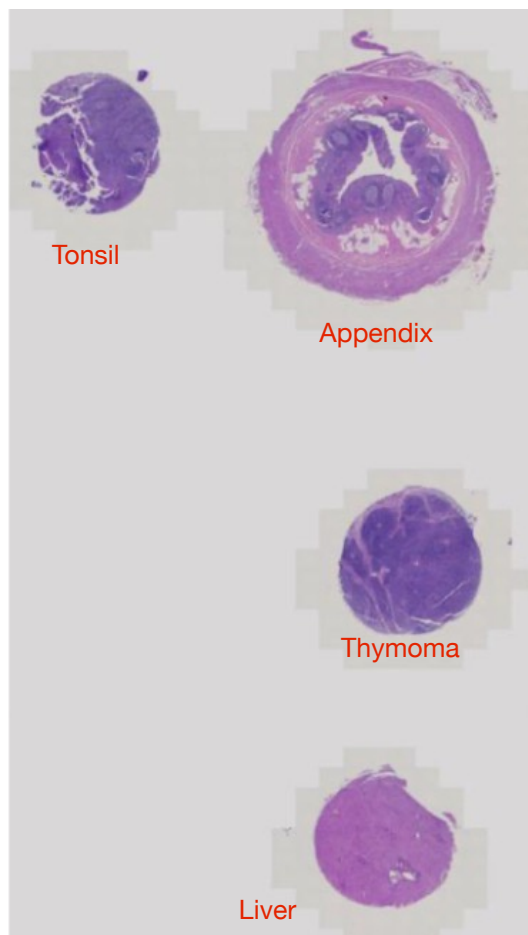
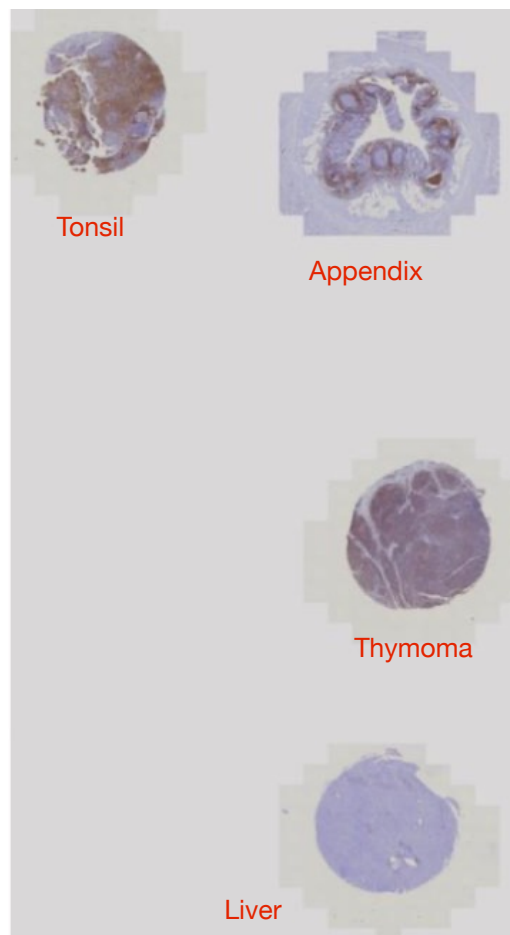




