR (2.1+, 13.8+)	12.7 (2.1+, 12.7)	NR (4.2, 13.8+)

NR= Not reached + Denotes a censored value

<sup>1</sup> PD-L1 expression in tumor-infiltrating immune cells (IC)

## TROUBLESHOOTING

Troubleshooting guidance is provided in Table 11. If a problem cannot be attributed to any of these causes, or if the suggested corrective action fails to resolve the problem, consult your local support representative.

**Table 11.** Troubleshooting guidance for VENTANA PD-L1 (SP142) Assay

Problem	Probable Cause	Suggested Action
Light or no staining of slides	Incorrect staining protocol selected	Verify that U OptiV DAB VENTANA PD-L1 (SP142) procedure was used.
		Verify that VENTANA PD-L1 (SP142) was selected for Primary Antibody
	Degradation of tissue	Verify tissue was stained within the recommended time frame following sectioning.
	Dispenser malfunction	Verify nozzle cap is removed.
		Ensure dispenser is primed
		Check the priming chamber for foreign materials or particulates, such as fibers or precipitate
		Refer to inline dispenser package insert associated with P/N 740-4859 located at <a href="http://www.ventana.com">www.ventana.com</a>
		Ensure that only recommended fixatives and fixation times are used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
Excessive background staining of slides	Incorrect staining protocol selected	Verify that U OptiV DAB VENTANA PD-L1 (SP142) procedure was used.
	Incorrect or missing bulk reagent	Ensure bulk reagents are correctly filled.
	Inappropriate fixation method used	Ensure that only recommended fixatives and fixation times are used.
Tissue detached from slides	Use of incorrect microscope slides	Ensure positively charged microscope slides are used.

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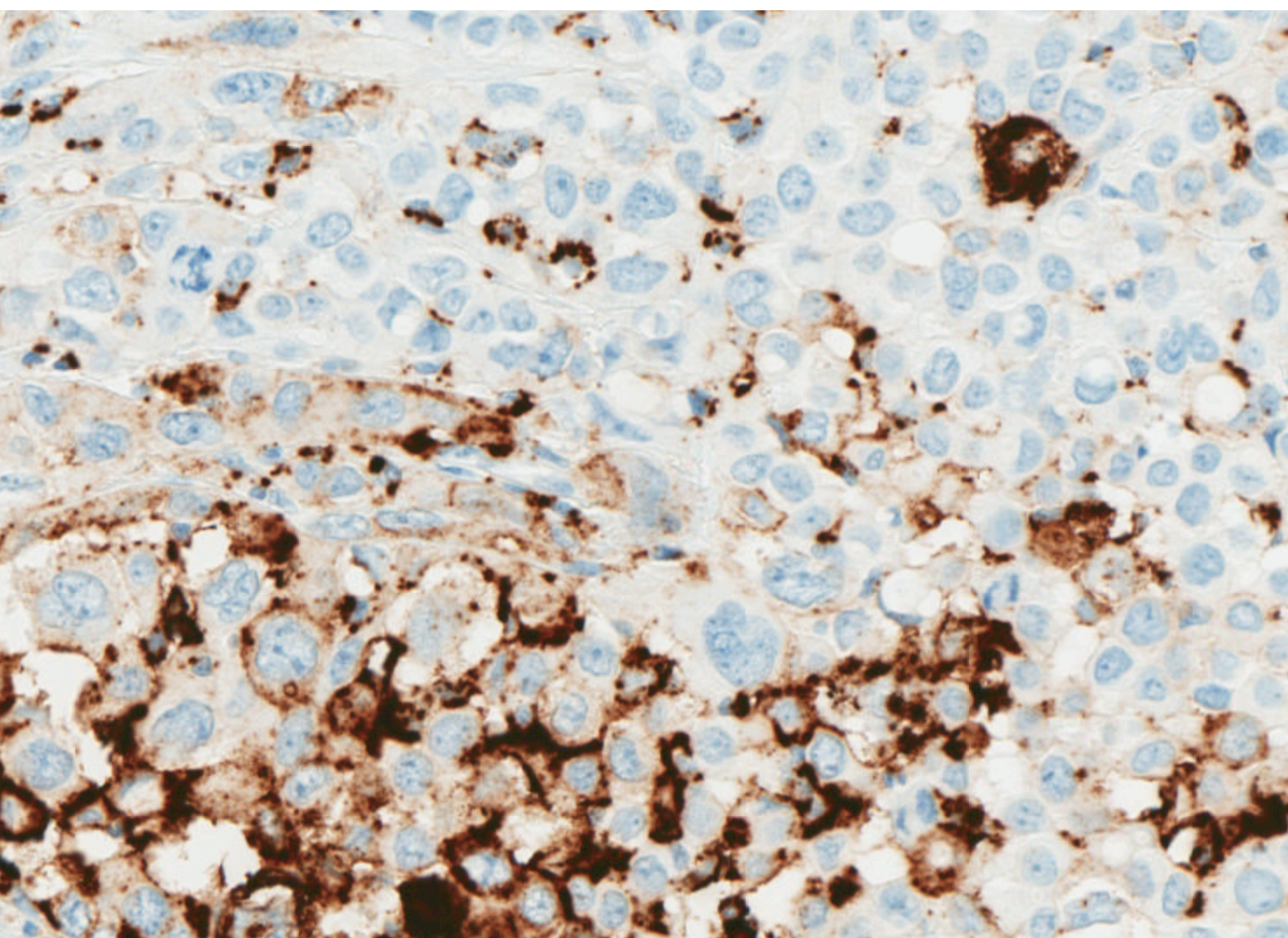


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# **VENTANA PD-L1 (SP142) Assay**

## *Interpretation Guide for Urothelial Carcinoma*







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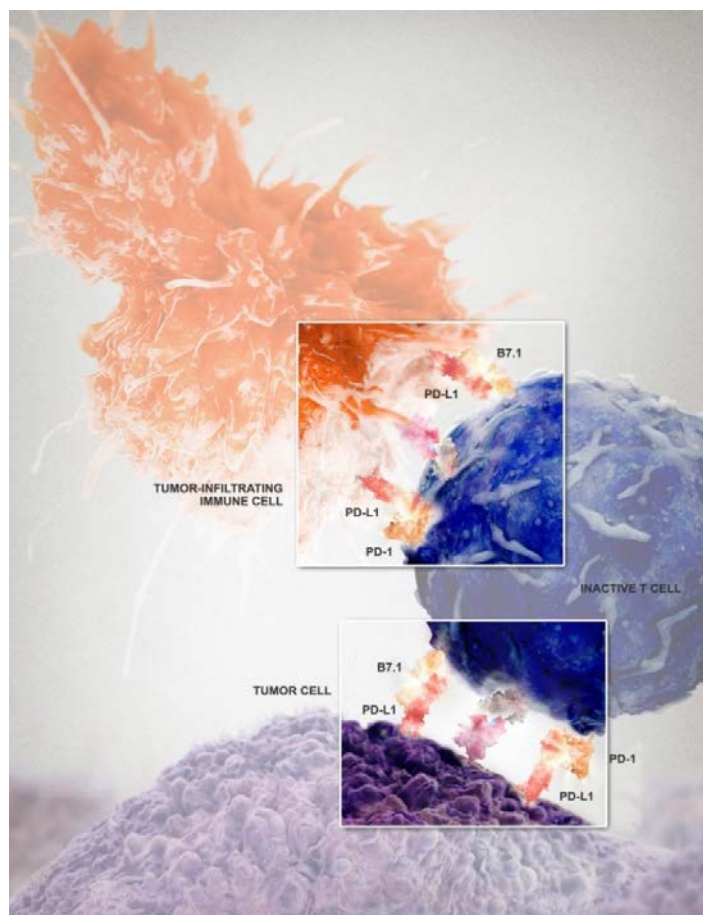
## Introduction

VENTANA PD-L1 (SP142) Assay is an immunohistochemical assay utilizing an anti-PD-L1 rabbit monoclonal primary antibody to recognize the PD-L1 protein. This assay was co-developed by Roche/Ventana Medical Systems, Inc. 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.<sup>1</sup>

Globally, there were an estimated 429,793 new cases of bladder cancer and 165,084 deaths in 2012.<sup>2</sup> 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.<sup>3</sup> Urothelial carcinoma presents as non-muscle-invasive, muscle-invasive, or metastatic disease. The 5-year relative survival rate for mUC is approximately 5.4%.<sup>4</sup>

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**).<sup>5,6</sup> 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.<sup>7</sup> 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.<sup>8</sup> PD-L1 expression has been observed in immune cells and tumor cells.<sup>5,9</sup> Aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion.<sup>7</sup> 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.<sup>10</sup> 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.<sup>9,10,11</sup> 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.<sup>13</sup>

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 tissue stained with OptiView DAB IHC Detection Kit and OptiView Amplification Kit on a VENTANA BenchMark ULTRA instrument. PD-L1 status is determined by the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity.

PD-L1 expression in  $\geq 5\%$  IC determined by VENTANA PD-L1 (SP142) Assay in urothelial carcinoma tissue is associated with increased objective response rate (ORR) in a non-randomized study of TECENTRIQ™ (atezolizumab).

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

## 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.

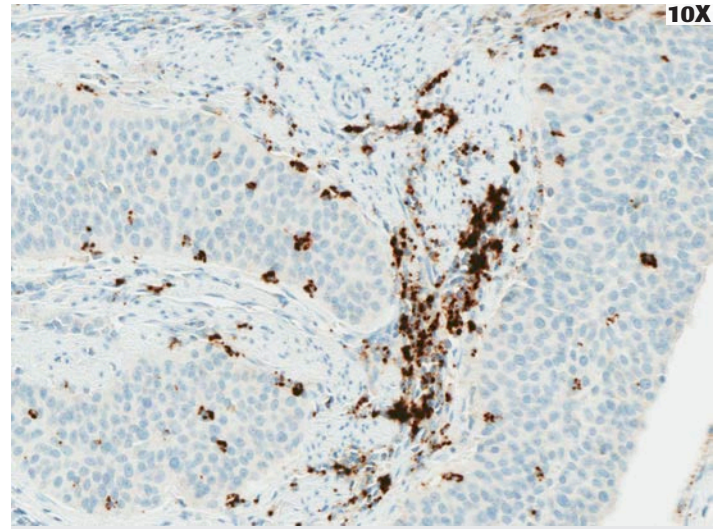
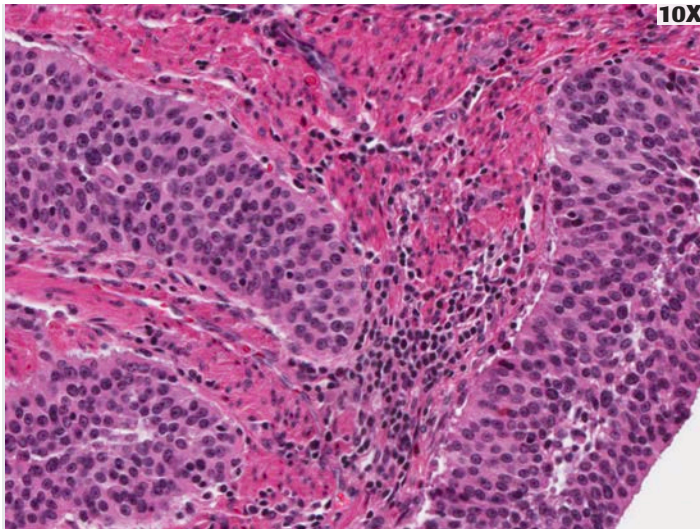
- The photomicrographs included as part of this training guide illustrate the staining patterns, as well as the range of PD-L1 scores, which may be present in urothelial carcinoma tissues stained with VENTANA PD-L1 (SP142) Assay.
- The use of tonsil as a tissue control in the context of PD-L1 evaluation, 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.



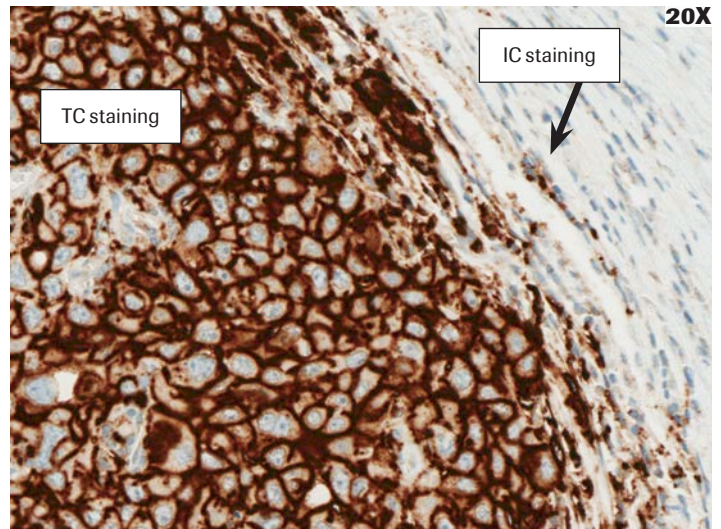
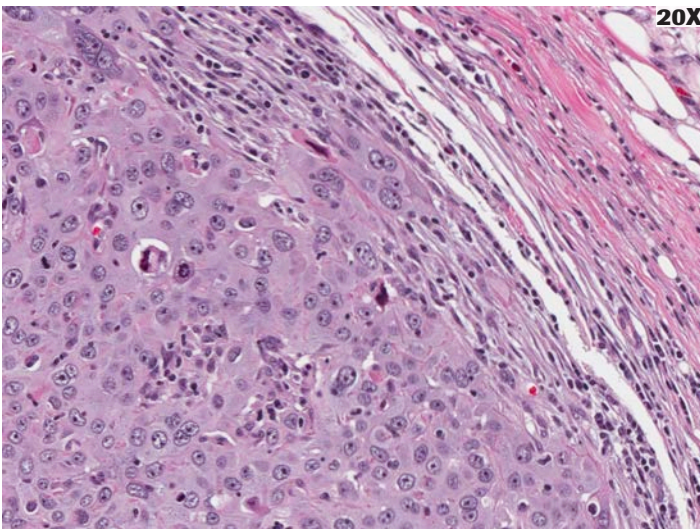
# Clinical Evaluation

## 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

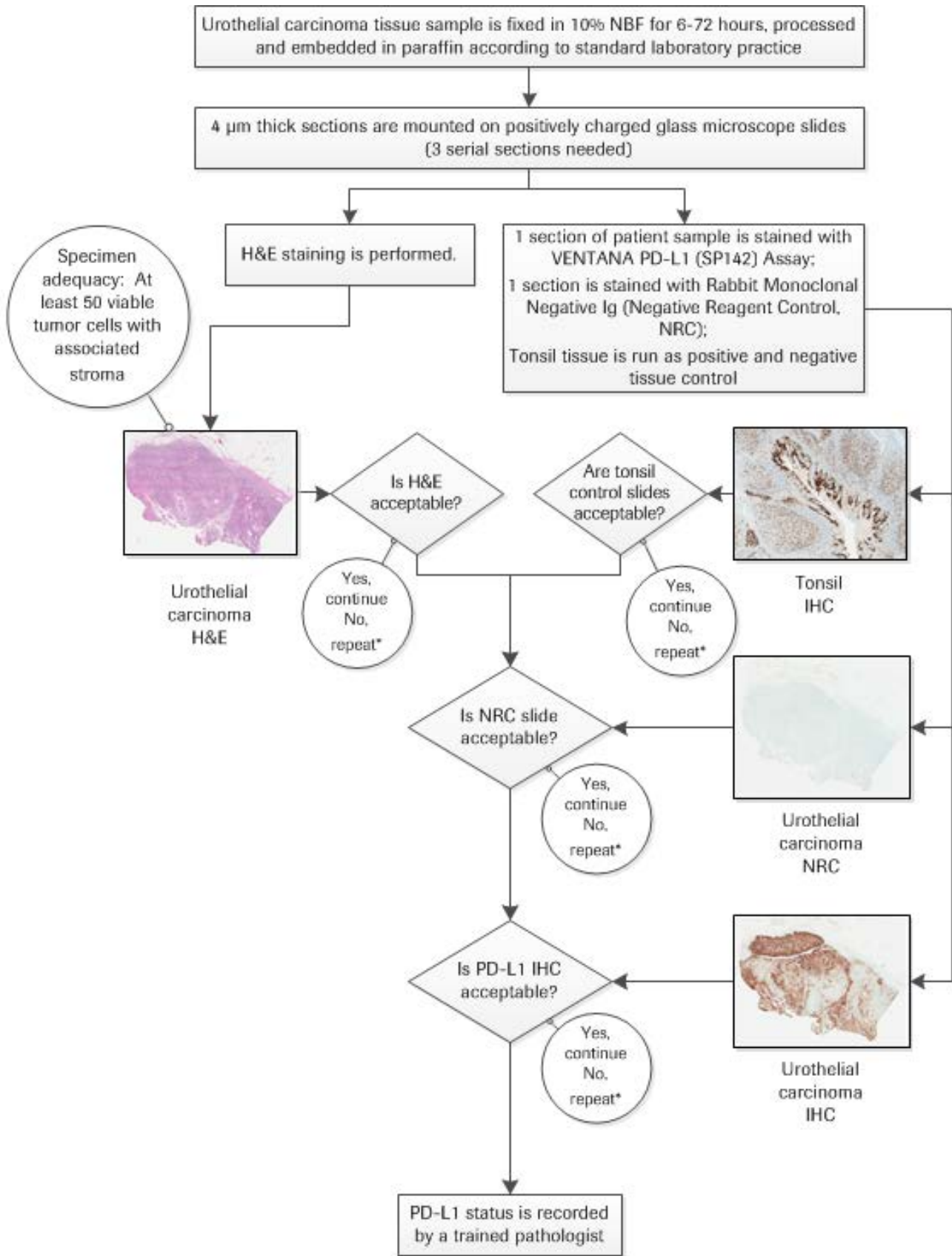
Urothelial carcinoma tissue stained with VENTANA PD-L1 (SP142) Assay will be scored according to the criteria in **Table 1**. IC are scored as the proportion of tumor area that is occupied by PD-L1 staining IC of any intensity. If the specimen contains PD-L1 staining of any intensity in tumor-infiltrating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

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**

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

Specimen Flow



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

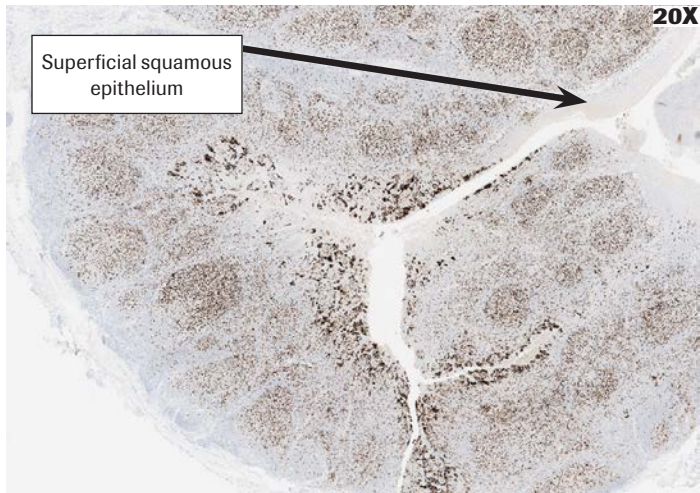
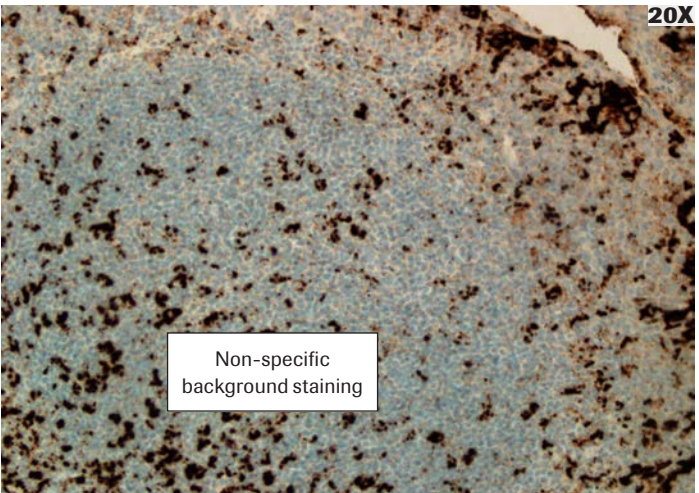
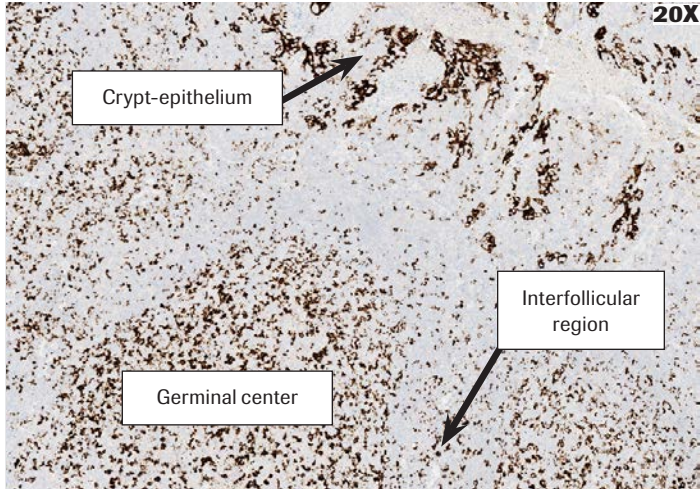
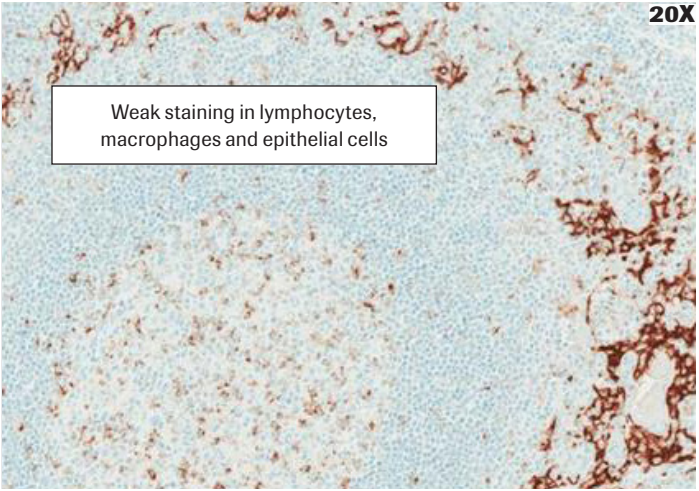


Controls

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 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.
	
