Tissue Biomarker Laboratory
Center for Immuno-Oncology
Department of Pathology
Dana-Farber Cancer Center

Director
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Immunohistochemistry (IHC) Analytical Validation

1. ***Analyte(s):*** **AE1/AE3**, **SOX10, CD3, CD8, CD4, CD31, Foxp3, CD68, CD163, PD-1, PD-L1**

2. ***Technical platform(s):***

The technical platform used for this assay is Bond RX by Leica Biosystems, an automated system for staining multiplex immunofluorescence paraffin sections. This automated machine allows for small volumes of reagents, which are applied in a uniform manner on each slide. We run batches of 15 slides per run/time-point.

3. ***Reagents, controls, and calibrators:***

Reagents:

- Bond Dewax Solution (AR9222)
- Secondary antibody conjugated to a polymer
- Post Primary (anti-mouse IgG)
- Polymer (anti-rabbit IgG)
- Bond Polymer Refine Detection Kit (DAB) (DS9800) (enhance staining quality).
- Horseradish Peroxidase (HRP)
- Epitope retrieval solution (ER1 Low pH (AR9961) or ER2 High pH (AR9640)
- DAB (diaminobenzidine) and Hematoxylin reagents
- 100% alcohol
- Wash solution (AR9590)
- Primary Antibodies (See Table below)

Marker	VENDOR (CAT#)	Clonality	CLONE	Epitope Retrieval	Dilution	Antibody Incubation Time
CD3	Dako (A0452)	Polyclonal Rabbit	N/A	ER2 for 30 min	1:250	30 min
CD4	Dako (M731029)	Monoclonal Mouse	4B12	ER2 for 30 min	1:80	30 min
CD8	Dako (M710301)	Monoclonal Mouse	C8/144B	ER2 for 30 min	1:200	30 min
CD68	Dako (M0876)	Monoclonal Mouse	PGM1	ER2 for 30 min	1:200	30 min
CD163	Leica (NCL-L-CD163)	Monoclonal Mouse	10D6	ER2 for 30 min	1:500	30 min
CD31	Abcam (ab28364)	Polyclonal Rabbit	N/A	ER2 for 30 min	1:300	30 min
Foxp3	Biolegend (320102)	Monoclonal Mouse	206D	ER2 for 30 min	1:50	30 min
PD-1	Cell Signaling (43248S)	Monoclonal Mouse	EH33	ER2 for 30 min	1:1,000	30 min
PD-L1	Cell Signaling (29122S)	Monoclonal Mouse	405.9A11	ER2 for 30 min	1:300	2 x 30 min
SOX10	Cell Marque (383R-16)	Monoclonal Rabbit	EP268	ER2 for 30 min	1:2,000	30 min
Cytokeratin	Dako (M351529-2)	Monoclonal Mouse	AE1/AE3	ER2 for 30 min	1:500ff	30 min

4. Quality control parameters for specimens:

In our laboratory, we perform quality control of specimens based on Hematoxylin and eosin (H&E) stains. Specifically, for each FFPE surgical resections and core needle biopsies, we confirm the following:

- Viable Tissue (% nucleated cells)
- Viable Tumor cells (% nucleated cells)
- Lymphocyte Influx (0- to 3+)
- Necrosis (% area)
- Fibrosis (% nucleated cells)

All H&E-stained sections from the formalin-fixed paraffin-embedded (FFPE) tissue diagnostic slides are scanned in the Aperio™ digital pathology scanner system. The pathologist evaluates these and depending

on viable tumor availability, he/she chooses a representative tissue block specimen to perform the different assays (in this case, panels 1 and 2).

Based on the criteria above, samples that do not pass quality control are excluded from IHC staining and analysis.

5. Any critical pre-analytic variables:

The pre-analytical variables that we carefully assess in our laboratory are the following:

a) Tissue Fixation: our laboratory has established SOPs for tissue fixation, which allows for eliminating pre-analytical variables that are related to tissue processing and analysis. These SOPs work well across different tissue types (melanoma, colorectal, head and neck, lung, Hodgkin lymphoma, tonsil, breast, glioblastoma, pancreas).

b) Run-to-run variation: In every run, positive and negative control slides from appropriate tissue types are included. The same tissue control blocks/slides are used across many runs. Staining of controls per antibody per run are assessed by the pathologists for staining patern, intensity and specificity. If the control tissues slides do not pass quality control, then the entire run is considered a "failed run" and the staining is repeated.

c) If two subsequent runs fail, the BOND RX recalibration is the first step that we investigate. Secondly, we also examine reagents used for the runs, such as product lot numbers and preparation of reagents.