CD-3



HE staining in scanner view



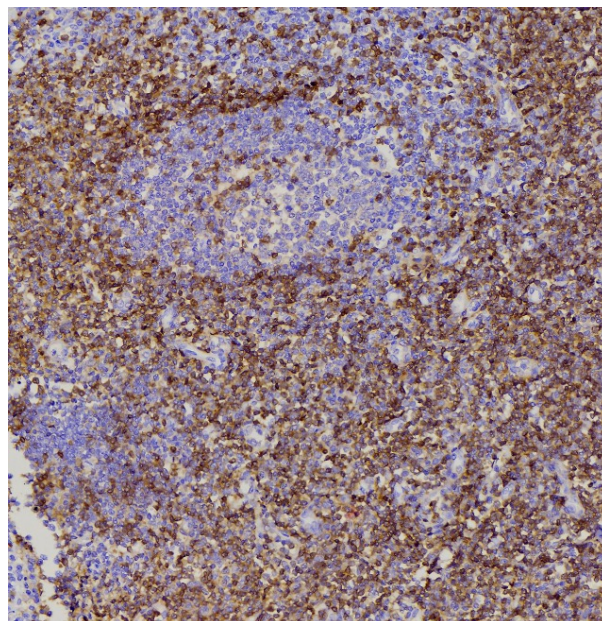
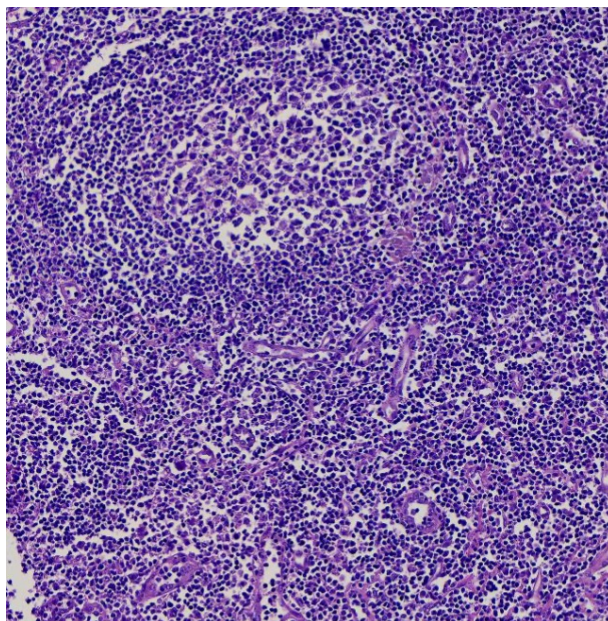
CD-3 immunostaining, scanner view

Provided sections in this run.

Tonsil, Appendix, thymoma and liver were circulated in this run. Tonsil and thymoma were rich in T cells that intensely expressed CD3. Appendix has good numbers of T cells alongside negative control in form of epithelial cells. Liver was rather a negative control, however with presence of small number of sinusoidal T cells. Those participants who have portal area represented can see good number of T cells there.

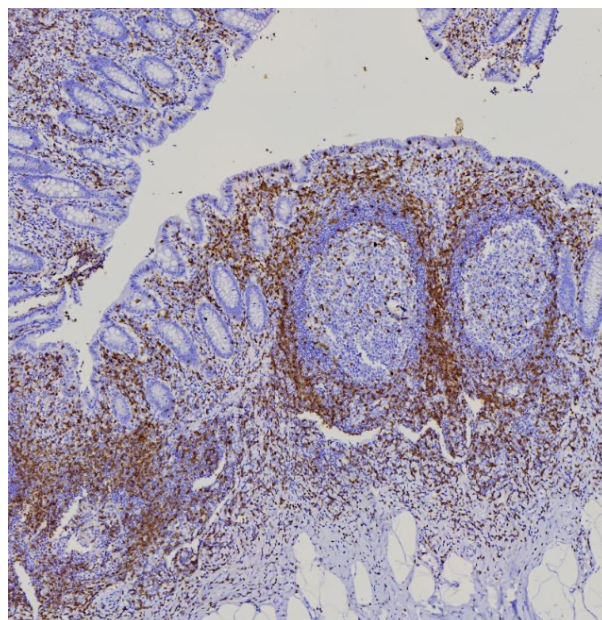
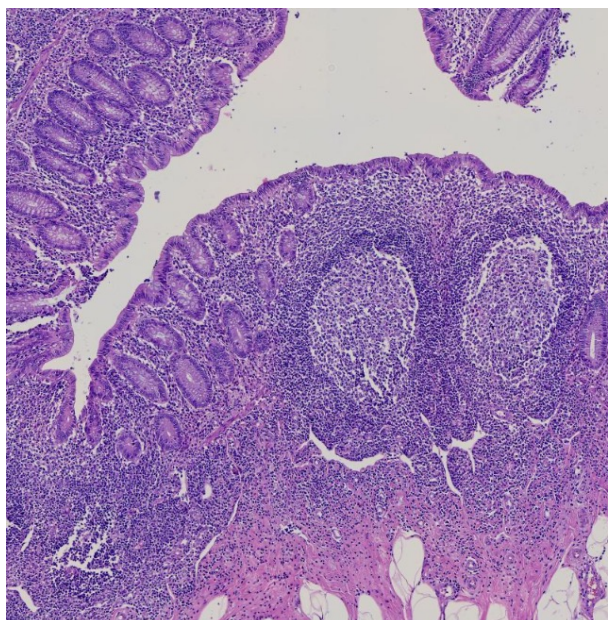