	

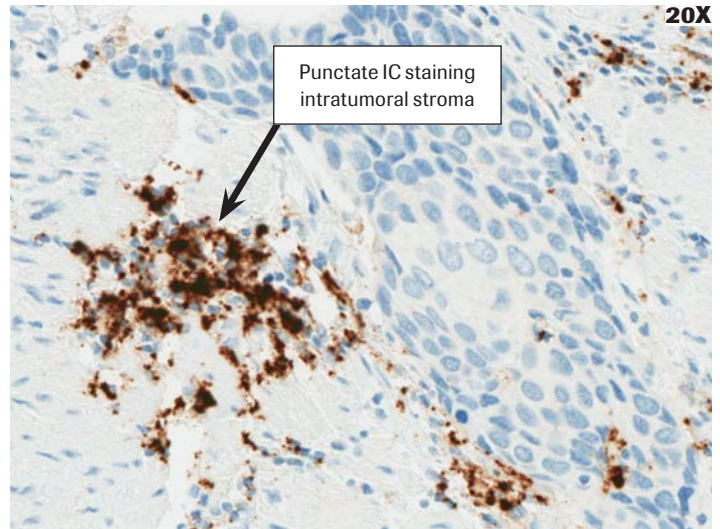
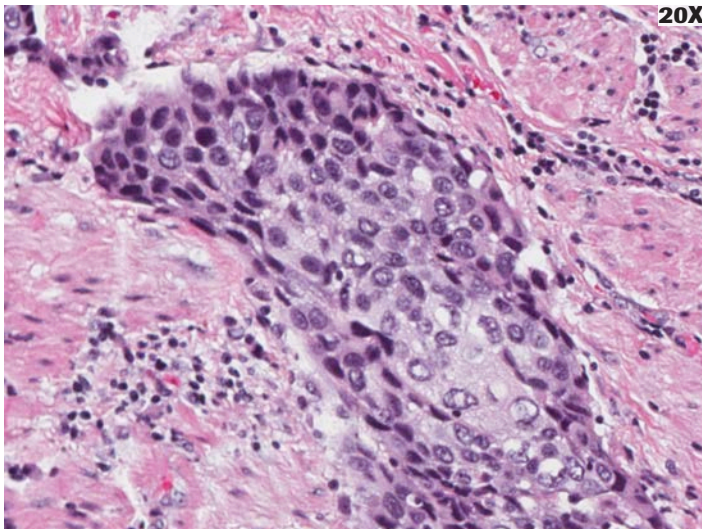


## Staining Characteristics – Urothelial Carcinoma

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.

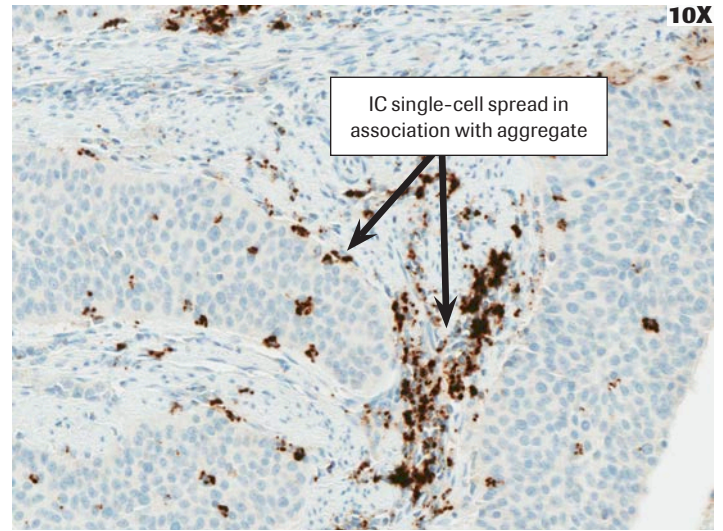
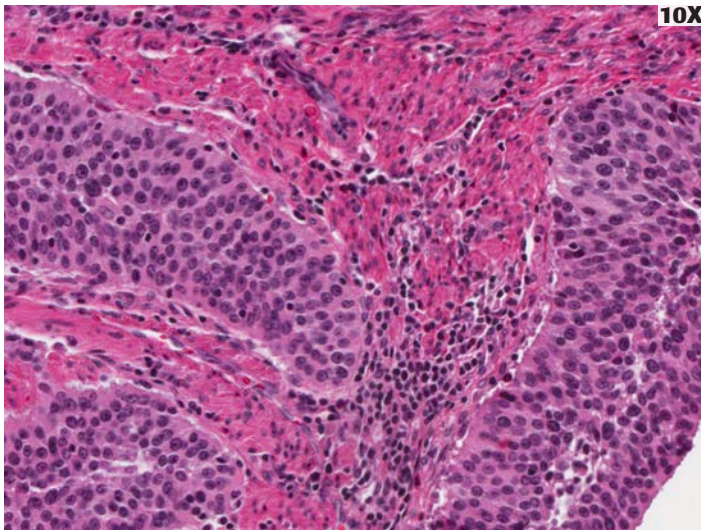
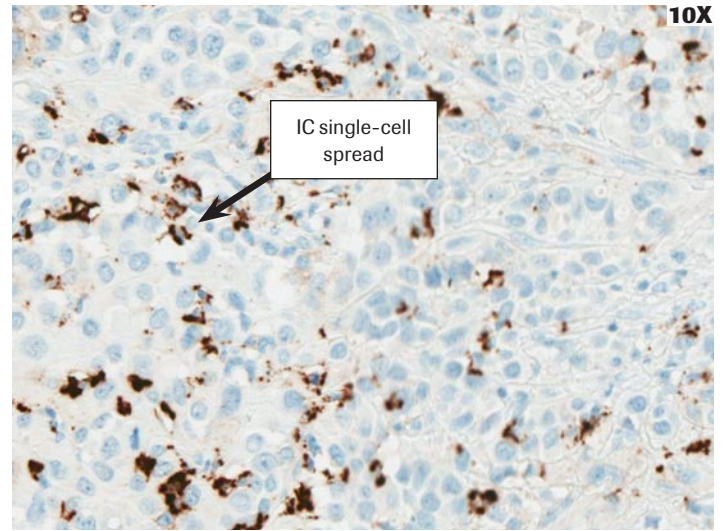
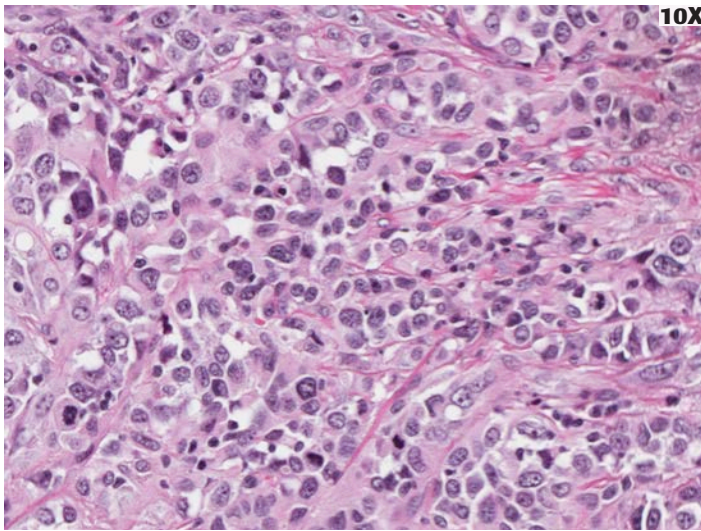
### IC Staining:

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.

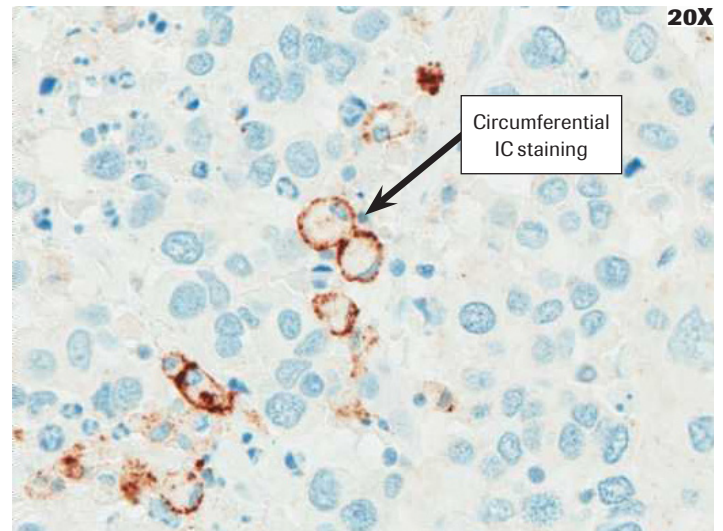
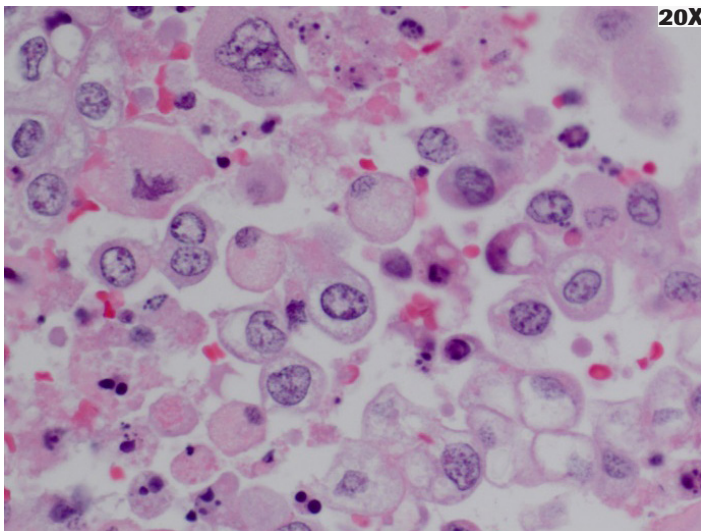


**Figure 4:** IC often show dark brown punctate or linear staining, which is the predominant IC staining pattern observed in the majority of tissues. IC staining is often seen as aggregates either in intratumoural or peritumoral stroma (invasive margin) or in both locations.



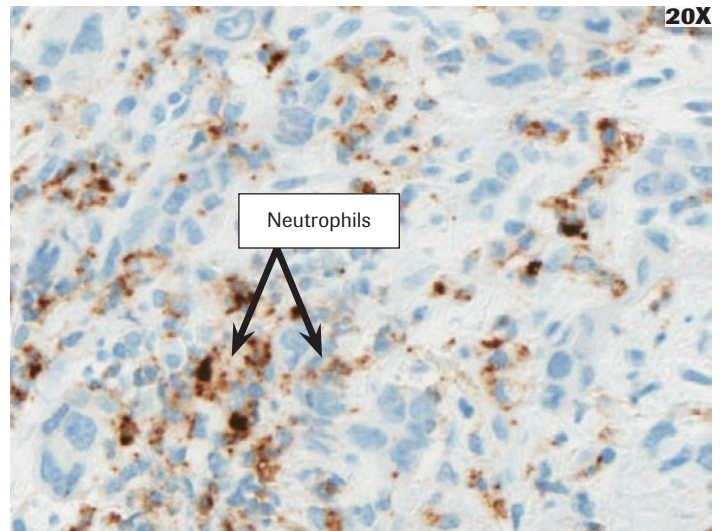
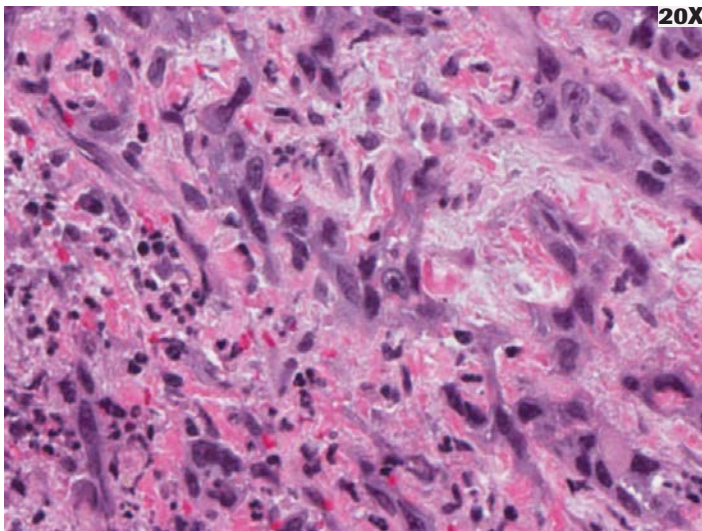


**Figure 5:** Occasionally, IC staining can also be observed in the form of focal or diffuse scattered single cells or small aggregates (single-cell spread) dispersed among tumor cells. This pattern may be seen in association with aggregates in tumor stroma. IC staining corresponds to the immune cells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



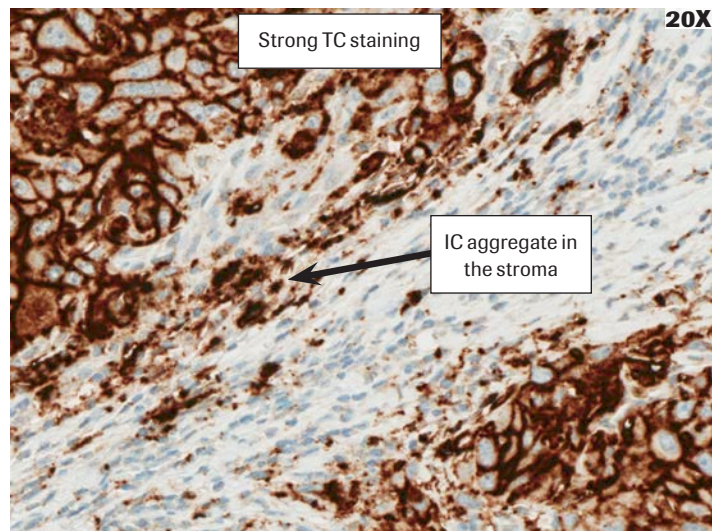
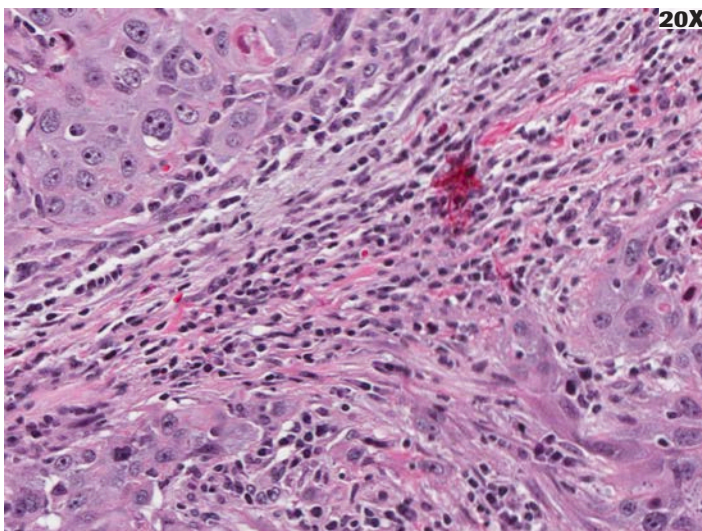


**Figure 7:** Rarely, IC staining can be observed in neutrophils, as fine punctate staining along with diffuse granular staining. Neutrophil staining can be seen dispersed among tumor cells, in the intratumoral or peritumoral stroma or as aggregates.

### Differentiation of IC from TC Staining

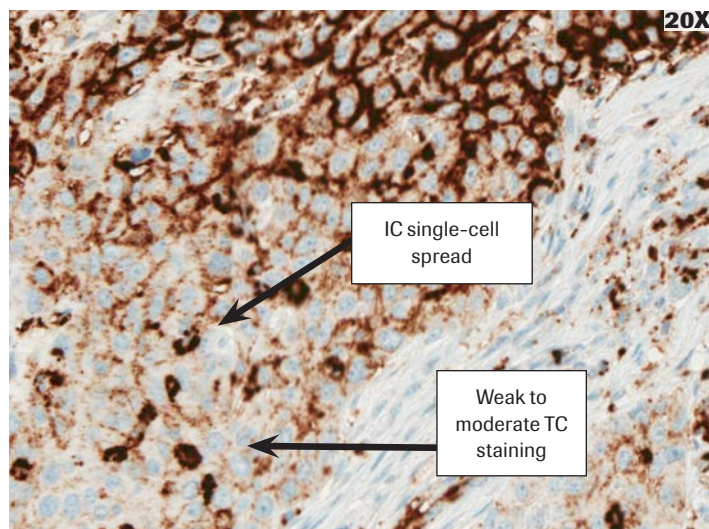
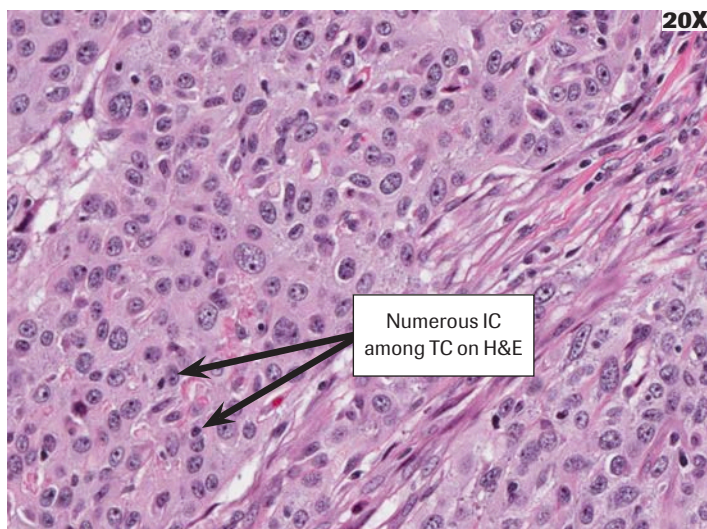
IC staining can be observed in association with TC staining. The PD-L1 stained slide will only be evaluated for IC staining; hence, differentiation of IC from TC staining is essential. Review of the corresponding H&E slide will help in identifying IC among TC. This, along with a high magnification review 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**).