6. Analytical Performance characteristics:

Antibody (Clone)	Parameter	Accuraffcy	Precision	Analytical sensitivity	Analytical specificity (including) interfering substances	Reportable range of assay results for the assay system	Establishment of appropriate quality control & improvement procedures
PD-L1 (405.9A11)	Programmed death ligand 1 protein expression by IHC in malignant cells membrane in head and neck squamous cells carcinomas (n = 10) and tonsil (n=3)	10/10 (100%) is the ratio of cases positive for >5% membrane staining in 10 HNSCCs	Inter-pathologist scoring concordanc e: 10/10 on >5% expression	a) Human tonsil controls known to have immune cells that are PD-L1 positive by IHC: Positive in 3/3 cases and 3 attempts; b)Human HNSCCs controls known to have malignant cells that are PD-L1 positive by	Human tonsil controls known to have immune cells that are PD-L1 positive by IHC: Positive in 3/3 cases and 3 attempts and human HNSCCs that are positive by IHC: Positive in 10/10 cases and 3 attempts	Pathologist scoring: range 0-100% immune cells and 0-100% squamous malignance cells in HNSCCs	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in PD-L1 expression

				IHC: Positive in 10/10 cases and 3 attempts;			positivity 3/3/ attempts for 10 samples in each attempt
CD3 (A045229-2)	CD3 positive T lymphocytes; We report: number of cells per 3-5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive lymphocytes type of cells in tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordance between pathologists was 0.95 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed and paraffin-embedded tonsil: Positive in 3/3 attempts, each attempt has 10 cases	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD3 as a control (ie: fat cells, fibroblast cells,)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD3 expression positivity 3/3/ attempts for 10 samples in each attempt
CD8 (144B)	CD8 positive T lymphocytes cytotoxic; number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive lymphocytes type of cells in tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordance between pathologists was 0.94 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed and paraffin-embedded tonsil: Positive in 3/3 attempts, each attempt has 10 cases	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD8 as a control (ie: fat cells, fibroblast cells, cells in the follicle B-cell rich areas)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD8 expression positivity 3/3/ attempts for 10 samples in each attempt

CD4 (4B12)	CD4 positive T lymphocytes helper; number of cells per 5 random square (1-$mm^{2)}$ using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive lymphocytes type of cells in tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordance between pathologists was 0.93 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed and paraffin-embedded tonsil: Positive in 3/3 attempts, each attempt has 10 cases	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD4 as a control (ie: fat cells, fibroblast cells)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD4 expression positivity 3/3/ attempts for 10 samples in each attempt
PD-1 (EH33)	Programmed death-1 (PD-1) positive inflammatory cells (majority of the cells are lymphocytes); number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive lymphocytes type of cells in tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordace between pathologists was 0.91 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed and paraffin-embedded tonsil: Positive in 3/3 attempts, each attempt has 10 cases	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for PD-1 as a control (ie: fat cells, fibroblast cells, epithelial cells)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in PD-1 expression positivity 3/3/ attempts for 10 samples in

PD-1 (EH33)	Programmed death-1 (PD-1) positive inflammatory cells (majority of the cells are lymphocytes); number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive lymphocytes type of cells in tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordace between pathologists was 0.91 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed and paraffin-embedded tonsil: Positive in 3/3 attempts, each attempt has 10 cases	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for PD-1 as a control (ie: fat cells, fibroblast cells, epithelial cells)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in PD-1 expression positivity 3/3/ attempts for 10 samples in each attempt
CD31 (ab28364)	CD31 positive endothelial cells; number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive endothelial type of cells on tonsil tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordace between pathologists was 0.89 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed an paraffin-embedded tonsil: Positive in 10/10 attempts	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD31 as a control (ie: fat cells, fibroblast cells, epithelial cells, cells in the follicle B-cell rich areas)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD31 expression positivity 3/3/ attempts for 10 samples in each attempt
Foxp3 (206D)	FOX-P3 positive T lymphocytes	10/10 (100%) is the ratio of	Inter-pathologist independen	Human formalin-fixed an	Human formalin-fixed and paraffin-	By Aperio image analysis,	1.Lot variation analysis of antibodies and