Tonsil, CD3, 10x



Many cells in the para cortical region of the lymphoid tissue and few cells in the germinal center show intense cytoplasmic staining

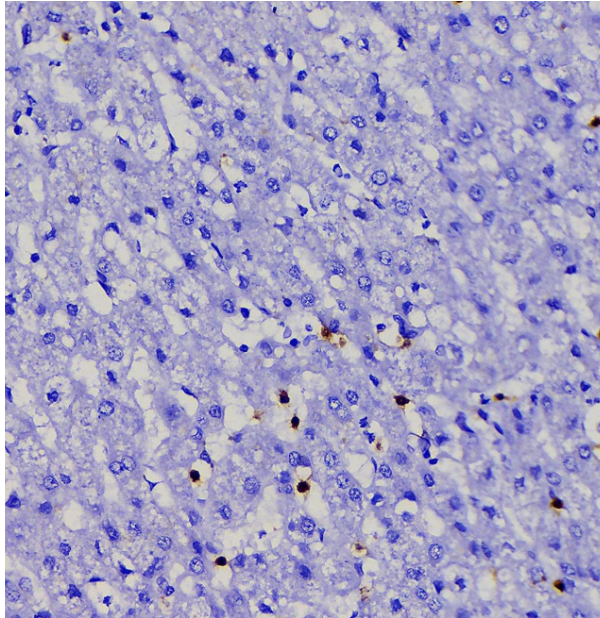
Appendix, CD3, 10x



Many cells in the para cortical region of the lymphoid tissue, few cells in the germinal center and mucosal lymphoid cells show intense cytoplasmic staining

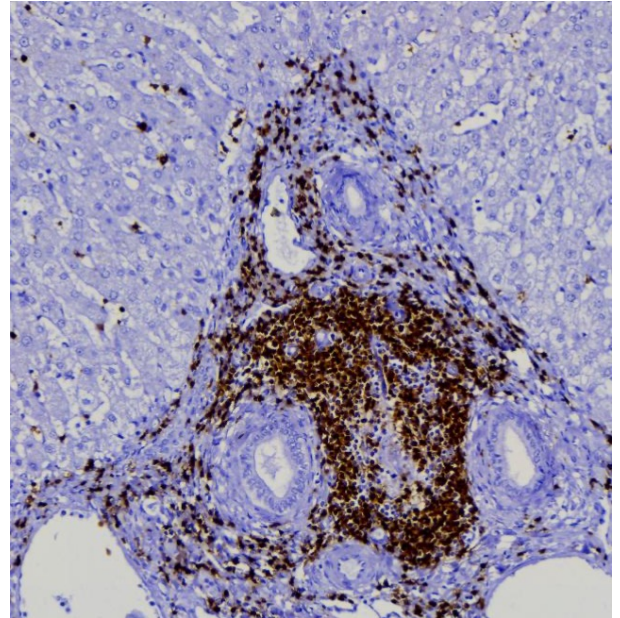


Normal liver, CD3, 10x



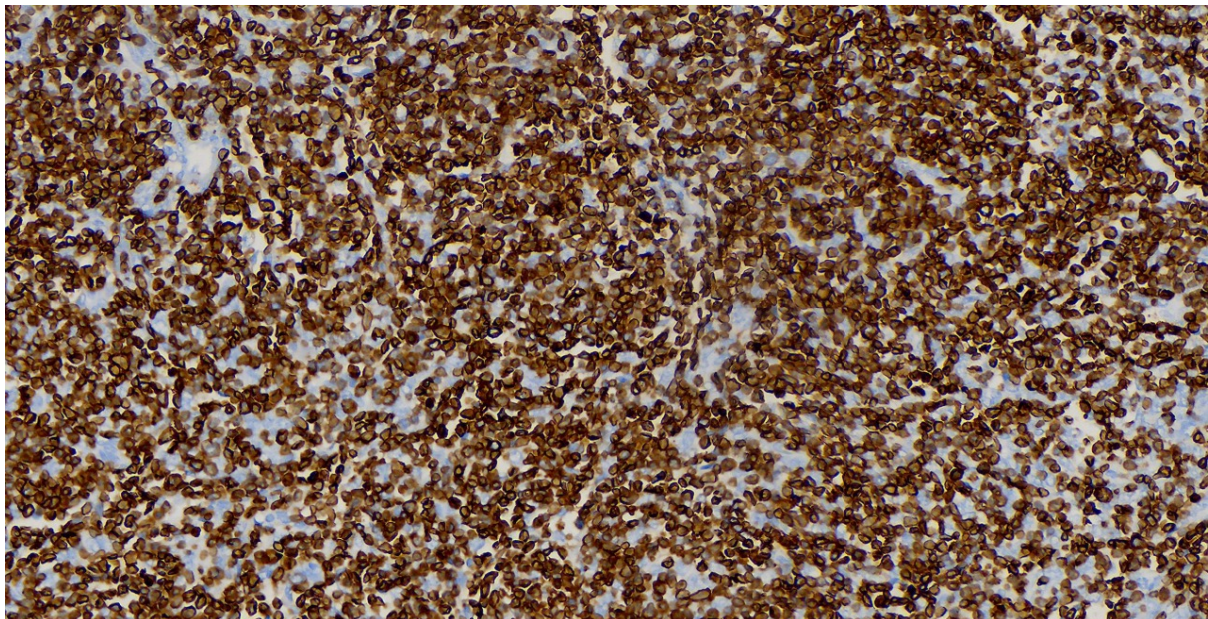
Sinusoidal T cells are intensely stained with CD3