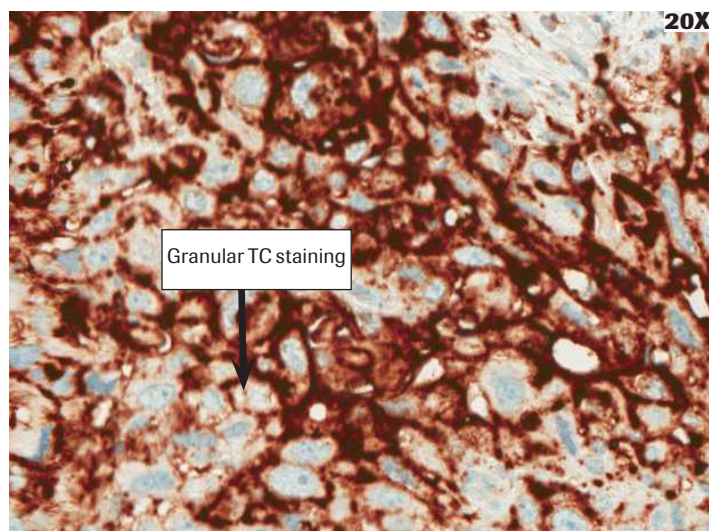
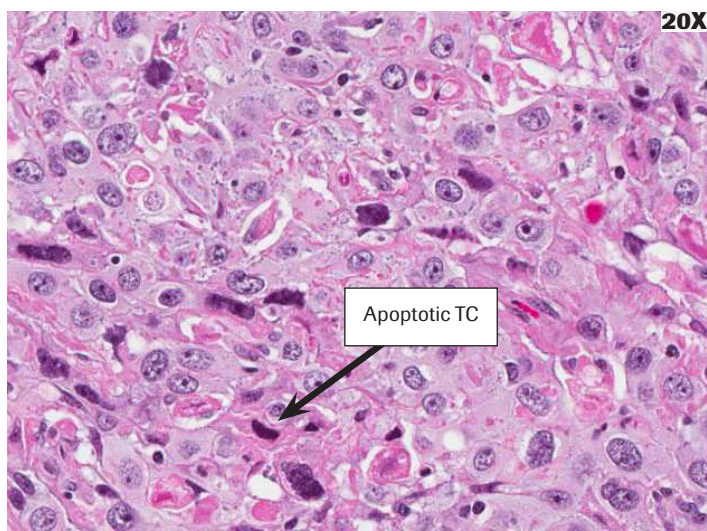
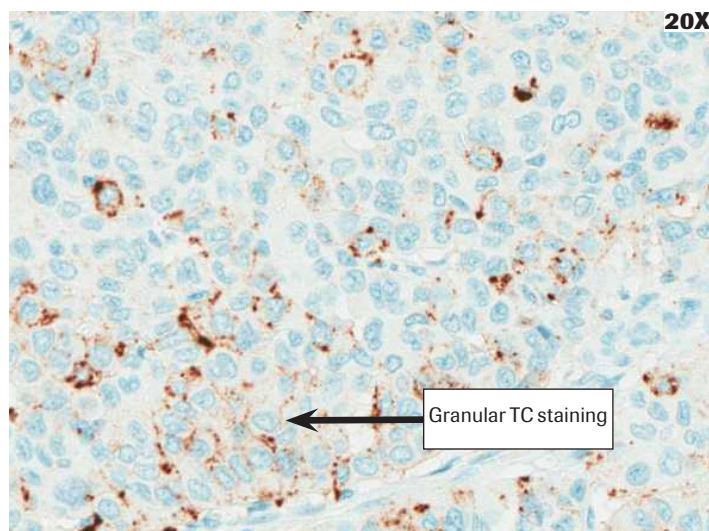
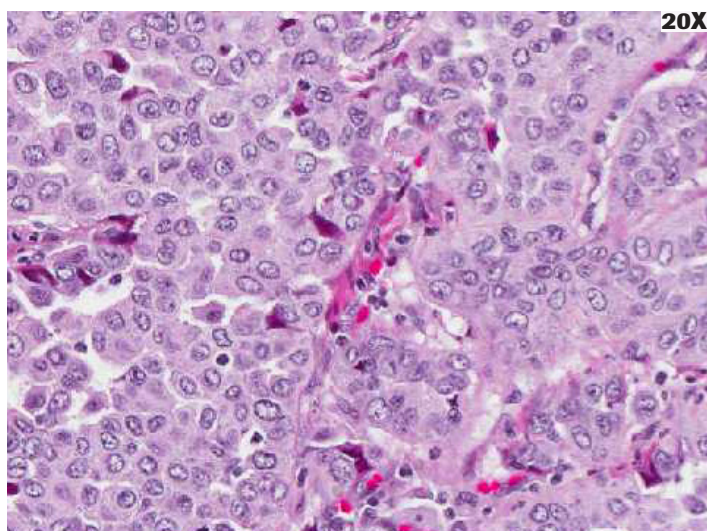


**Figure 8:** TC show strong membrane staining, with rare IC among the TC identified on the H&E slide; focus on evaluating IC staining in intratumoral and contiguous peritumoral stroma.





**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.

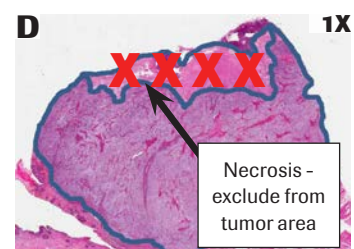
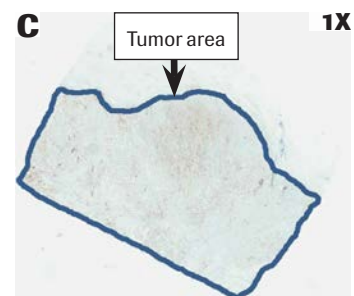
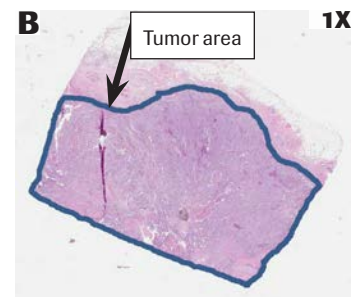
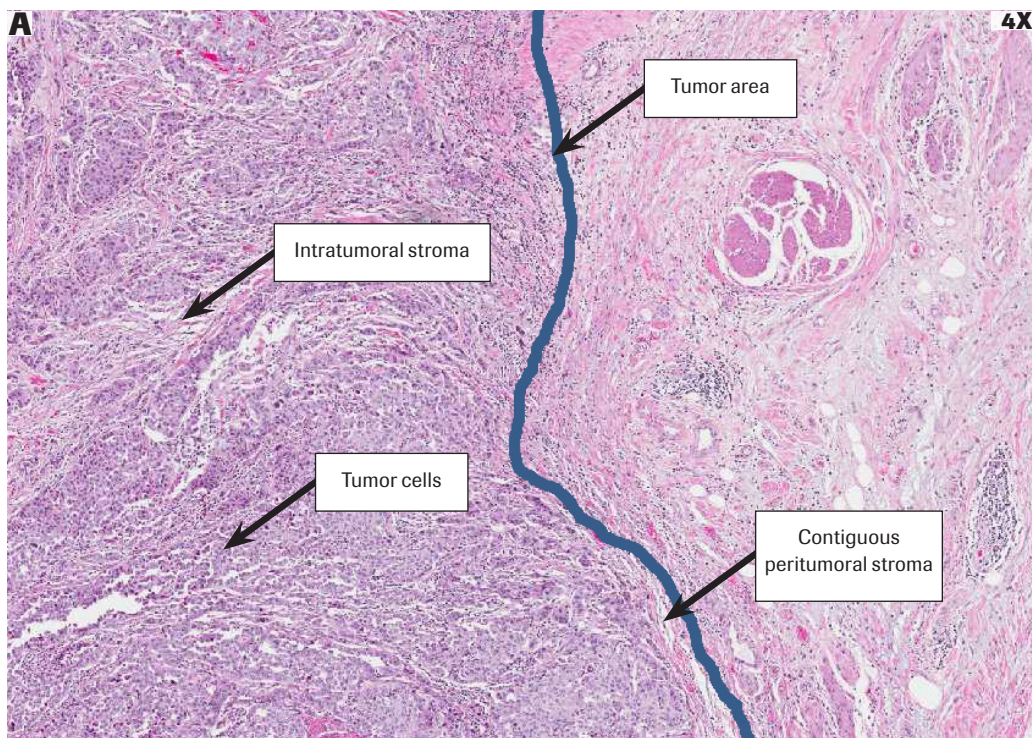


## Scoring Method

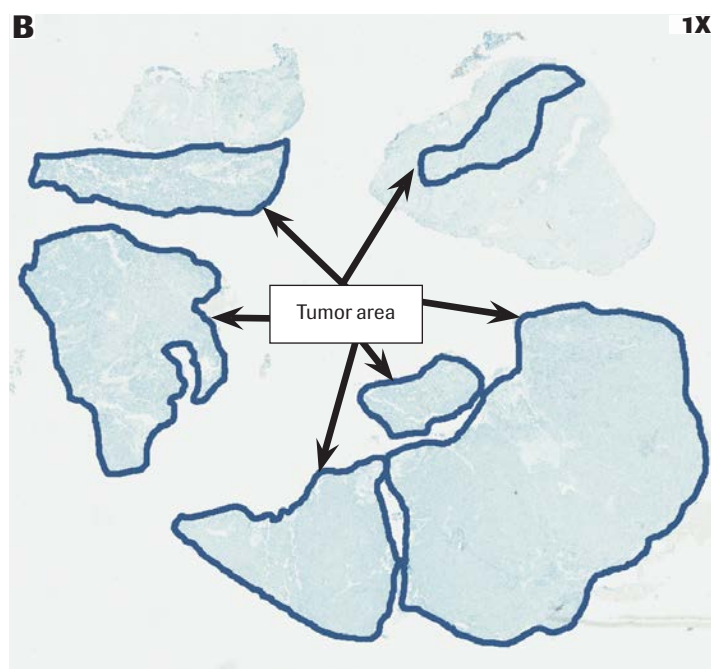
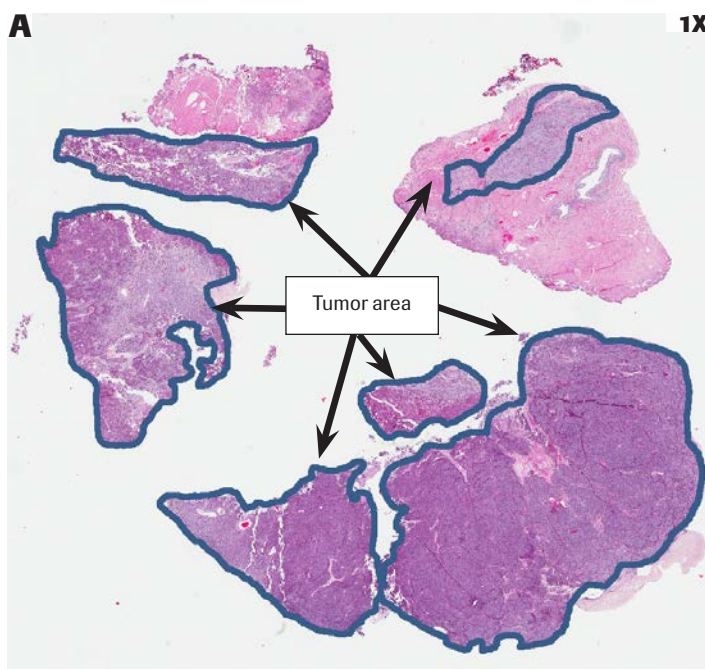
VENTANA PD-L1 (SP142) Assay stained urothelial carcinoma tissue will only be evaluated for IC staining.

**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 12A & B**). Necrotic tumor is excluded from this definition of tumor area (**Figure 11D**).
- 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 tissue fragment, but not contiguous to viable tumor, is excluded (**Figure 12A & B**).



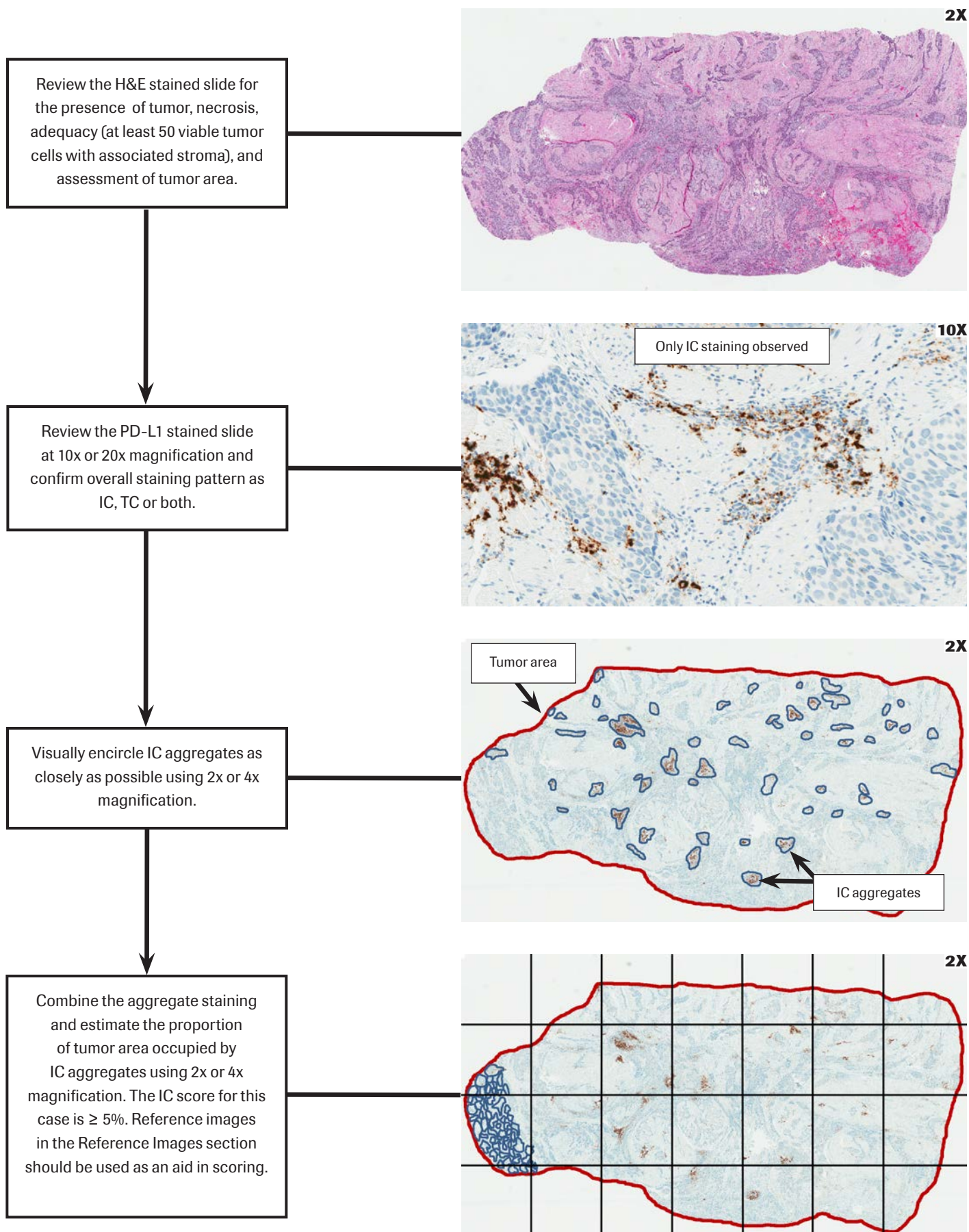
**Figure 11: A** Tumor Area; **B** (H&E), and **C** (PD-L1 IHC): Tumor area in a resection specimen; **D** Tumor area in a specimen containing necrosis.



**Figure 12: A** (H&E), **B** (PD-L1 IHC) Tumor area in a transurethral resection of bladder tumor (TURBT) specimen

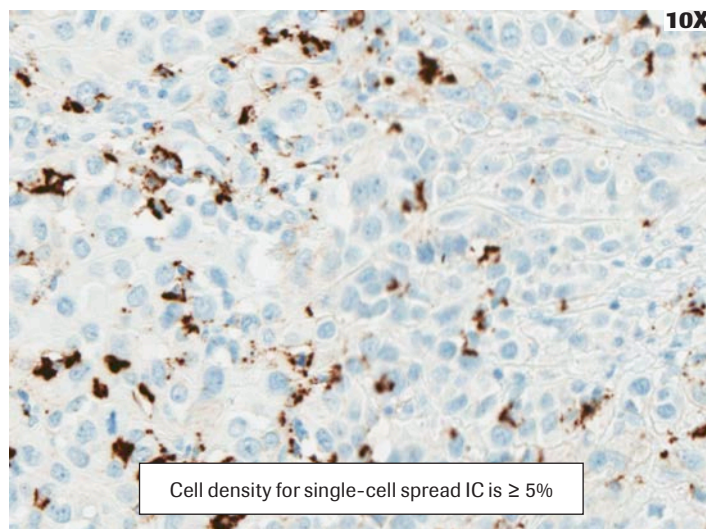
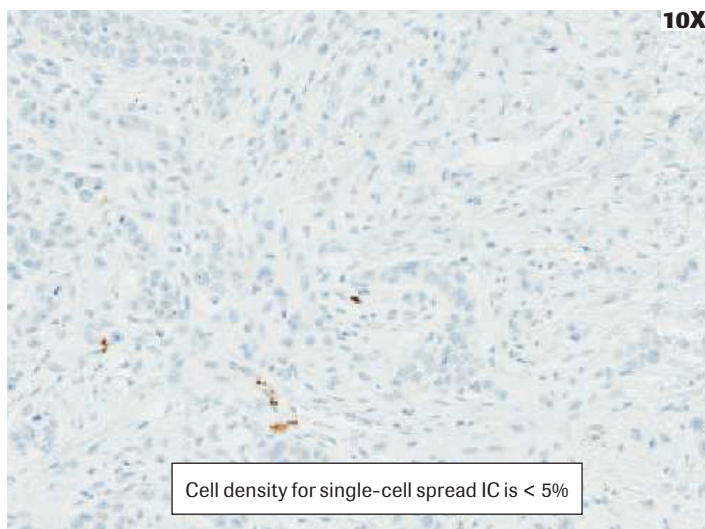


## Scoring of PD-L1 IC aggregate staining:



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

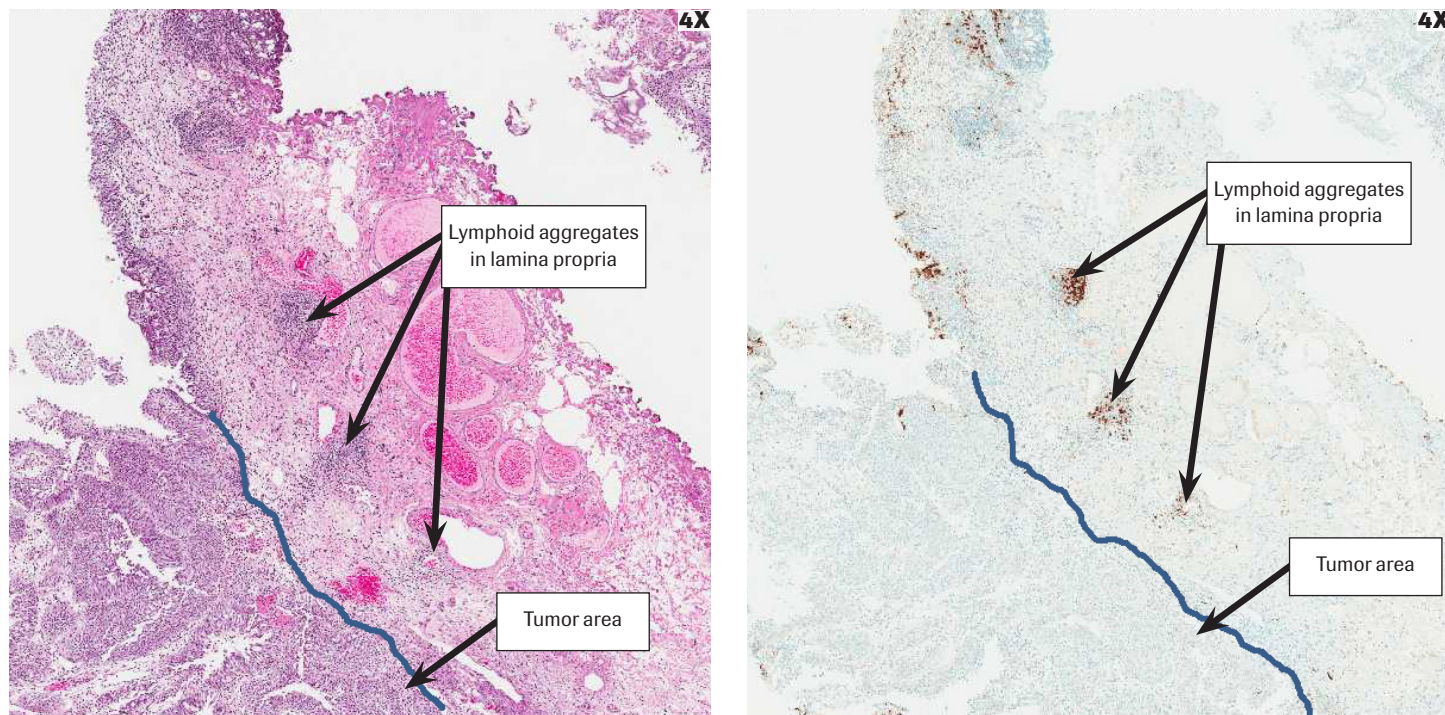
Single-cell spread IC is scored based on the density of single cell spread, using the Reference Images section of this guide.





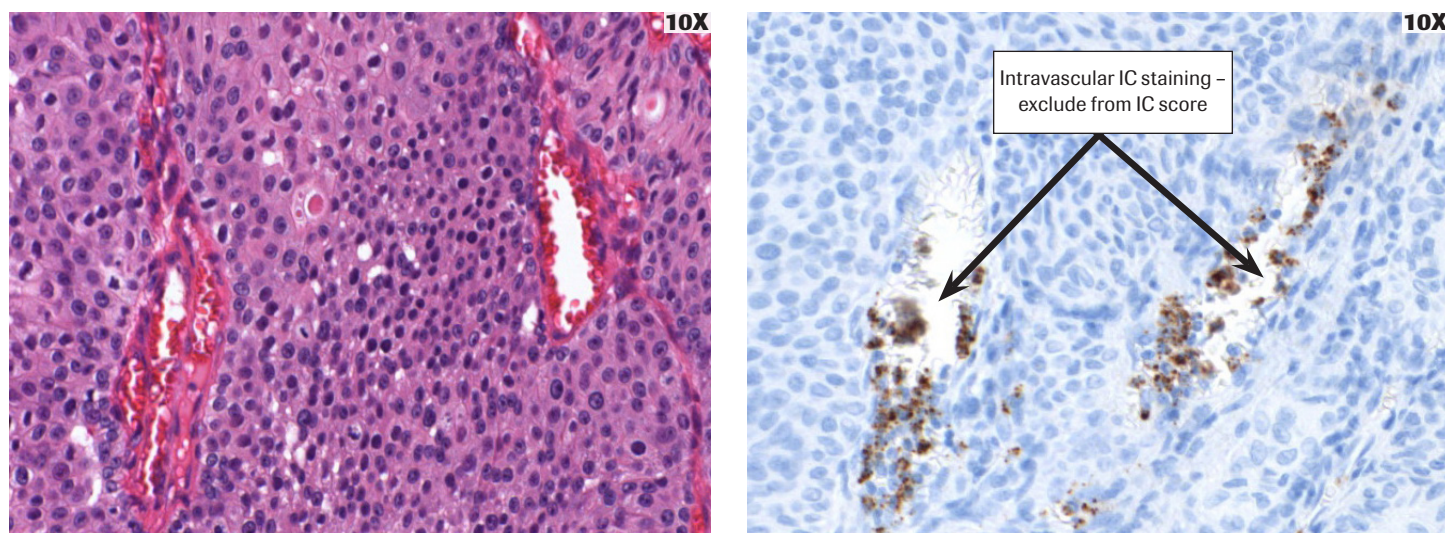
## Scoring Methods: Challenges and Pitfalls

**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 13**).



**Figure 13:** Lymphoid aggregates in the lamina propria are excluded from IC scoring in a papillary urothelial carcinoma.