CD163 (10D6)	CD163 positive macrophages ; number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive macrophage s type cells in tonsil tissues	Inter-pathologist independen t scoring using Aperio image analysis algorithm. Concordac e between pathologists was 0.89 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed an paraffin-embedded tonsil: Positive in 10/10 attempts	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD163 as a control (ie: B-cell rich mantle zone)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD163 expression positivity 3/3/ attempts for 10 samples in each attempt
CD68 (PG-M1)	CD68 positive macrophages ; number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	10/10 (100%) is the ratio of cases expressing positive macrophage s type cells in tonsil tissues	Inter-pathologist independen t scoring using Aperio image analysis algorithm. Concordac e between pathologists was 0.91 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed an paraffin-embedded tonsil: Positive in 10/10 attempts	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for CD68 as a control (ie: T-cells, endothelial cells, B-cell rich mantle zone)	By Aperio image analysis, range observed in tonsils tissues is 0-5,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in CD68 expression positivity 3/3/ attempts for 10 samples in each attempt

SOX10 (EP268)	Sox10 positive melanoma tumor cells; number of cells per 5 random square (1-mm^2) using Aperio (Leica) nuclear algorithm	3/3 (100%) is the ratio of cases expressing positive melanoma cells in melanoma tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordace between pathologists was 0.97 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed an paraffin-embedded melanoma: Positive in 3/3 attempts	Human formalin-fixed and paraffin-embedded melanoma. We use intraspecimen cell populations that are negative for SOX10 as a control (ie: T-cells, endothelial cells, stromal regions)	By Aperio image analysis, range observed in meanoma tissues is 0-10,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in SOX10 expression positivity 3/3/ attempts for 3 samples in each attempt
Cytokeratin AE1/AE3	AE1/AE3 positive colorectaltum or cells; number of cells per 5 random square (1-mm^2) using Aperio (Leica) cytoplasmic algorithm	3/3 (100%) is the ratio of cases expressing positive epithelial carcinoma cells in colorectal cancer tissues	Inter-pathologist independent scoring using Aperio image analysis algorithm. Concordace between pathologists was 0.98 in 10/10 cases for cells averaged three- to-five 1 mm^2 squares	Human formalin-fixed an paraffin-embedded colorectal cancer: Positive in 3/3 attempts	Human formalin-fixed and paraffin-embedded tonsil. We use intraspecimen cell populations that are negative for Cytokeratin as a control (ie: B-cell rich and T-cell rich areas such as germinal centers and mantle zones)	By Aperio image analysis, range observed in colorectal cancer tissues is 0-10,000 cells	1.Lot variation analysis of antibodies and kits; 2. calibration of instruments; 3.Three independent run of tissue controls in 3 different days 4. Inter-technologist (100% concordance); 5.external validation performed with another lab (PI: Dr. Loda/DFCI); Concordance in AE1/AE3 expression positivity 3/3/ attempts for 3 samples in each attempt

Analytical Data

ANALYTE	PD-L1 (Clone 405.9A11)		
Date	Testing Tissue Cases	Results	Comment
01/08/2015	Head and Neck SCC 1	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 2	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 3	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 4	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 5	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 6	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 7	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 8	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 9	+ Tumor cells and + immune cells	
01/08/2015	Head and Neck SCC 10	+ Tumor cells and + immune cells	
01/08/2015	Tonsil #1	+ Crypt epithelium + Germinal Center	
01/08/2015	Tonsil #2	+ Crypt epithelium + Germinal Center	
01/08/2015	Tonsil #3	+ Crypt epithelium + Germinal Center	
ANALYTE	**CD3**		
03/05/2015	Tonsil #1	+ T- Cells pattern	
03/05/2015	Tonsil #2	+ T- Cells pattern	
03/05/2015	Tonsil #3	+ T- Cells pattern	
03/05/2015	Tonsil #4	+ T- Cells pattern	
03/05/2015	Tonsil #5	+ T- Cells pattern	
03/05/2015	Tonsil #6	+ T- Cells pattern	
03/05/2015	Tonsil #7	+ T- Cells pattern	
03/05/2015	Tonsil #8	+ T- Cells pattern	
03/05/2015	Tonsil #9	+ T- Cells pattern	
03/05/2015	Tonsil #10	+ T- Cells pattern	
ANALYTE	**CD8 (144B)**		
03/05/2015	Tonsil #1	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #2	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #3	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #4	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #5	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #6	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #7	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #8	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #9	+ T- Cells pattern around the germinal centers	
03/05/2015	Tonsil #10	+ T- Cells pattern around the germinal centers	
ANALYTE	**CD4 (4B12)**		
03/10/2015	Tonsil #1	+ T- Cells pattern	
03/10/2015	Tonsil #2	+ T- Cells pattern	
03/10/2015	Tonsil #3	+ T- Cells pattern	
03/10/2015	Tonsil #4	+ T- Cells pattern	
03/10/2015	Tonsil #5	+ T- Cells pattern	
03/10/2015	Tonsil #6	+ T- Cells pattern	
03/10/2015	Tonsil #7	+ T- Cells pattern	
03/10/2015	Tonsil #8	+ T- Cells pattern	
03/10/2015	Tonsil #9	+ T- Cells pattern	
03/10/2015	Tonsil #10	+ T- Cells pattern	