Normal liver, CD3, 10x



Some participants have portal area represented that shows many T cells with intense cytoplasmic staining.

Thymoma, CD3, 20x



Intense cytoplasmic staining of practically all the lymphoid cells in this case of thymoma.



Protocol used by the top 3 in this run

Variable	1	2	3
Technique	Manual	Automated	Manual
Clone	PS1	2GV6	QR4
Vendor	BiogeneX AM322-5M	VENTANA, 790-4341	quartett, P-C002-70
Format	RTU	RTU	RTU
Batch/Year	AM3220620	H04553, 04.03.2021	413930, NA
Expiry	July 2022	03.03.2023	2023-10
Dewaxing temperature	60-62 degree Celsius	76 DEGREE CELSIUS	65°C
Retrieval	HIER	HIER	HIER
Enzyme	NA	NOT APPLICABLE	NA
HIER	Microwave Oven	COMPANY SYSTEM VENTANA,BMXT	Microwave oven
Peak T and Time	At 900 Watt-20 minutes At 540 Watt-20 minutes Temperature: 95 degree celsius	100 DEGREE CELSIUS, 30 MINUTES	110°C, 15 mins (5+5+5)
Peak Pressure and Time	NA	NOT APPLICABLE	NA
Retrieval Buffer	In-House	COMPANY ,CC1	Company provided, EnVision FLEX TARGET RETRIVAL SOLUTION.
pH	pH=8	8.5	9.0
Blocking	3%H2O2 for 10 minutes	3% H2O2 SOLUTION,ULTRAVIEW UNIVERSAL DAB INHIBITOR	EnVision FLEX PEROXIDASE-BLOCKING REAGENT, 10 mins.
Wash sol	Phosphate buffer saline solution PBS (pH=7.2-7.6)	REACTION BUFFER ,TRIS BASED BUFFER	Tris buffered saline with saponifying agent.
Dilution of RTU	No	NO	NA
Dilution of conc	NA	NOT APPLICABLE	NA
Diluent	NA	NOT APPLICABLE	NA
Inc time of Primary	1 hour covered in Humidity chamber	32 MINUTES	30 mins.

Variable	1	2	3
Detection	Polymer based system	POLYMER BASED	Polymer based system
Cat No	Supersensitive Polymer HRP, IHC Detection system/Megavolume BiogeneX QD430-XAKEN, LOT: QD4300821, Expiry: January 2023	ULTRAVIEW DAB DETECTION VENTANA KIT,760-500, H19915,13.10.2021, 14.4.2023	EnVision FLEX/ HRP, Dako, K8023, 41293161, NA, 2022-07-31.
Inc time of Sec	Polymer HRP-30 minutes Superenhancer-20 minutes At room temperature	8 MINUTES	30 mins
Chro-substrate	DAB-10 minutes at room temperature	8 MINUTES	8 mins.
Post-treatment	No	YES, ULTRAVIEW UNIVERSAL COPPER (CuSO4 VIA ACETATE BUFFER,04 MINUTES)	NA
Counterstain	Harris Hematoxylin	HEMATOXYLINE	Harris hematoxylin.