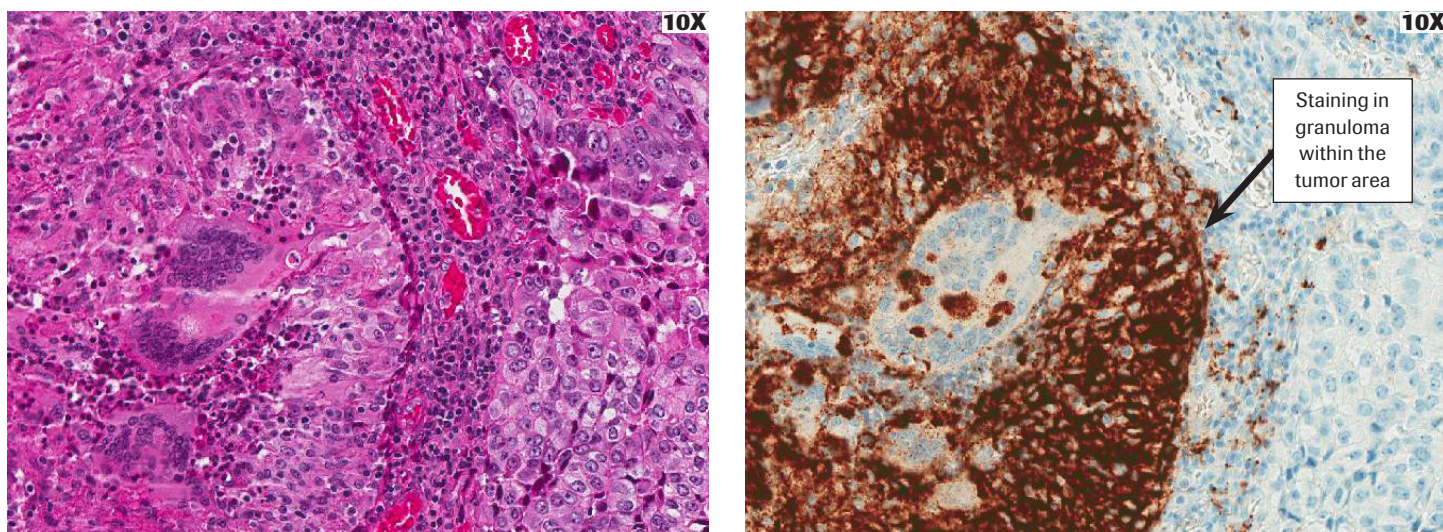
**2. Intravascular Immune cells:** Vasculature in tumor stroma may show PD-L1 positive immune cells (**Figure 14**). This is observed most often in fibrovascular cores of papillary urothelial carcinoma. These are not considered towards IC scoring.



**Figure 14:** Intravascular immune cell staining – exclude from IC scoring.

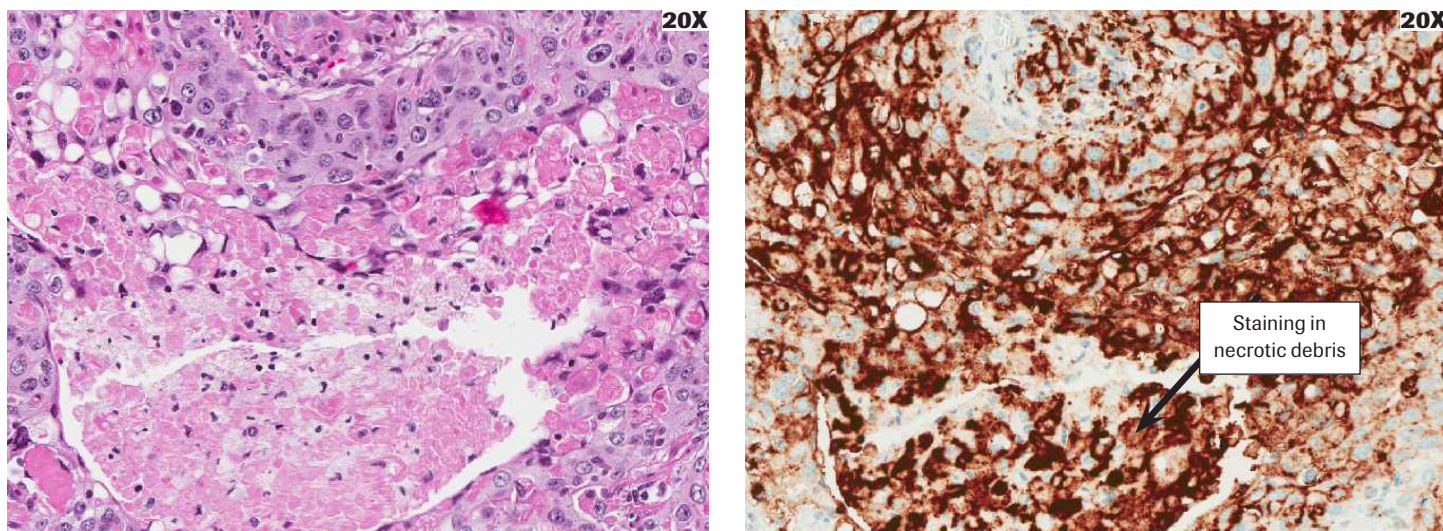


**3. Staining in granulomas:** Some urothelial carcinoma tissues can show granulomas, possibly due to treatment effect. Staining of PD-L1 can be seen in macrophages and giant cells in these granulomas and can be mistaken for tumor cell staining (**Figure 15**). Review of the corresponding H&E slide will facilitate 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

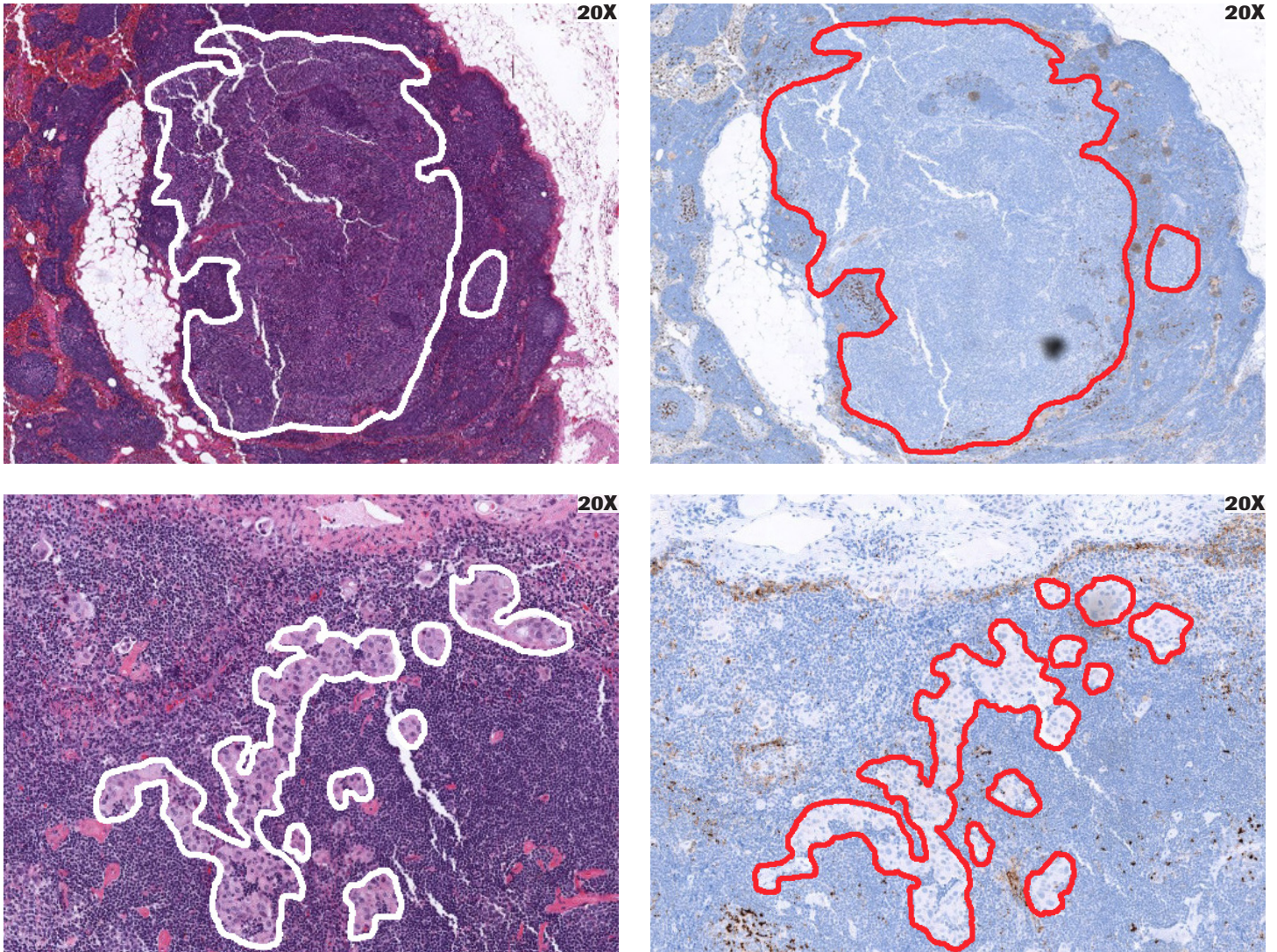
**4. Staining in necrotic debris:** Necrotic debris or immune cells in the periphery of necrotic or apoptotic regions can show PD-L1 staining. This staining may be granular and can be mistaken for IC staining. This staining, as well as the neutrophil staining observed as aggregates, should be excluded from scoring (**Figure 16**).



**Figure 16:** Necrotic debris showing PD-L1 staining (H&E on the left and the PD-L1 stained on the right.) 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 17**).



**Figure 17: A & B:** Lymph node with well circumscribed metastasis. **C & D:** Lymph node with multiple small tumor cell nests (H&E on the left and PD-L1 stained slide on the right.) Tumor area is outlined both on H&E and PD-L1 images.



Reference Images

VENTANA PD-L1 (SP142) Assay: IC Scoring

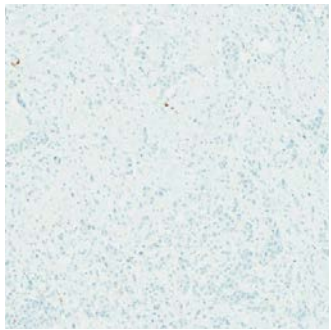
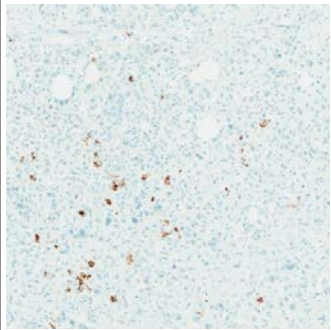
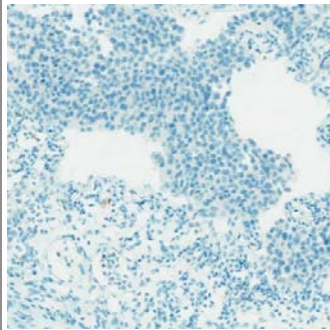
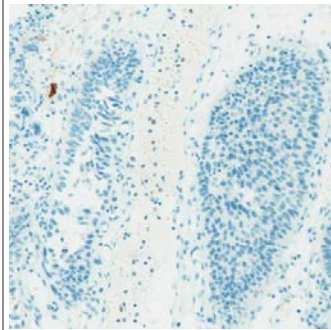
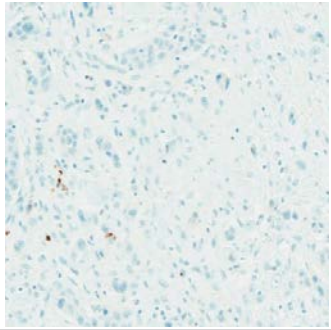
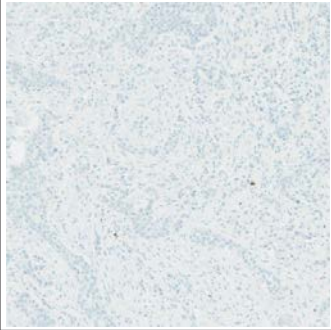
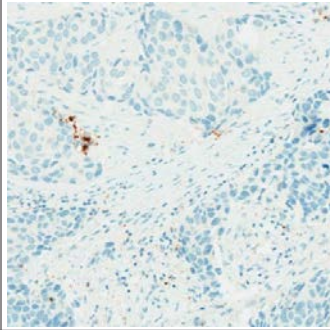
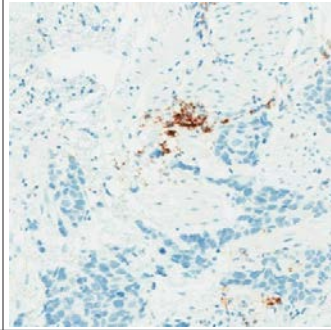
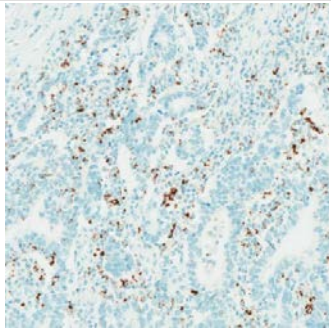
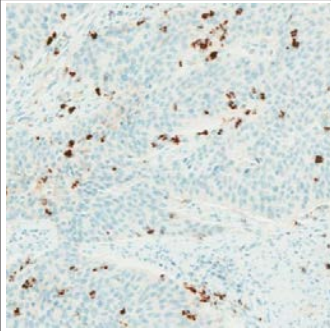
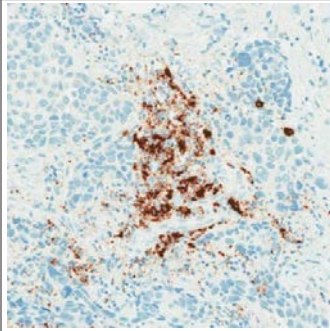
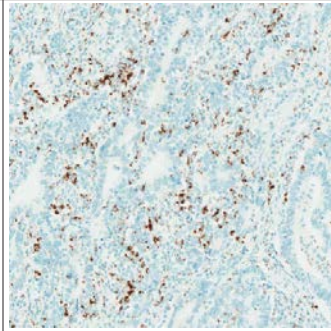
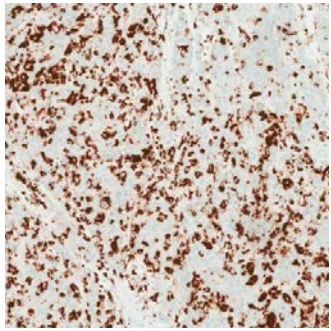
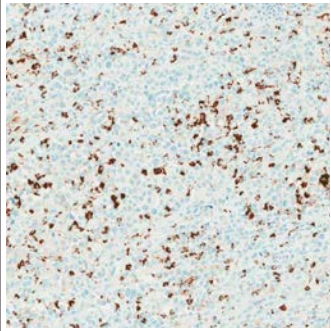
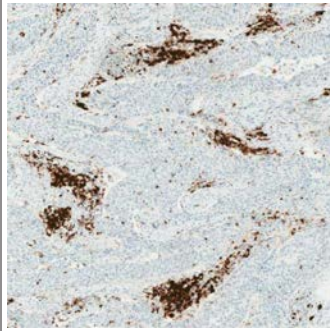
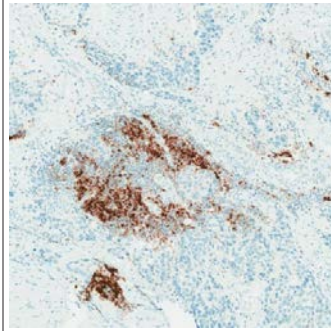
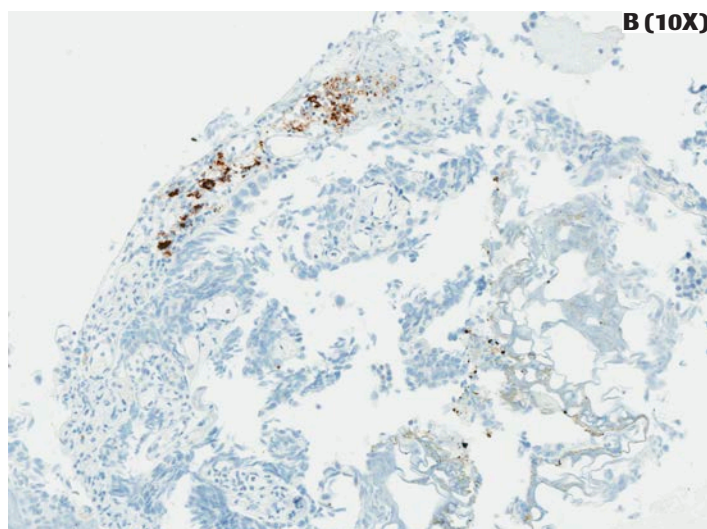
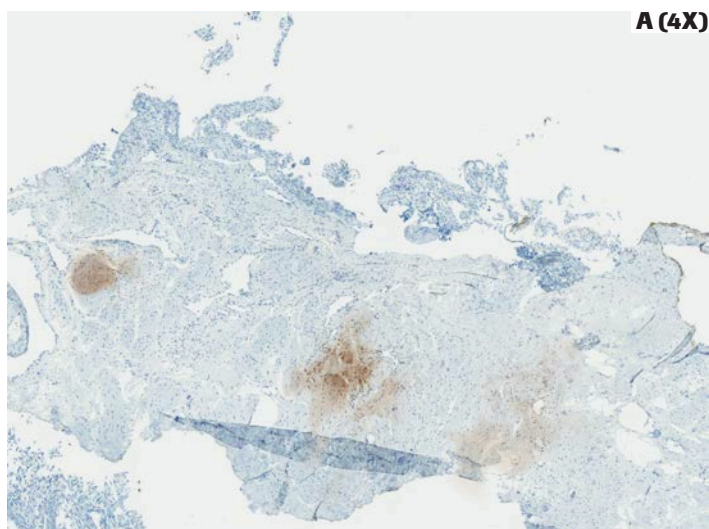
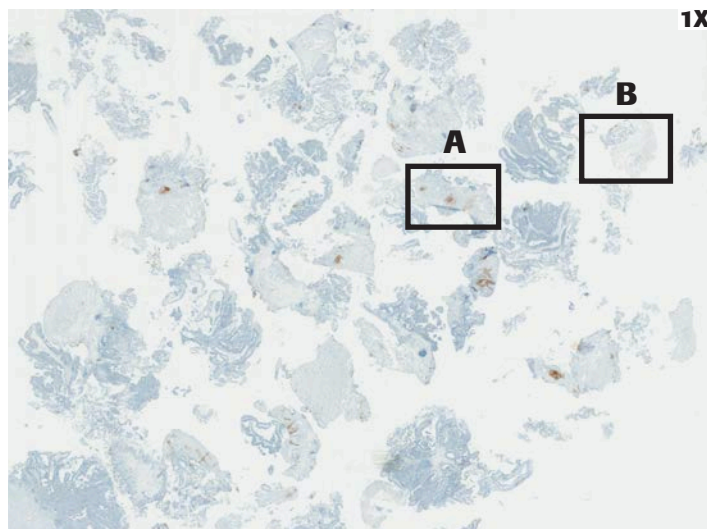
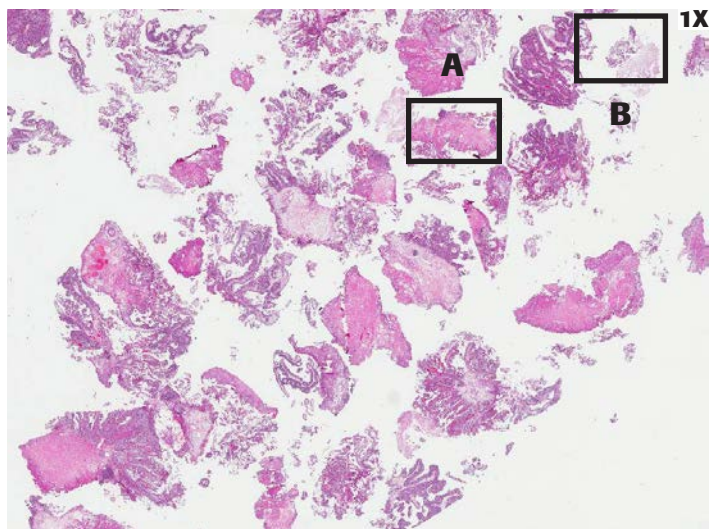
All images 10X magnification				
IC Single-cell spread			IC Aggregates	
IC < 5%				
				