ANALYTE	**CD31**		
04/08/2015	Tonsil #1	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #2	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #3	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #4	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #5	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #6	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #7	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #8	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #9	+ Endothelial cell/ vessel pattern	
04/08/2015	Tonsil #10	+ Endothelial cell/ vessel pattern	
ANALYTE	**PD-1 (EH33)**		
04/15/2015	Tonsil #1	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #2	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #3	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #4	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #5	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #6	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #7	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #8	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #9	+ T-cell pattern inside the germinal center	
04/15/2015	Tonsil #10	+ T-cell pattern inside the germinal center	
ANALYTE	**Foxp3 (Clone: 206D)**		
05/06/2015	Tonsil #1	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #2	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #3	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #4	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #5	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #6	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #7	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #8	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #9	+ nuclear expression in a subset of lymphocytes in T-cell region	
05/06/2015	Tonsil #10	+ nuclear expression in a subset of lymphocytes in T-cell region	
ANALYTE	**CD68 (Clone: PG-M1)**		
03/03/2015	Tonsil #1	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #2	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #3	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #4	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #5	+ macrophages-tingible bodies in the germinal center region	

03/03/2015	Tonsil #6	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #7	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #8	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #9	+ macrophages-tingible bodies in the germinal center region	
03/03/2015	Tonsil #10	+ macrophages-tingible bodies in the germinal center region	

ANALYTE	**CD163 (Clone: 10D6)**		
03/04/2015	Tonsil #1	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #2	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #3	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #4	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #5	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #6	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #7	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #8	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #9	+ macrophages-tingible bodies in the germinal center region	
03/04/2015	Tonsil #10	+ macrophages-tingible bodies in the germinal center region	

ANALYTE	**Cytokeratin (Clone: AE1/AE3)**		
01/22/2015	Tonsil #1	+ crypt epithelium cells	
01/22/2015	Tonsil #2	+ crypt epithelium cells	
01/22/2015	Tonsil #3	+ crypt epithelium cells	
01/22/2015	Tonsil #4	+ crypt epithelium cells	
01/22/2015	Tonsil #5	+ crypt epithelium cells	
01/22/2015	Colorectal ca #1	+ expression in highly differentiated carcinoma cells	
01/22/2015	Colorectal ca #2	+ expression in highly differentiated carcinoma cells	
01/22/2015	Colorectal ca #3	+ expression in highly differentiated carcinoma cells	
01/22/2015	Colorectal ca #4	+ expression in highly differentiated carcinoma cells	
01/22/2015	Colorectal ca #5	+ expression in highly differentiated carcinoma cells	

ANALYTE	**SOX10 (Clone: EP268)**		
01/23/2015	Melanoma #1	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #2	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #3	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #4	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #5	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #6	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #7	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #8	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #9	+ nuclear expression in melanoma cells	
01/23/2015	Melanoma #10	+ nuclear expression in melanoma cells	

Data Analysis

Data analysis was performed by performing analysis on the malignant cells and on the immune cells using APERIO as an image analysis software. Analysis is performed by two pathologists independently, who then meet and reconcile their score and harmonize their nuclear and cytoplasmic algorithms.

SCORING:

Analyte	Standard Microscopy Evaluation	Digital Image Analysis	Analysis in Malignant Cells	Analysis in Immune Cells
PD-L1 (405.9A11)	Performed	Routinely performed	Percentage of positive cells	Routinely performed/ Percent positive
CD3	Performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
CD8 (Clone 144B)	Performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
CD4 (Clone 4B12)	Performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
CD31 (ab28364)	Not routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
PD-1 (EH33)	Performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
CD68 (PGM1)	Not routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
CD163 (10D6)	Not routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
FOXP3 (Clone 206D)	Routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
SOX10 (EP268)	Not routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)
Cytokeratin (AE1/AE3)	Not routinely performed	Performed (Aperio)	n/a	cell density (number of positive cells/mm^2)

n/a: not applicable