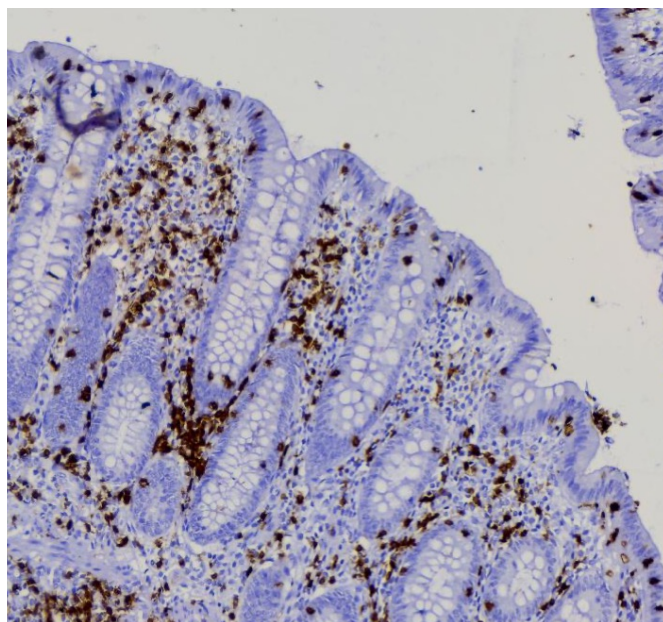


Fig.1: Adequate HIER and RTU monoclonal antibody used by one participant has produced crisp staining without any background staining.

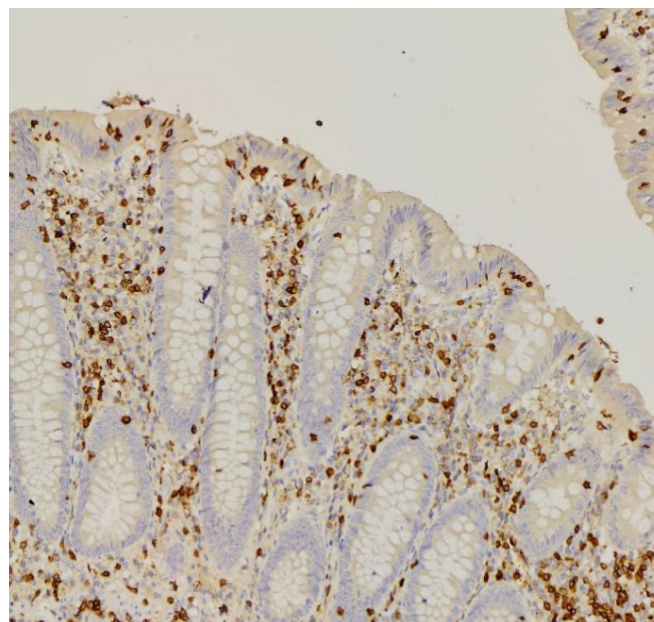


Fig. 2 Background staining in another participant using polyclonal antibody.

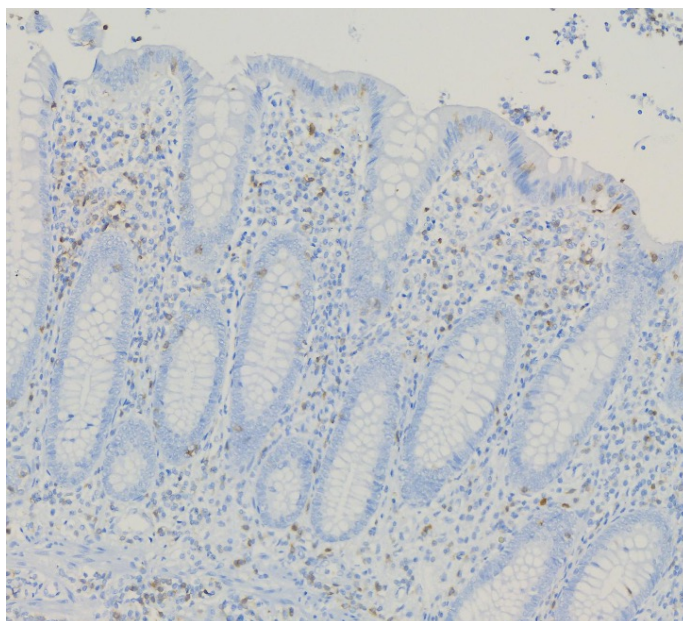


Fig.3 weak staining of mucosal lymphoid cells in participant with inadequate HIER