IC ≥ 5%				
				

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

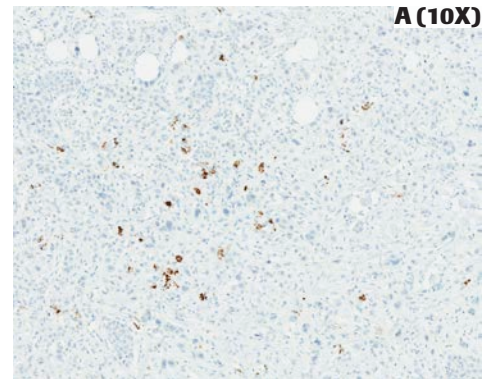
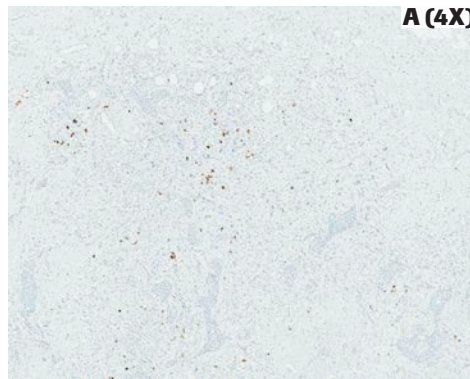
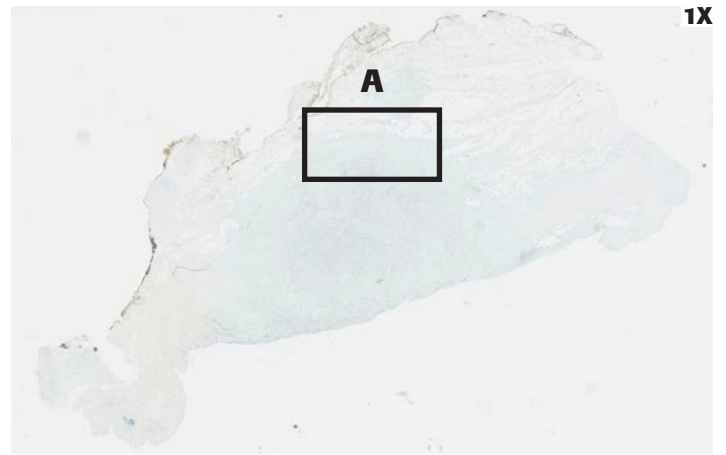
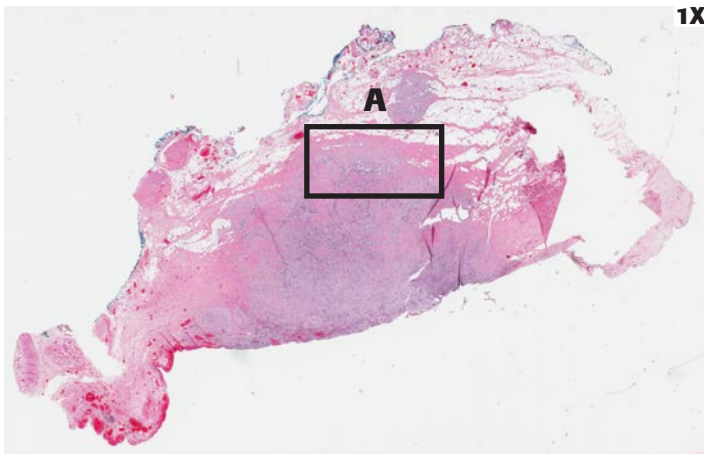


**VENTANA PD-L1 (SP142) Assay: IC < 5%**

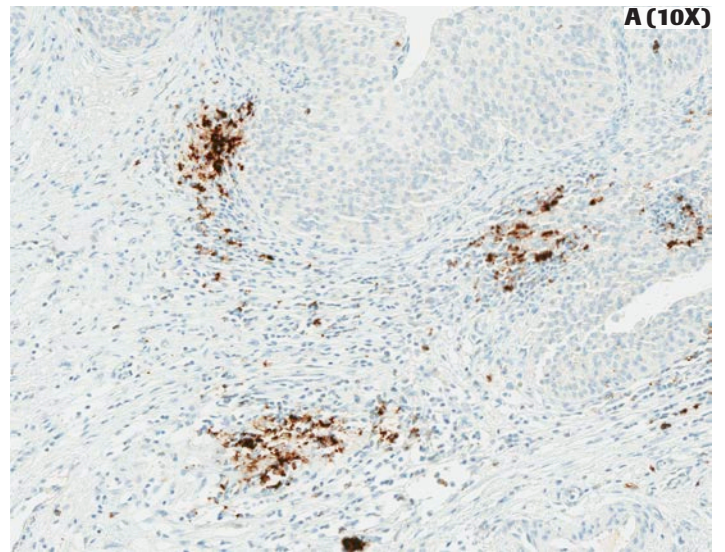
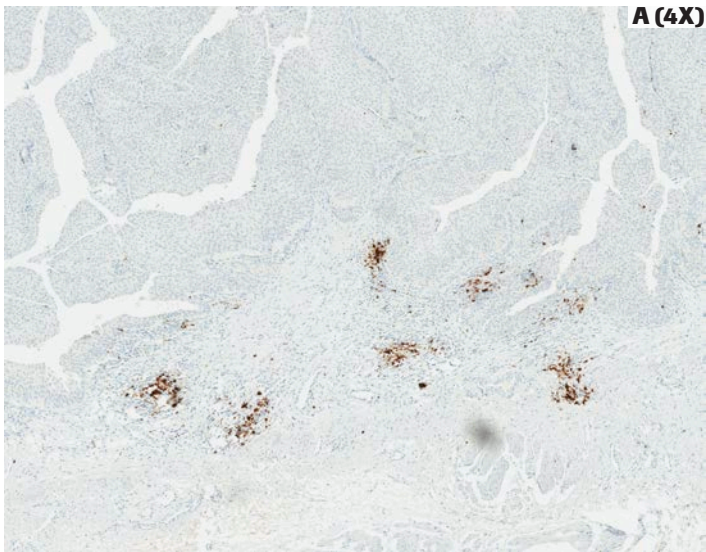
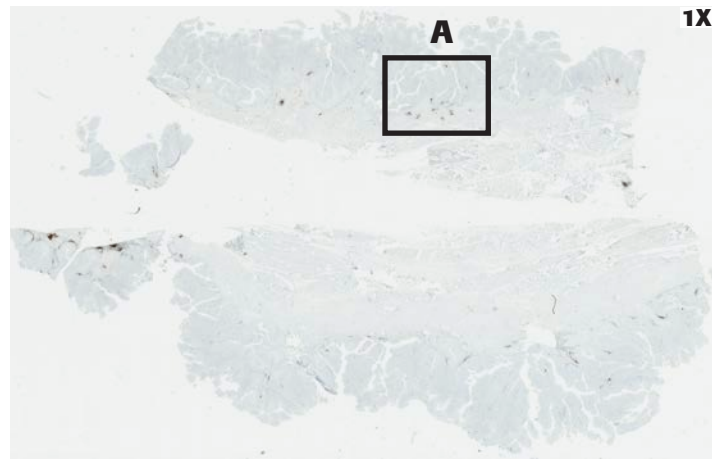
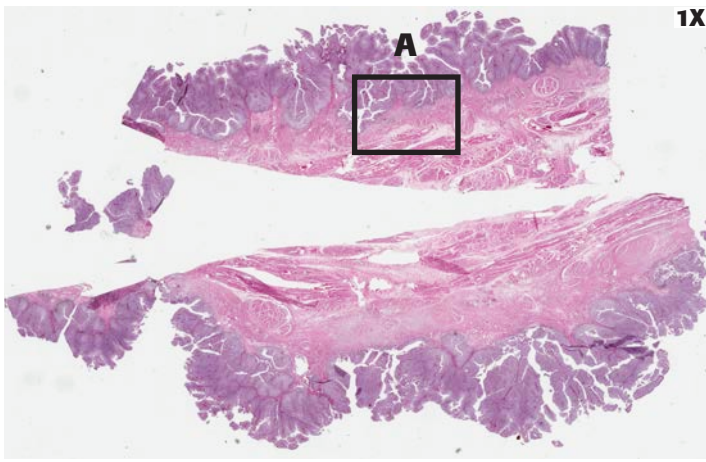


**Case 1:** This case is IC < 5%. Score: IC: <1%; TC 0%. At low magnification, this case exhibits several regions of brown staining. When examined at high magnification most of the brown staining appears to be due to trapping of reaction products (dye) under the folded tissue (dye trapping). It is important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





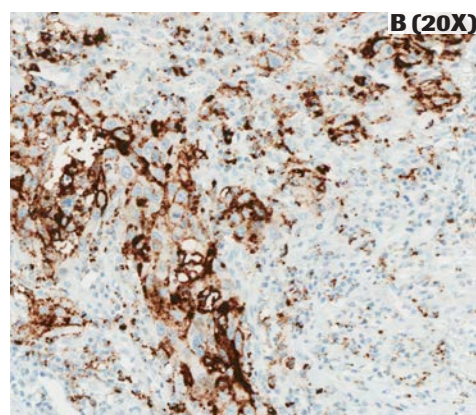
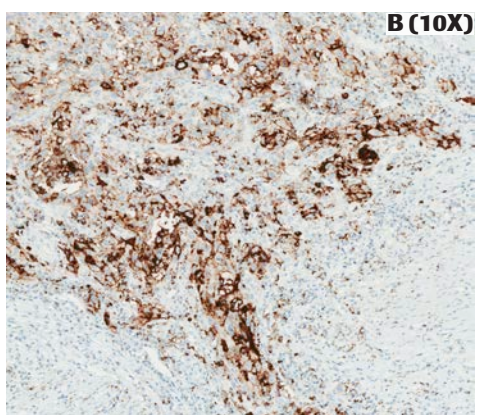
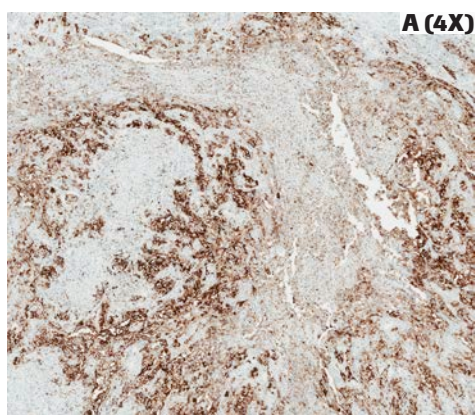
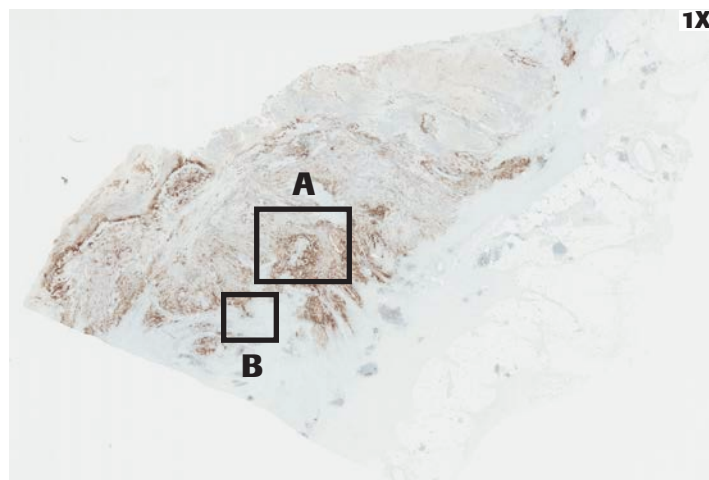
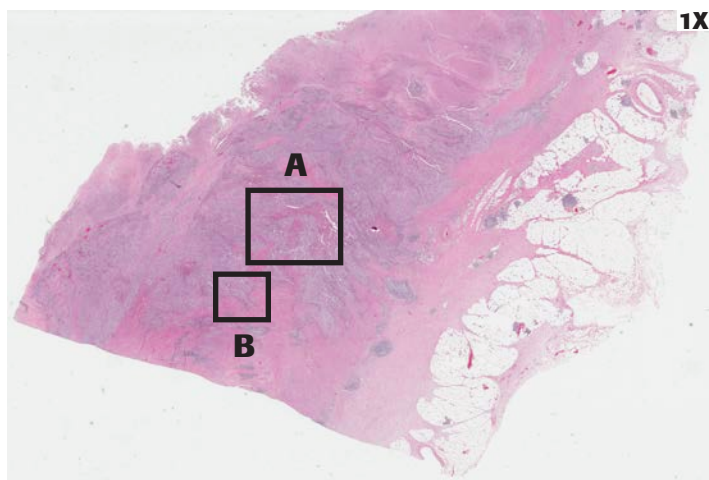
**Case 2:** This case is IC < 5%. Scores: IC: 1%; TC: 0%. Note the presence of focal IC single cell spread of PD-L1 staining IC, which when normalized to the entire tumor area is < 5%.



**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.

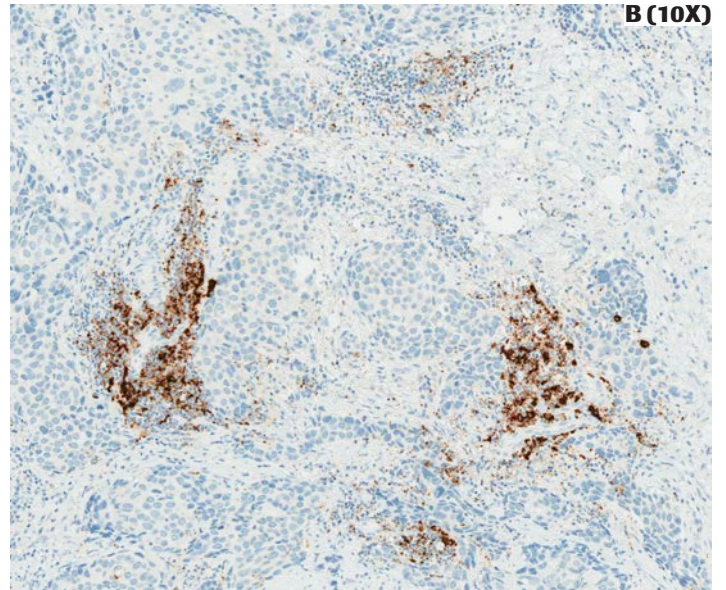
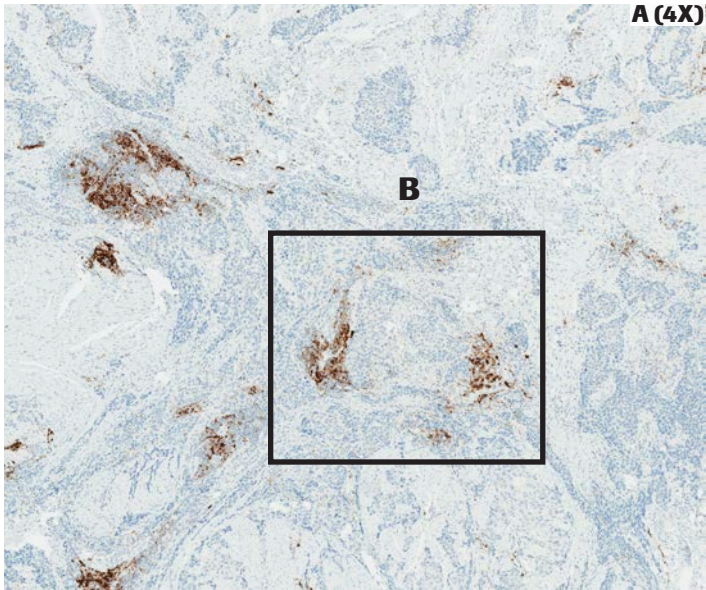
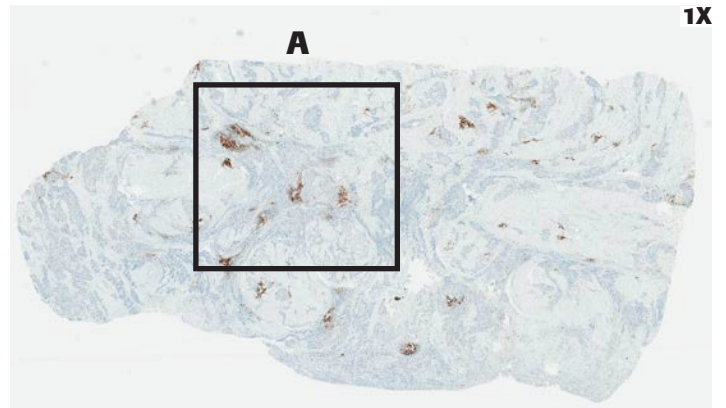
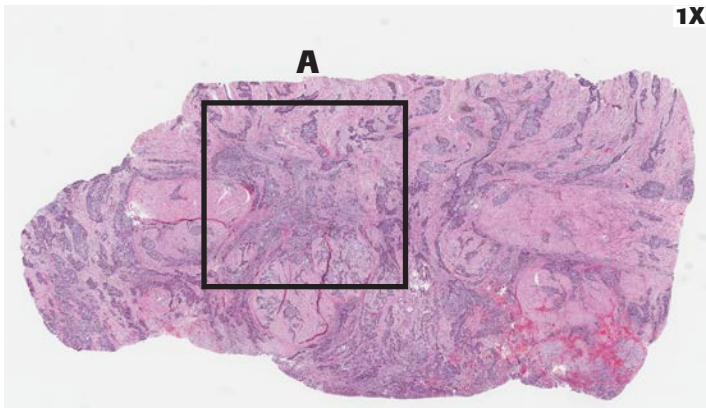


**VENTANA PD-L1 (SP142) Assay: IC  $\geq$  5%**



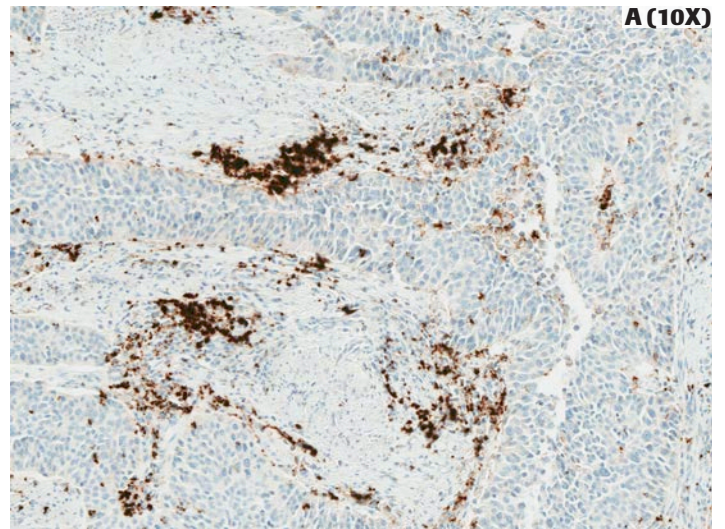
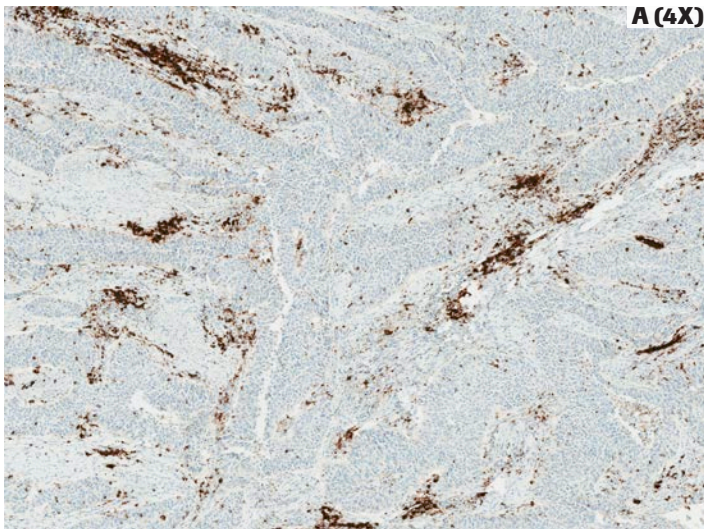
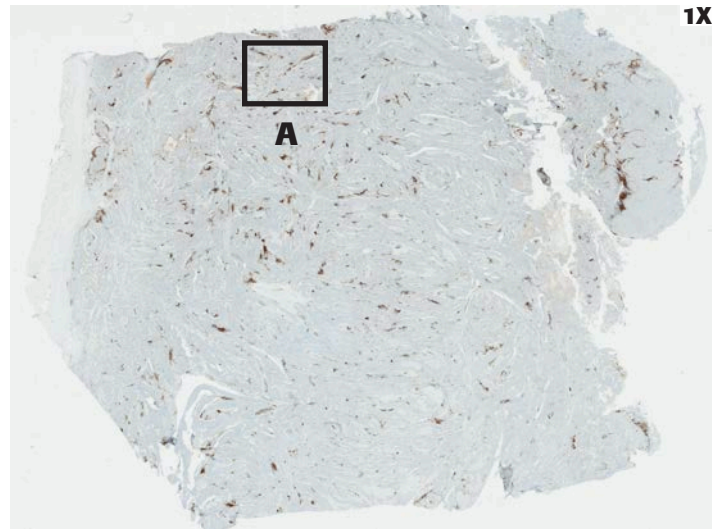
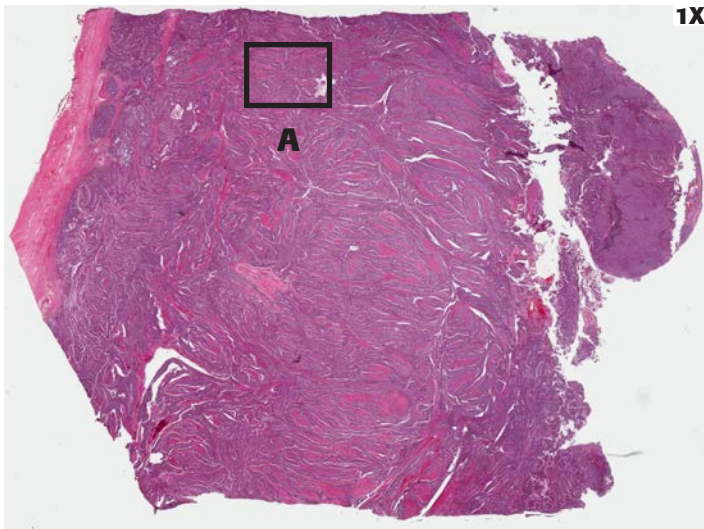
**Case 4:** This case is IC  $\geq$  5%. Scores: IC: 10%; TC: 90%. This case illustrates IC staining along with TC staining. At low magnification it is difficult to distinguish these 2 cell types, but when examined at a higher magnification (10X), punctate IC staining can be distinguished from circumferential 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  $\geq$  5%. Scores: IC: 5%; TC: 0%. Note the presence of IC aggregate staining.

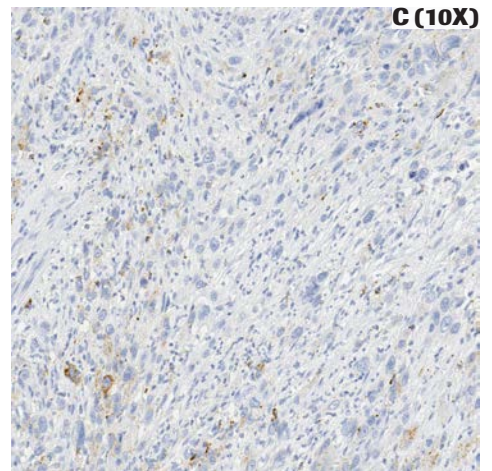
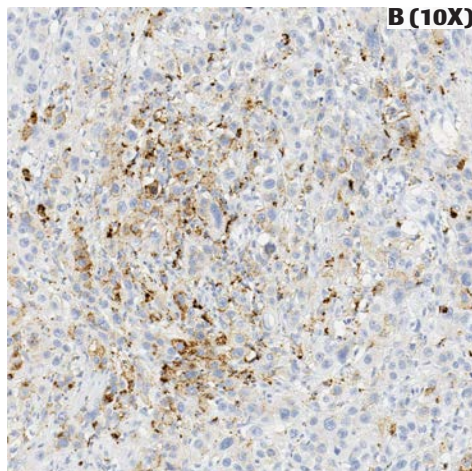
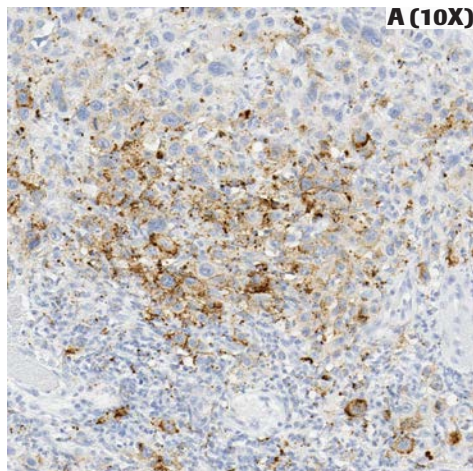
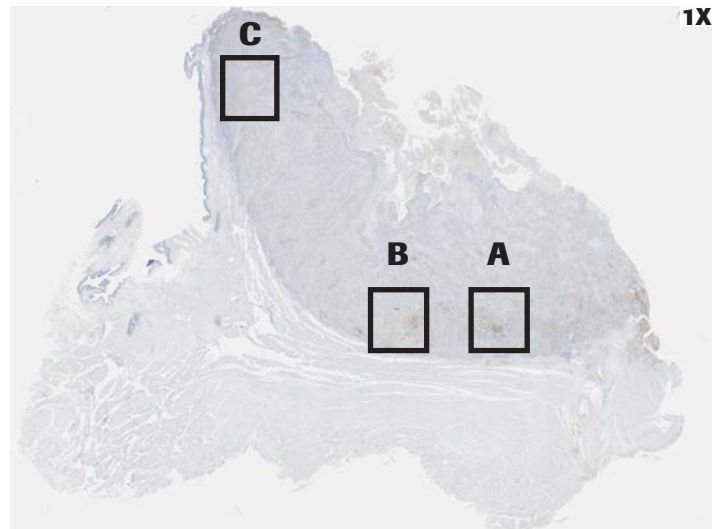
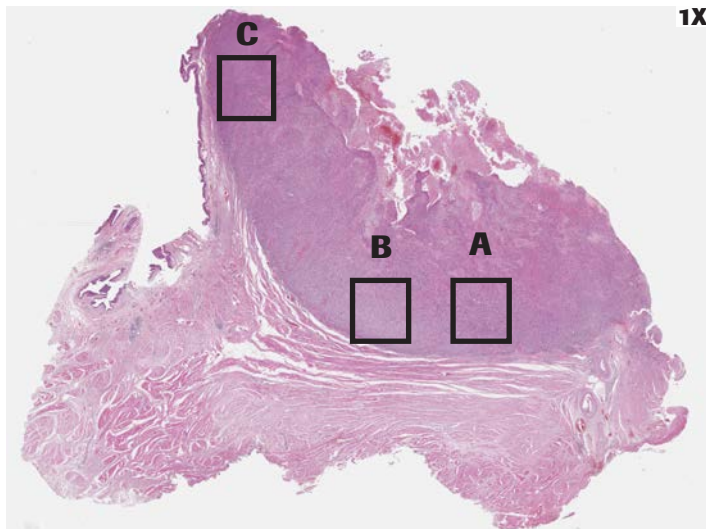




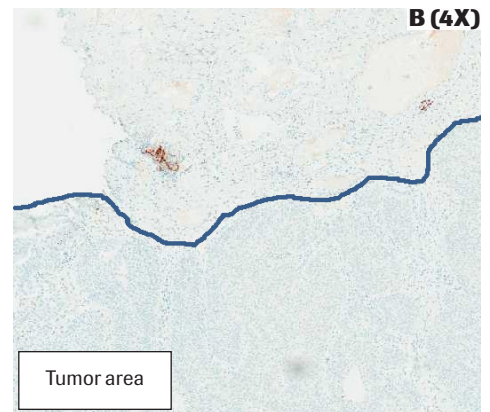
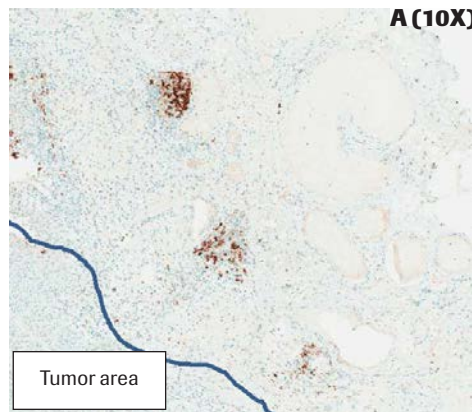
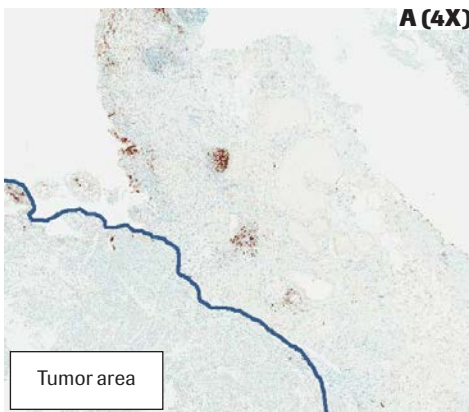
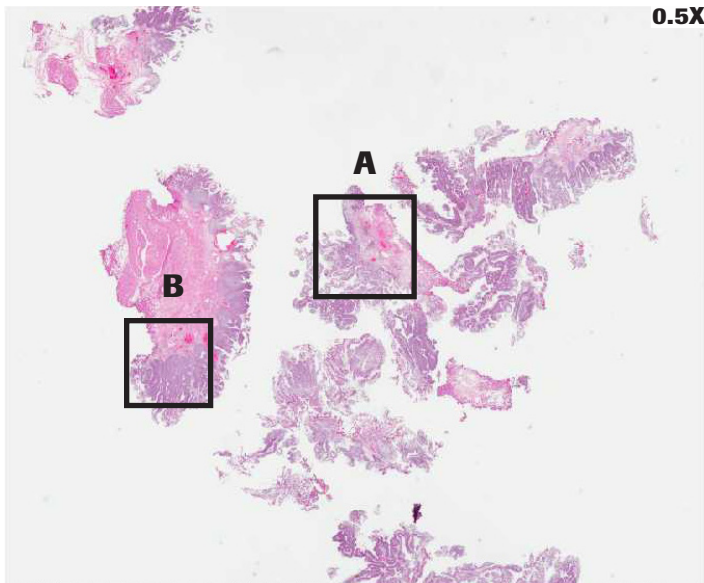
**Case 6:** This case is IC  $\geq$  5%. Scores: IC: 20%; TC 0%. Note the presence of IC aggregates along with foci of single-cell spread.



## 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.



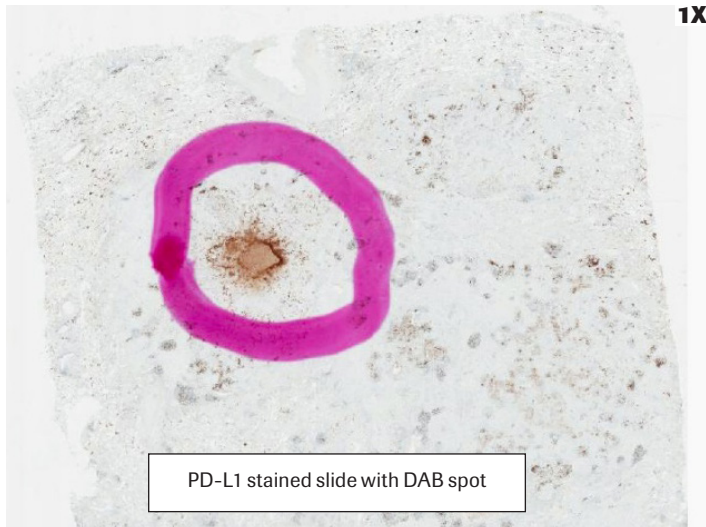
**Case 8:** This case is IC < 5%. Scores: IC: 2%; TC: 0%. Note the presence of papillary urothelial carcinoma in a TURBT specimen. Highlighted in **Regions A & B** are lymphoid aggregates in lamina propria that are not contiguous with the tumor and can be mistakenly included in scoring IC. The line defines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



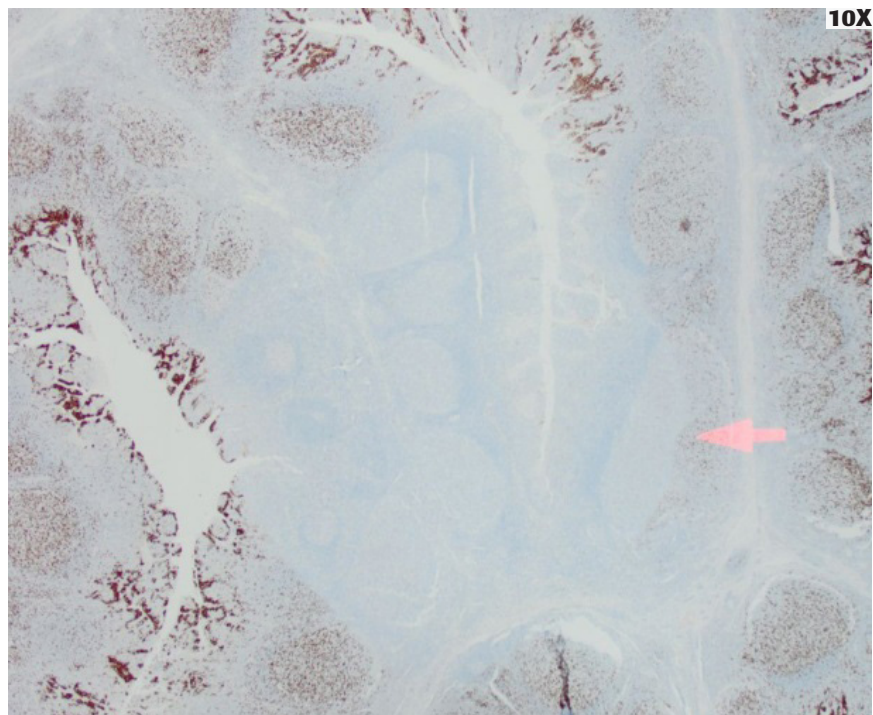
## Staining Artifacts with VENTANA PD-L1 (SP142) Assay

Artifacts noted in this section can be observed on NRC 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

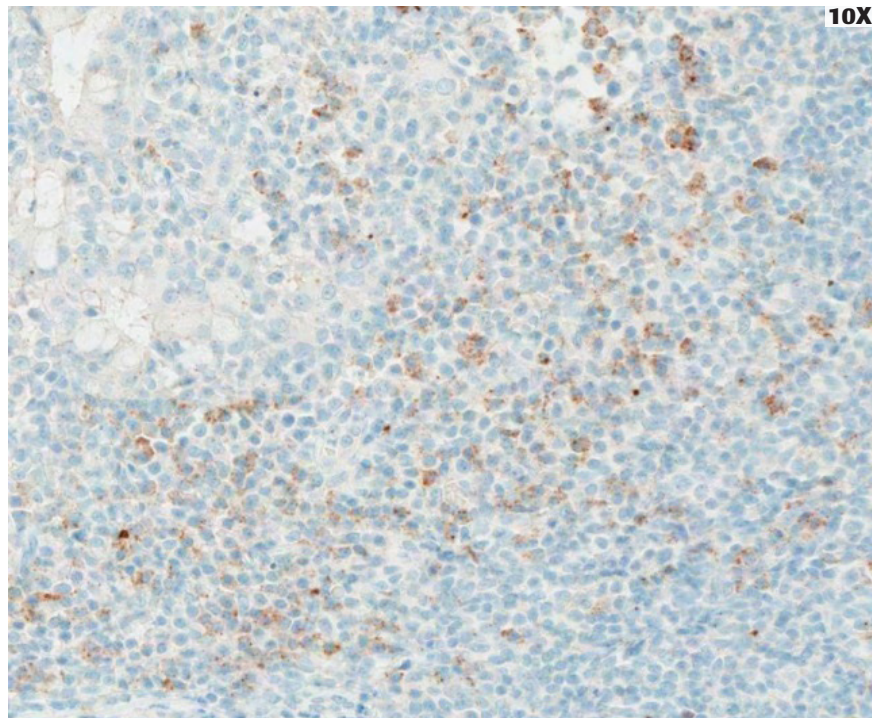
review the corresponding NRC slide to ensure that non-specific background staining is within acceptable limits.



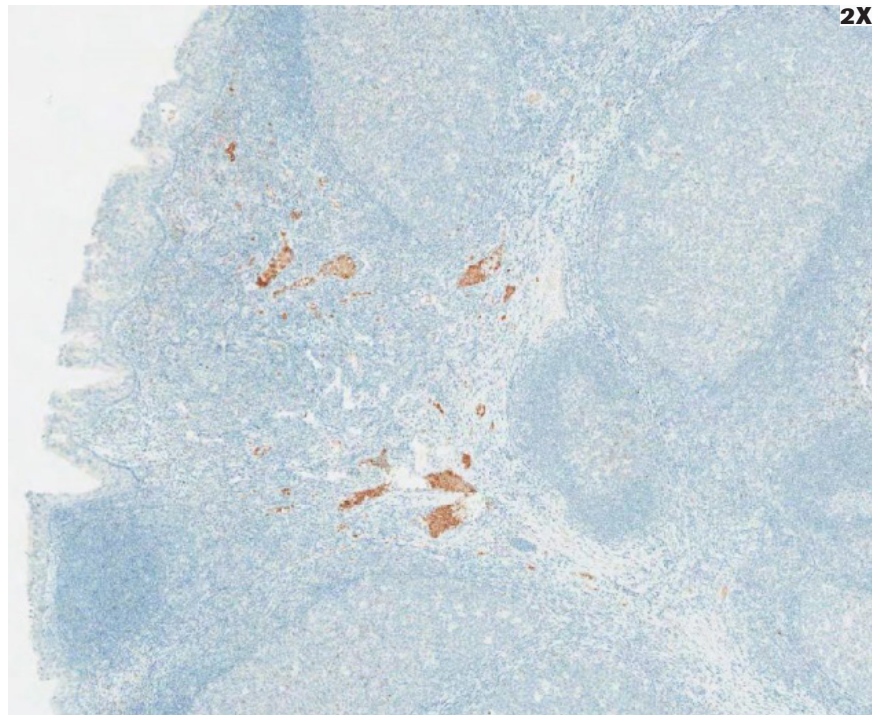
**DAB Spots:** DAB spots are circular spots due to a static bubble formed during the staining procedure. If this artifact interferes with the interpretation of the PD-L1 stained slide, repeat the stain with a fresh unstained slide. In the images shown, the DAB spot was not present with repeat staining of a serial section (NSCLC tissue shown).



**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. If the blank spot interferes with interpretation of the PD-L1 stained slide, a repeat run may be required.

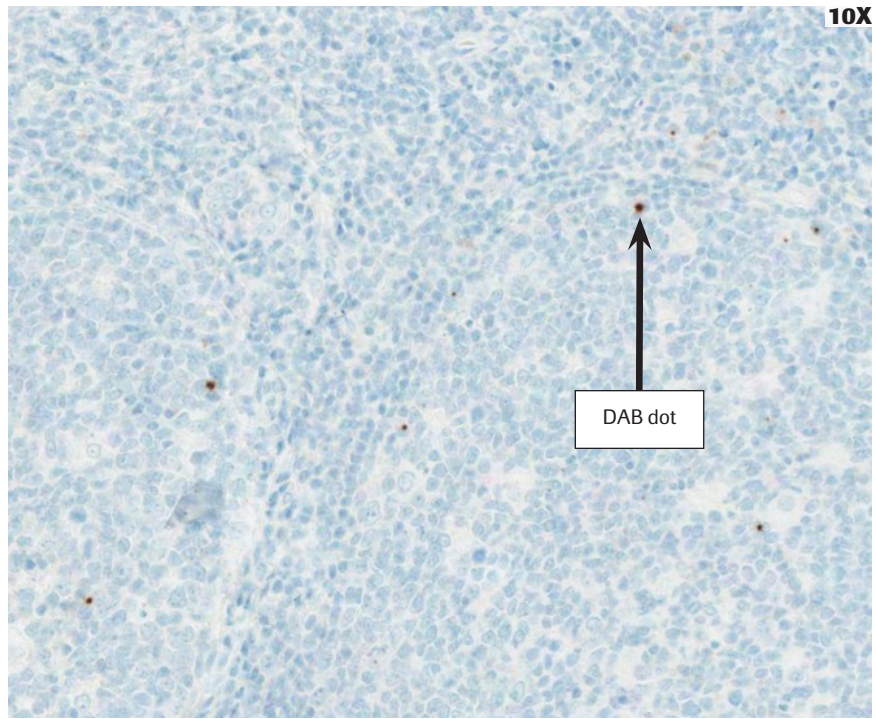


**Speckling:** Speckling is non-specific staining that appears as a uniformly distributed fine granular precipitate most often in the cytoplasm.

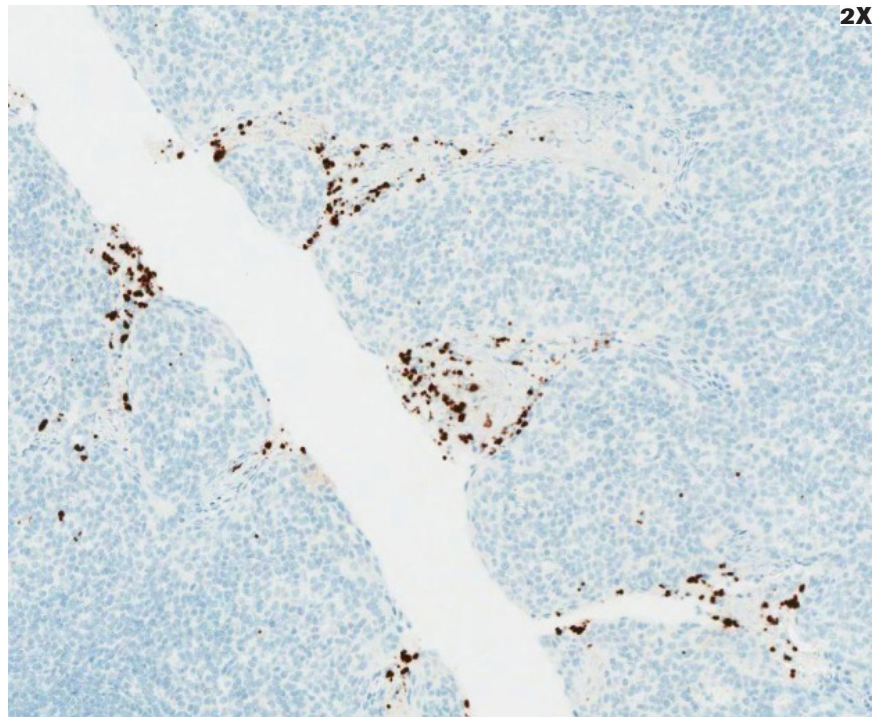


**Serum Background:** Serum background is non-specific staining in vascular spaces and serum extravasates.





**DAB Dots:** Punctate dots may be observed on tonsil or other lymphoid organs and are non-specific background from the detection system.