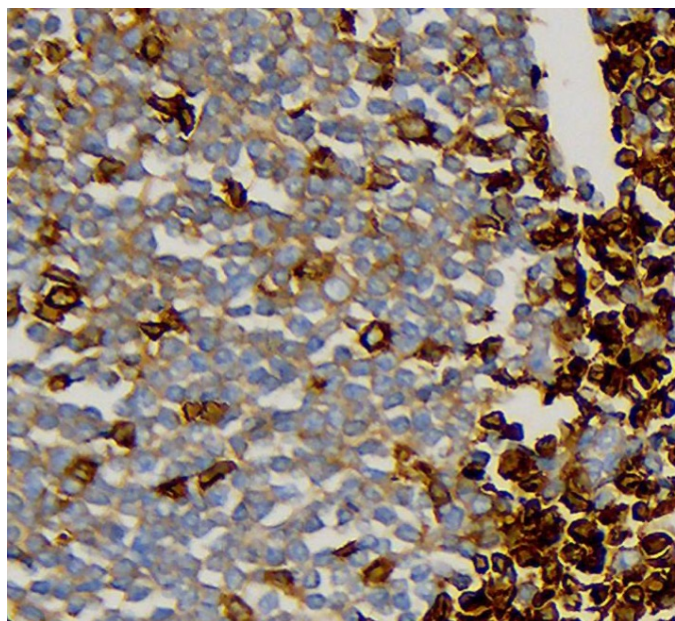


Fig.4 Non-specific weak membranous staining of B cells in germinal center is observed in some participants.

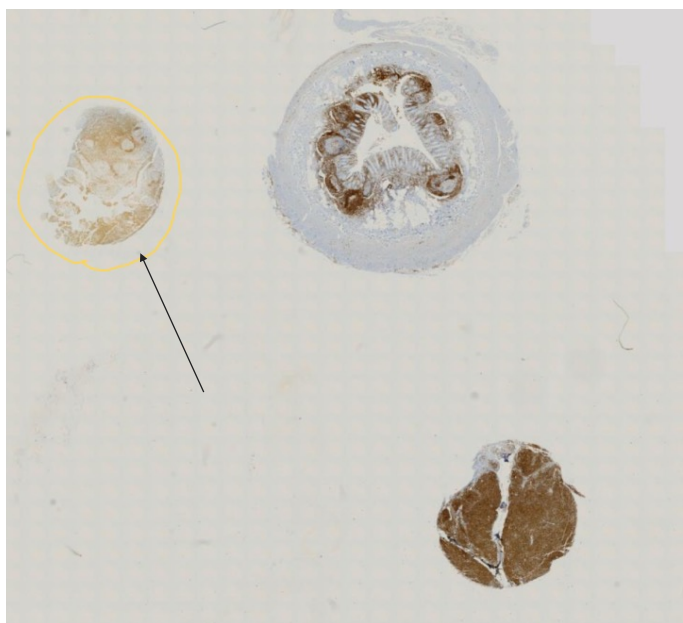


Fig.5 Effect of slope on staining rack results in non-staining or weaker staining of the area that is inadequately immersed in the reagents.

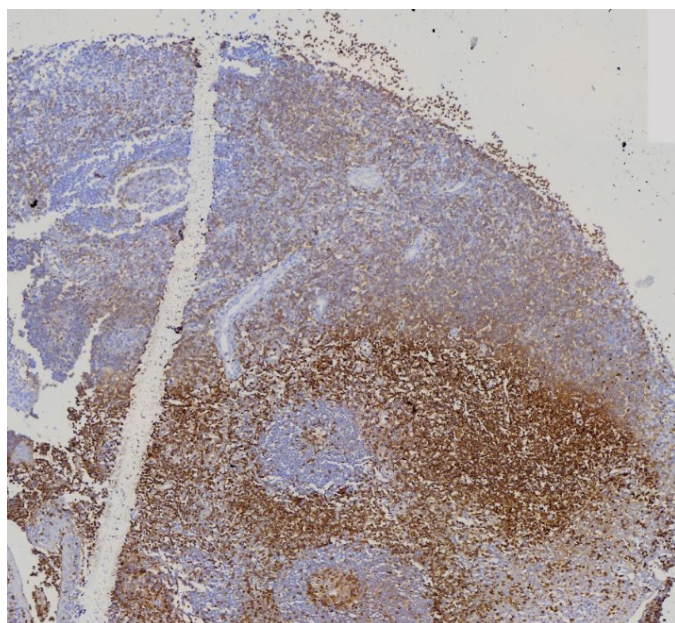


Fig.6 Drying artefact results in suboptimal staining