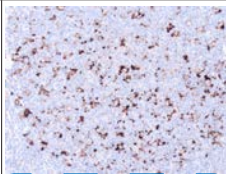
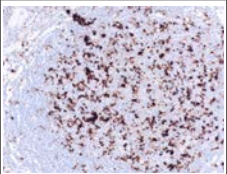
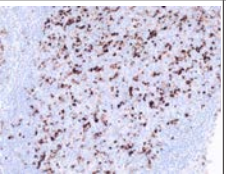
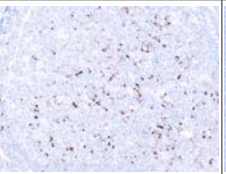
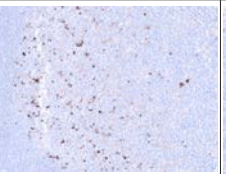
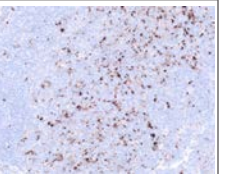
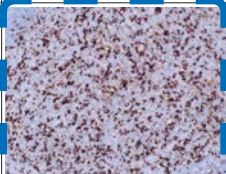
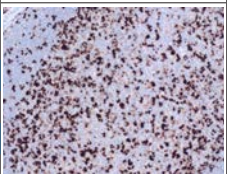
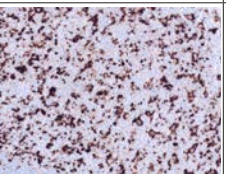
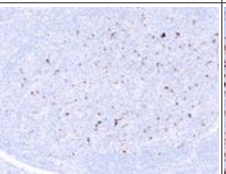
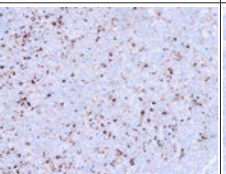
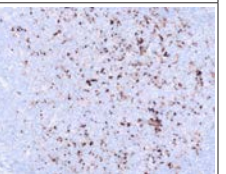
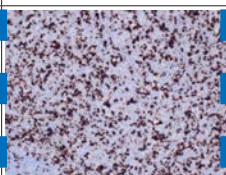
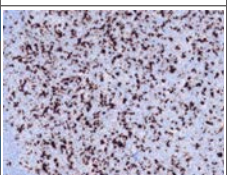
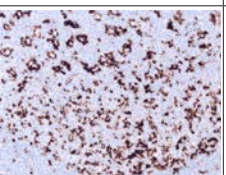

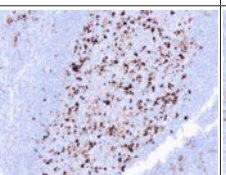
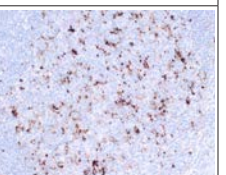
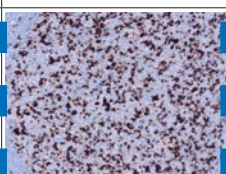
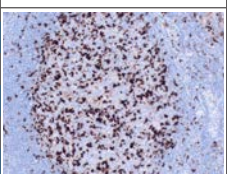
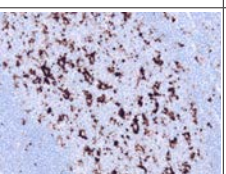
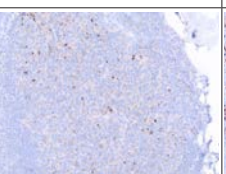
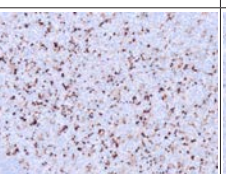
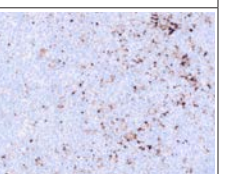
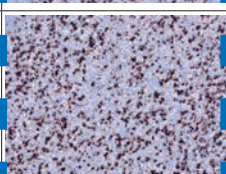
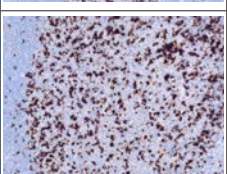
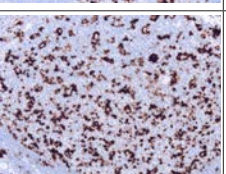
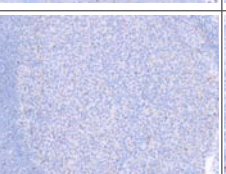
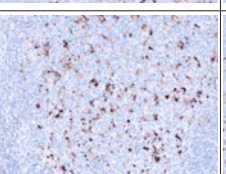
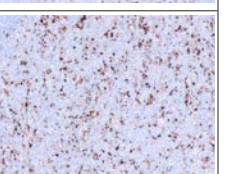
**Luminal Debris:** Luminal staining in tonsil stained with NRC is likely due to a cross reactivity with an unknown antigen and is of uncertain significance. Tonsil tissue exhibiting this staining should be excluded at the time of qualification.



# 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

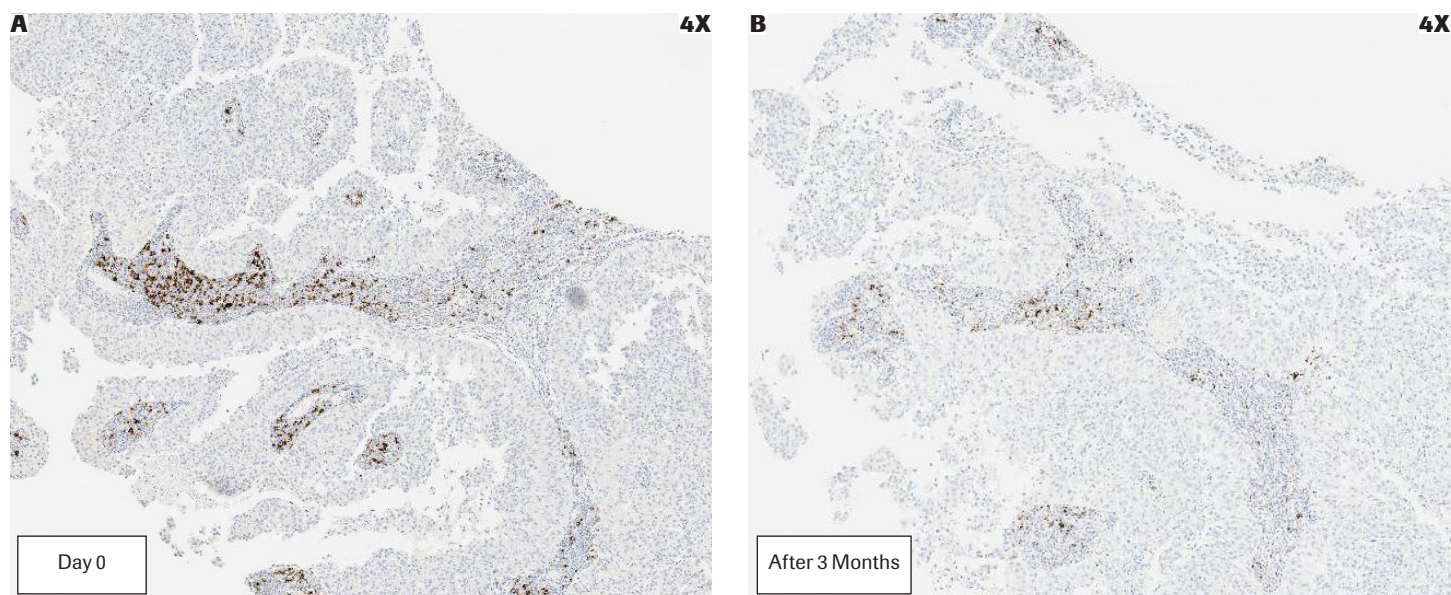
\*Not recommended

(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**).

## References

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13. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014;515(7528):558-562.





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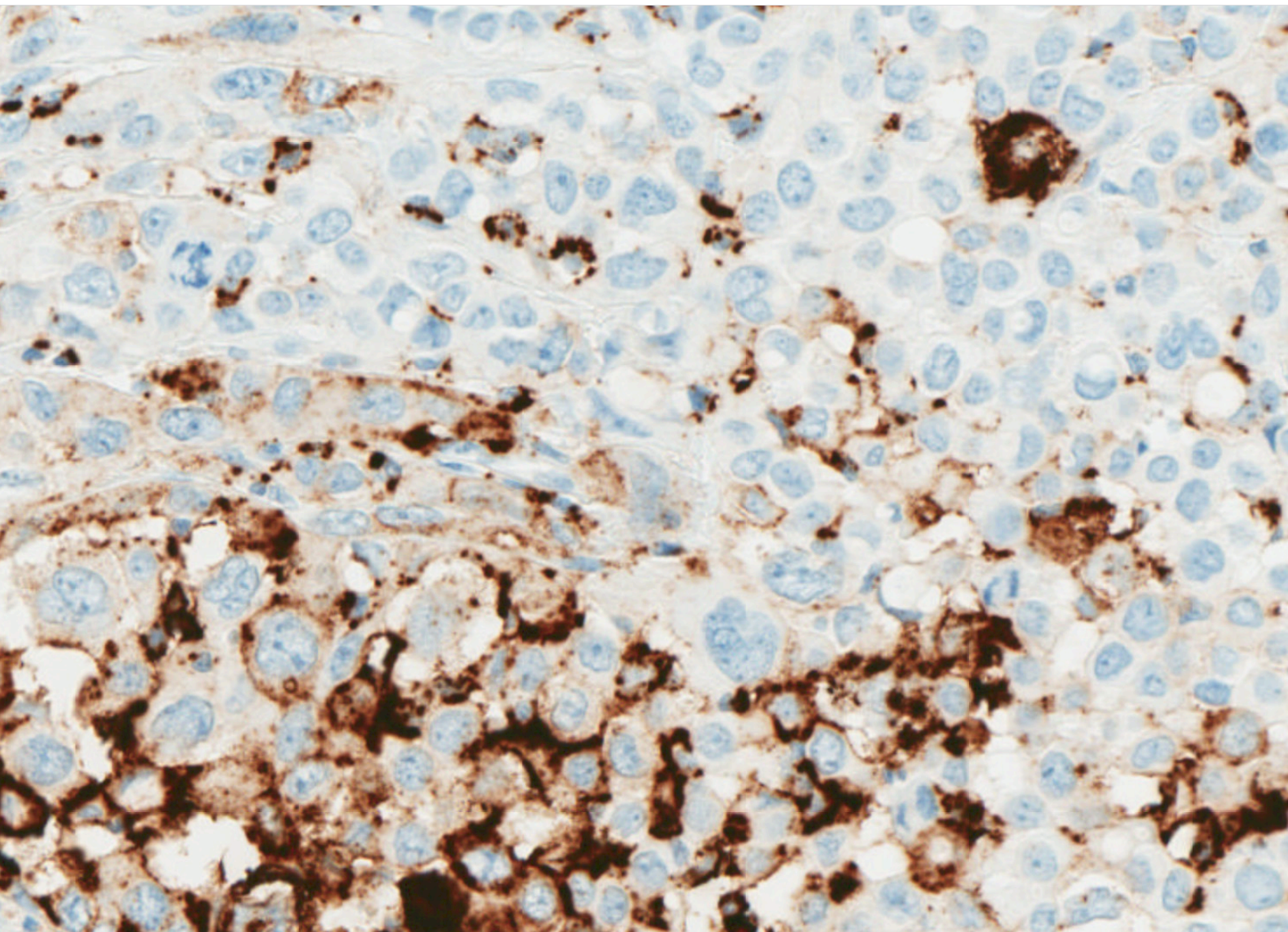
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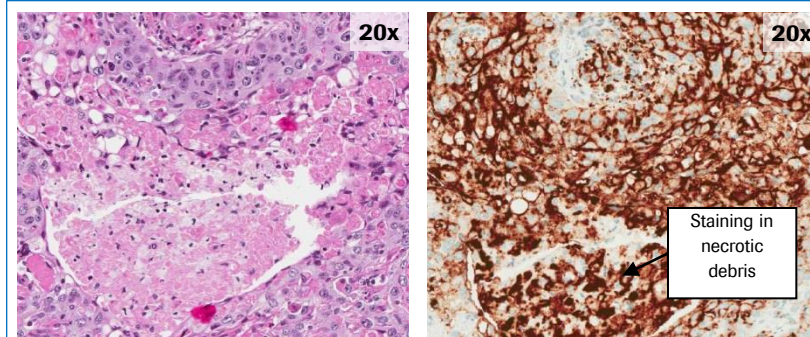
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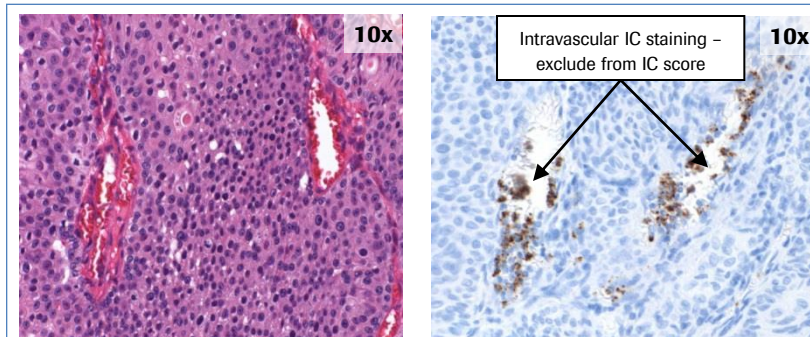




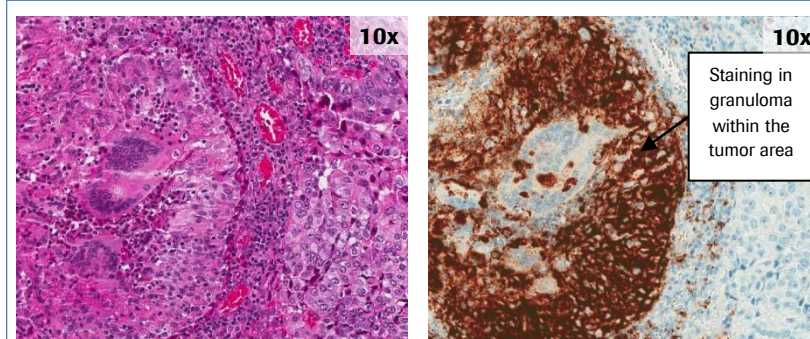
## Scoring Methods: Challenges and Pitfalls



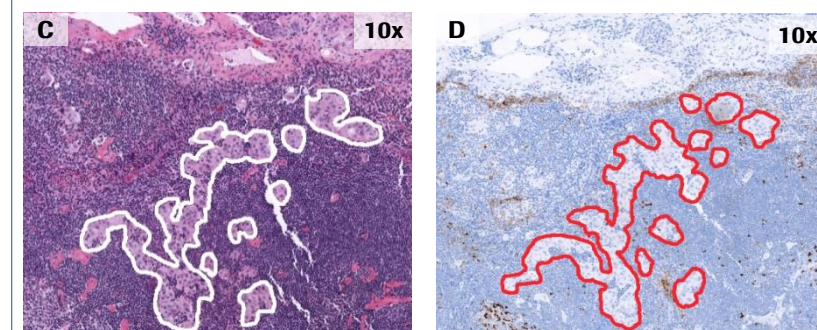
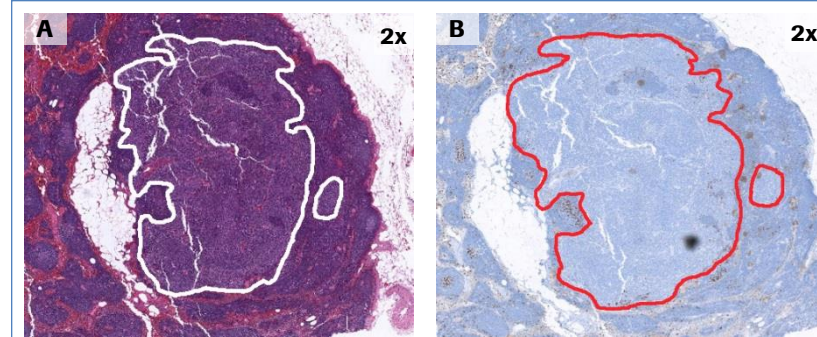
Necrotic debris showing PD-L1 staining (H&E on the left and the PD-L1 stained on the right.) This should not be included in IC scoring. Note the presence of TC staining, which is not scored.



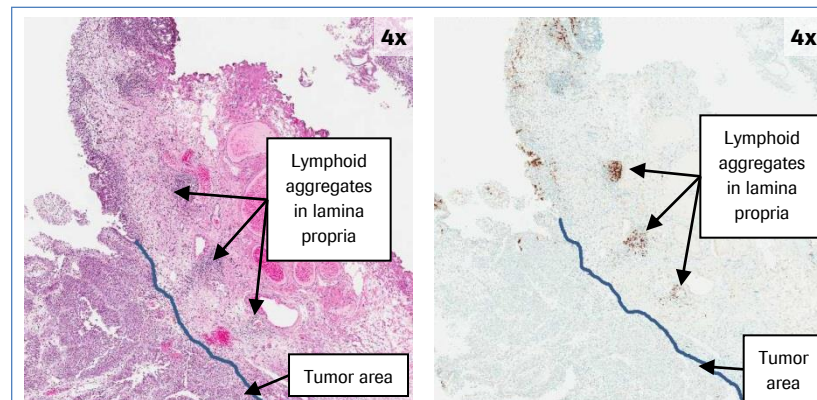
Intravascular immune cell staining – exclude from IC scoring



Staining in granuloma – consider towards IC if present in tumor area

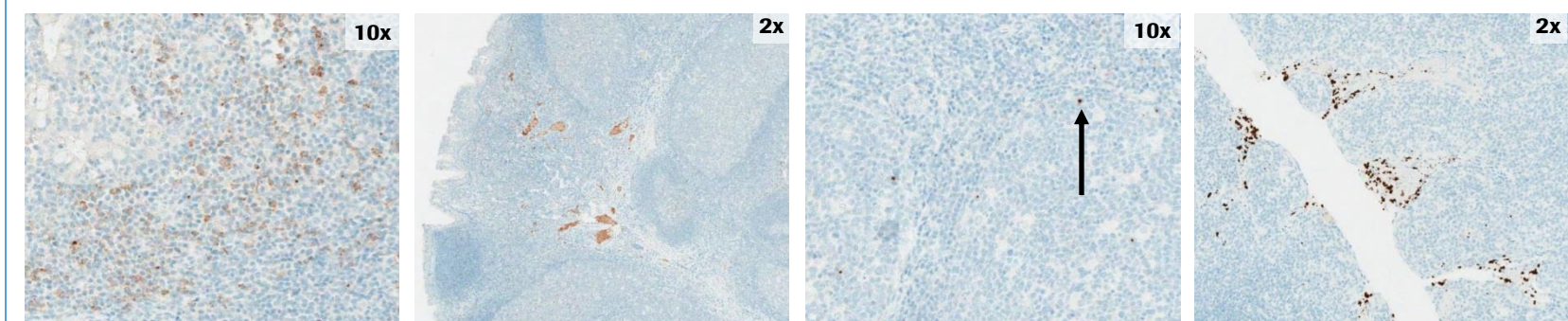


**A & B:** Lymph node with well circumscribed metastasis. **C & D:** Lymph node with multiple small tumor cell nests (H&E on the left and PD-L1 stained slide on the right.) Tumor area is outlined both on H&E and PD-L1 images.



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

## Staining Artifacts



Speckling

Serum background

DAB dots

Luminal debris (tonsil)



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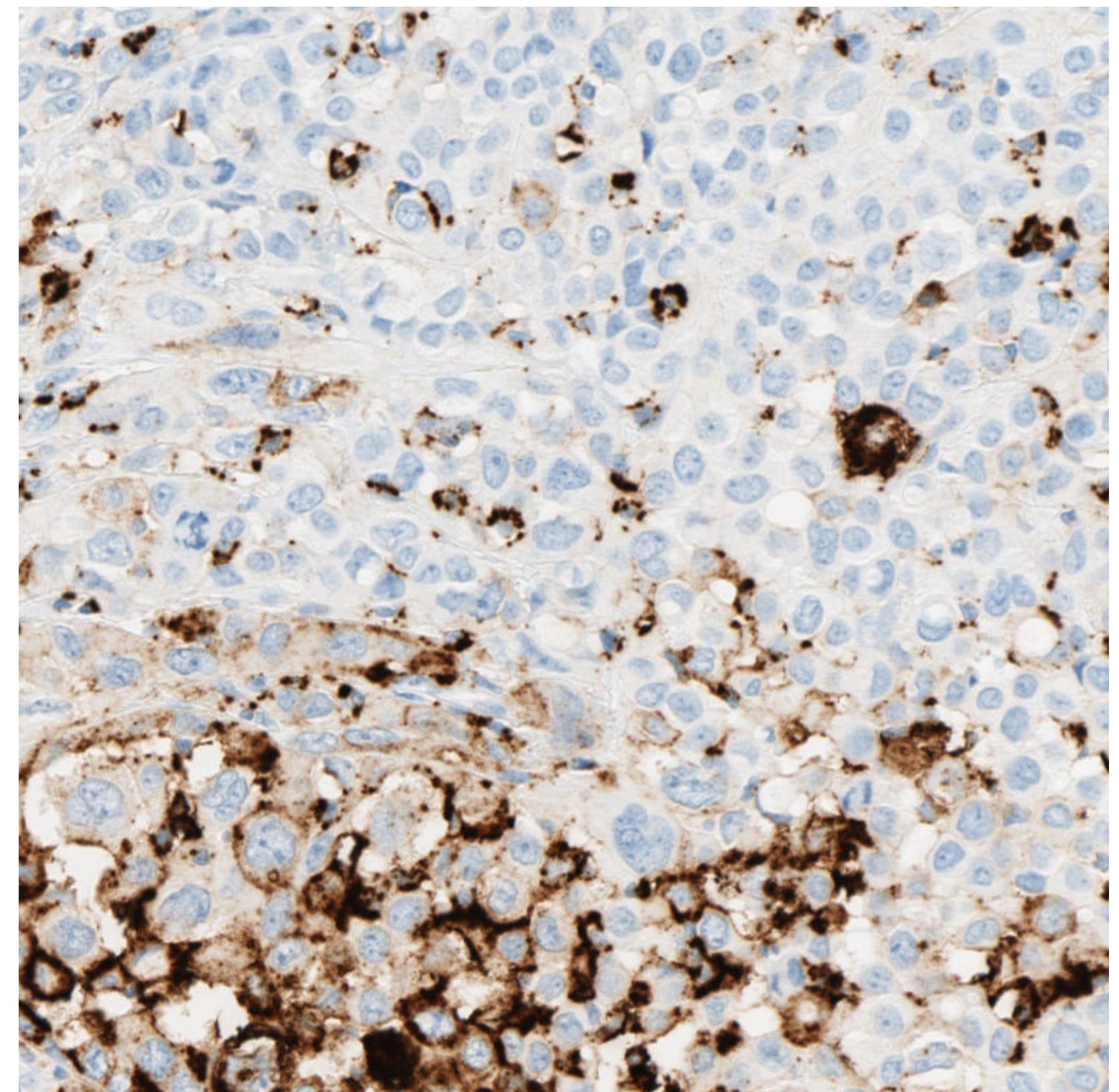
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# VENTANA PD-L1 (SP142) Assay Quick Reference Guide for Urothelial Carcinoma

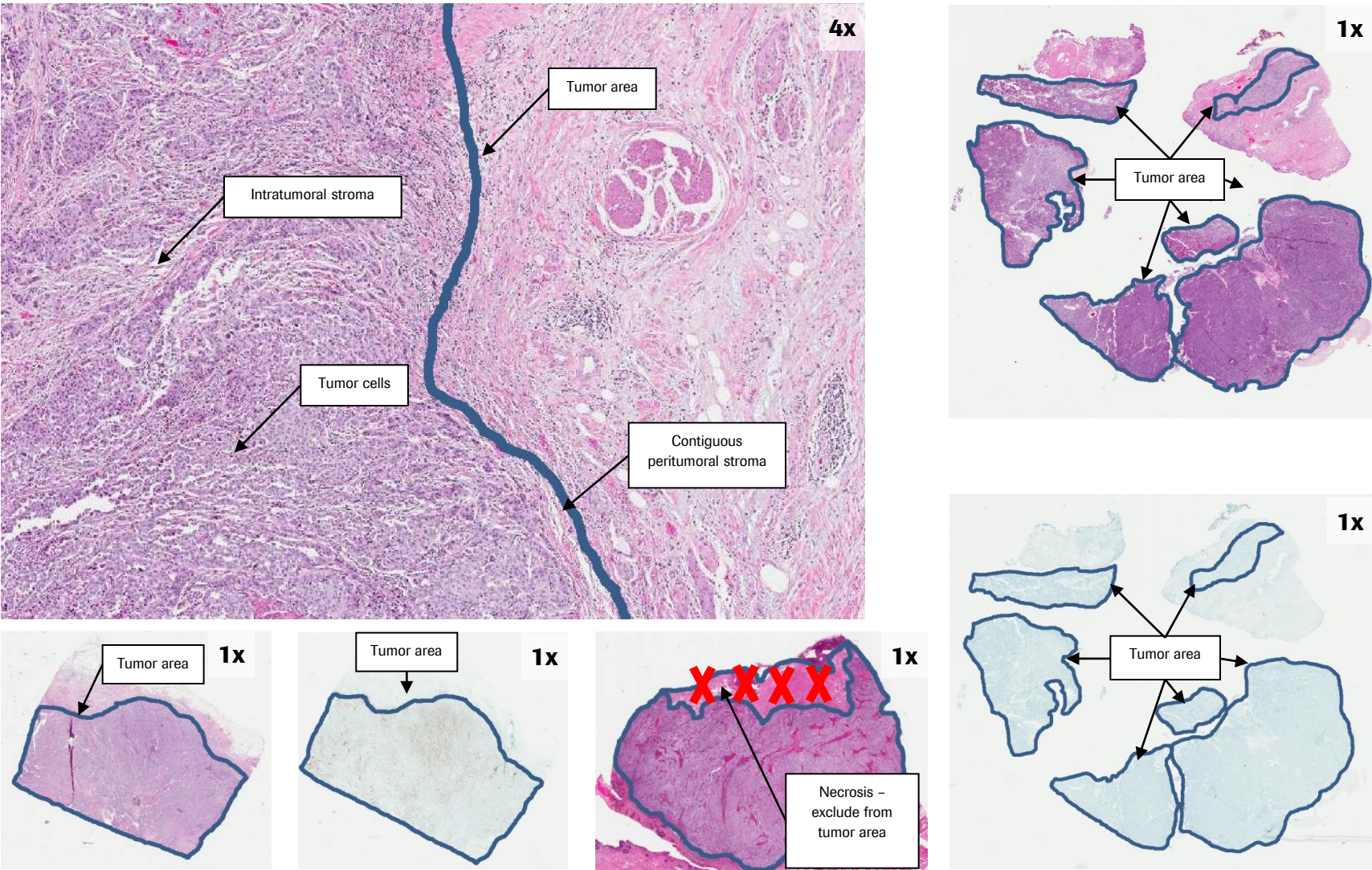




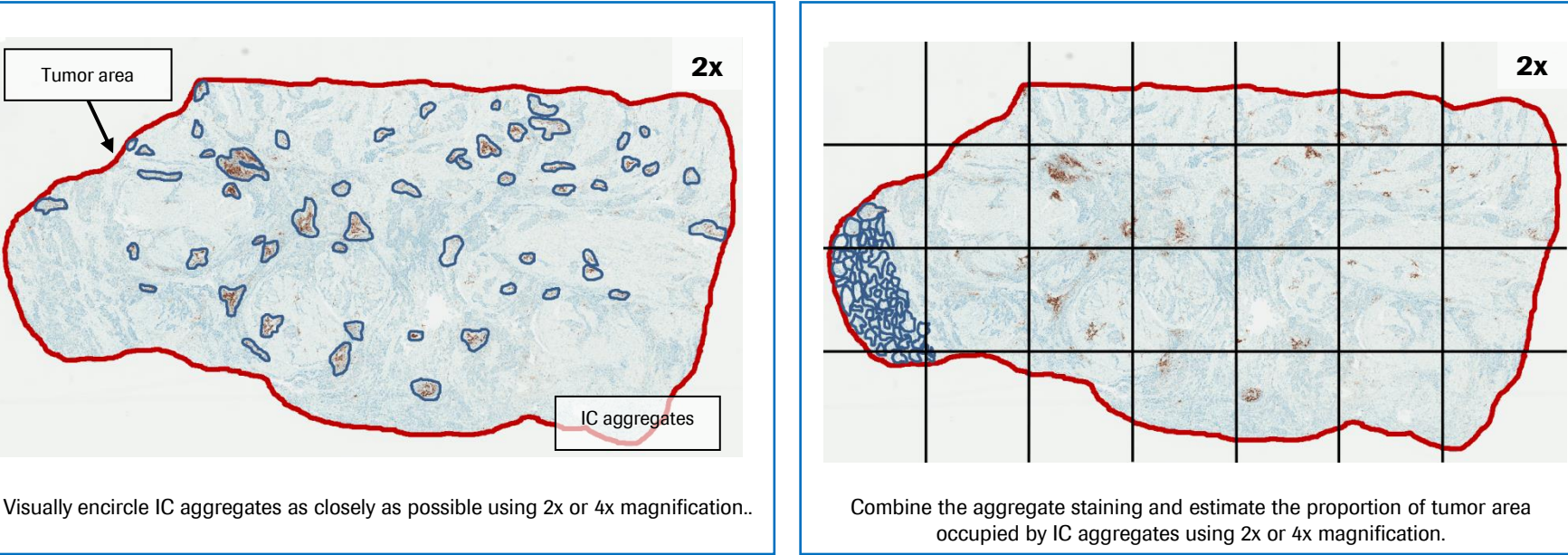
VENTANA PD-L1 (SP142) Assay Scoring Algorithm

Tumor-Infiltrating Immune Cell (IC) Staining Assessment	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%
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%

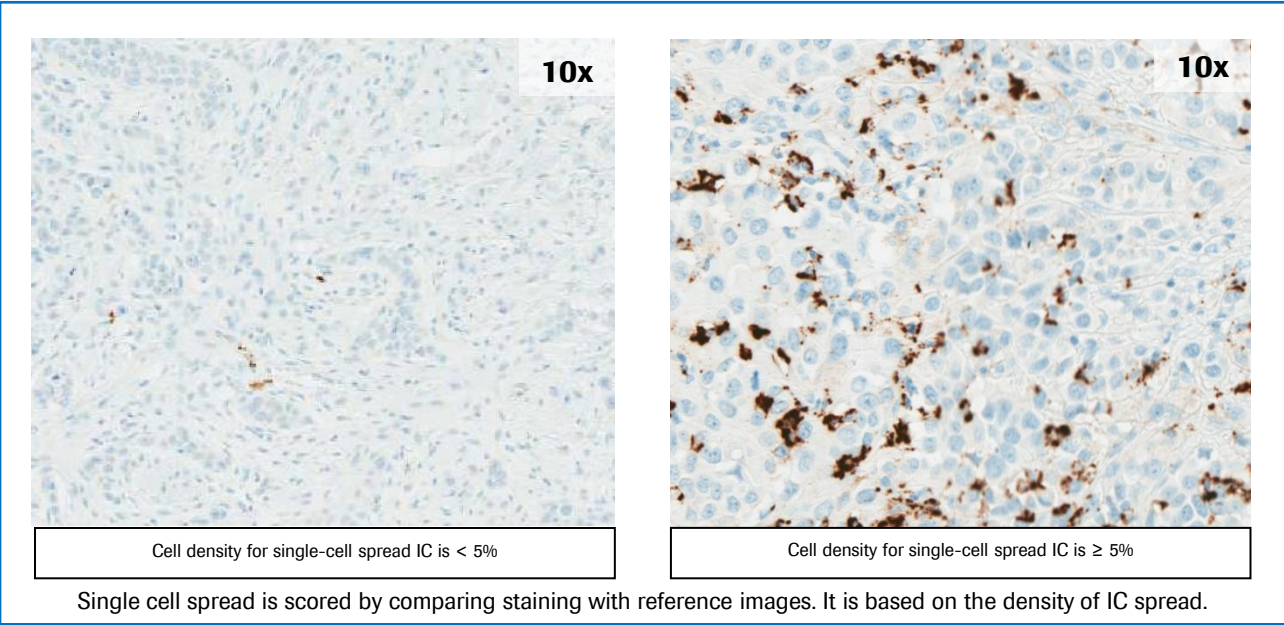
Tumor Area



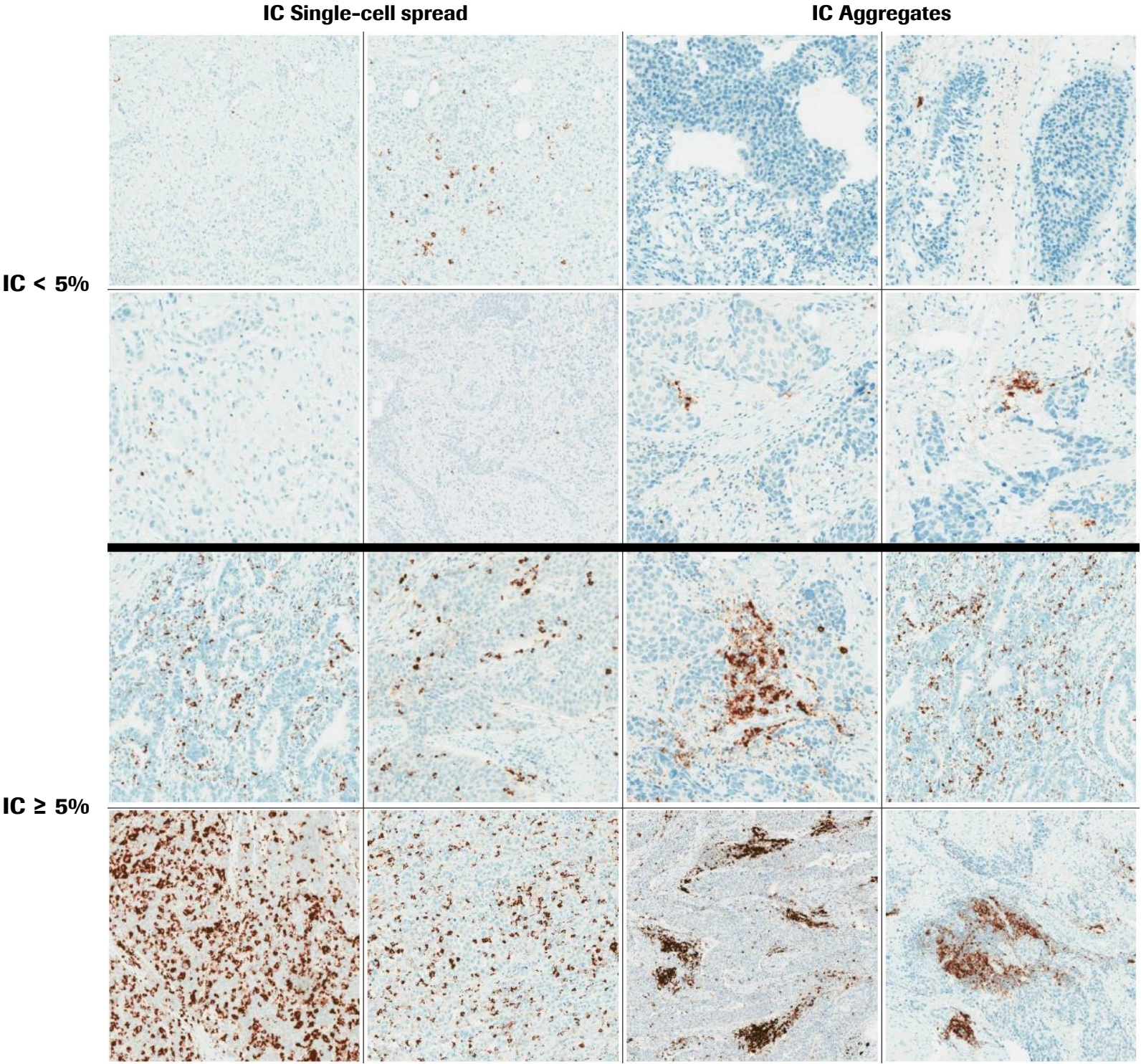
IC Scoring Method: Aggregates



IC Scoring Method: Single-Cell Spread



IC Scoring Method: Reference Images



(all images 10X magnification)





VENTANA®

### VENTANA PD-L1 (SP142) Assay

5 mL (~7 µg/mL)  
Rx Only

**REF** 740-4859

LOT A12345



2015-12-28



50



IVD

**GTIN** 04015630967155

(240)07709374001 -Roche #



740-4859A12345 0001



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Tucson, Arizona 85755 USA

 VENTANA®**VENTANA PD-L1  
(SP142) Assay**

5 mL (~7 µg/mL)  
Rx Only



REF 740-4859

LOT A12345



IVD



2015-12-28

GTIN

04015630967155



7 50

2°C 8°C

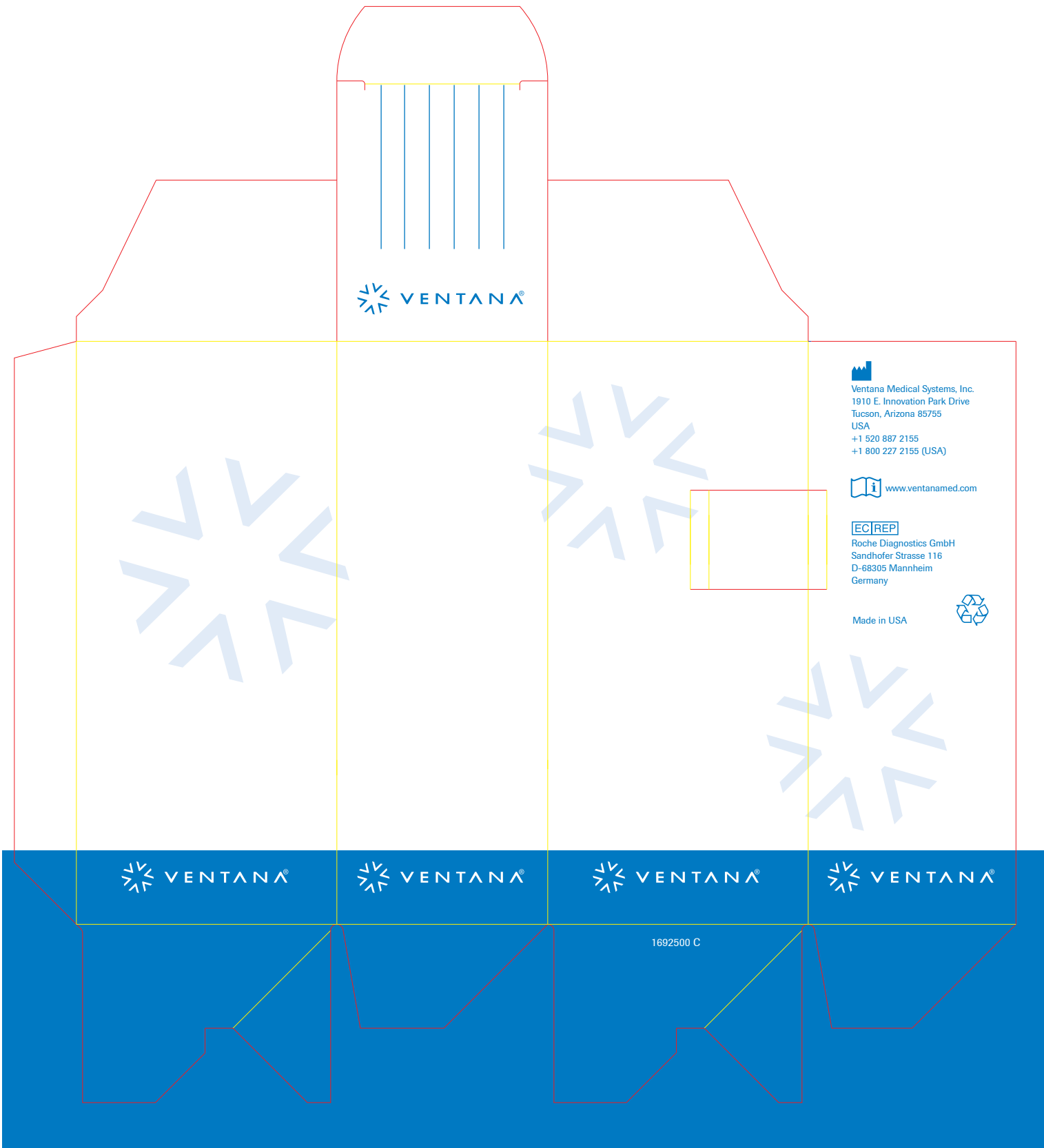
2°




NOTES:

1. COLOR SWATCHES

a.PMS 300



C	UPDATE REV FOR SPEC CHANGE ON VARNISH	SEE AGILE	GV	C007663
B	UPDATE ARTWORK WITH BLUE BORDER	SEE AGILE	PM	C003389
A	RELEASED DRAWING	SEE AGILE	GA	10682
REV	DESCRIPTION	Effective Date	RP	CO
<div><div> VENTANA®</div><div>Ventana Medical Systems Inc. Tucson, AZ</div></div>				
TITLE				
ARTWORK, SINGLE DISPENSER, BOX PN 1692500				
SCALE DO NOT SCALE	SHEET 1 OF 1	SIZE B	DWG NUMBER 000014900	REV C