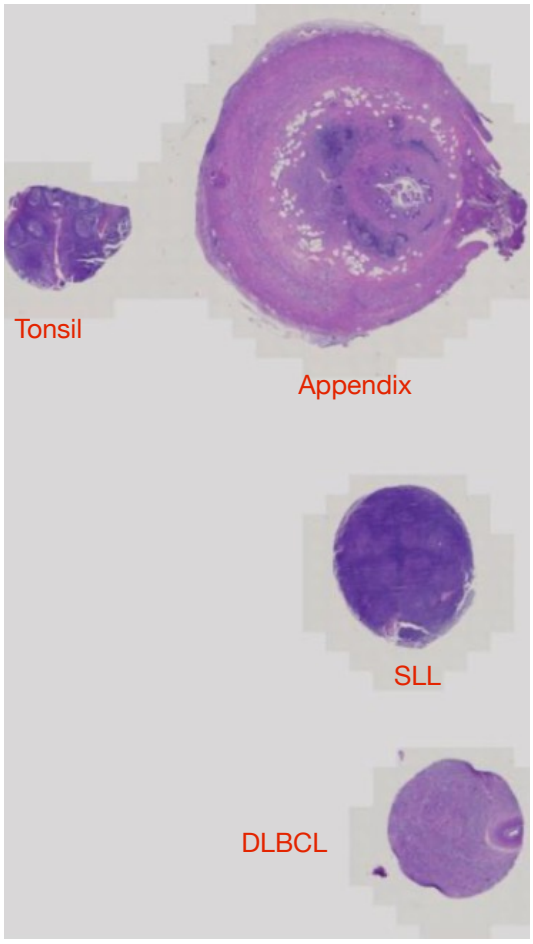
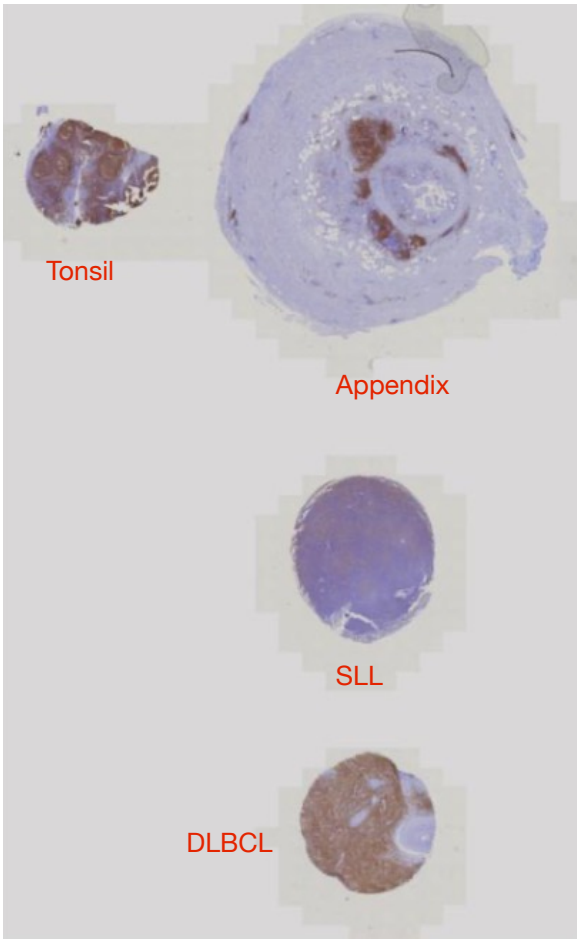
CD-20



HE staining in scanner view

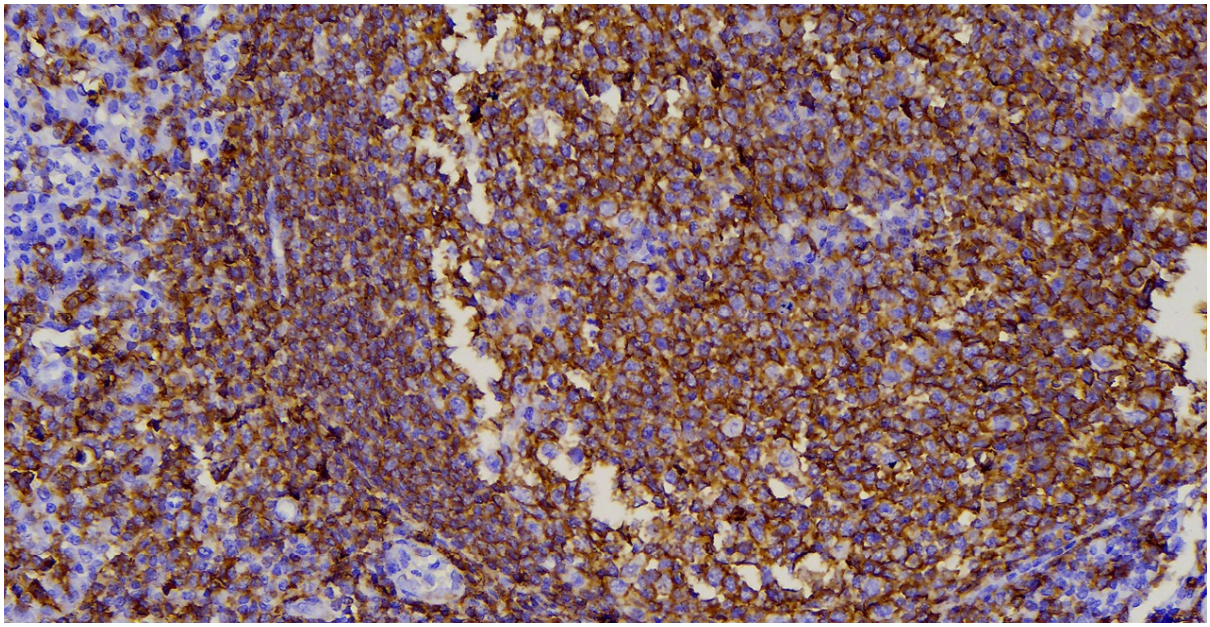


CD-20 immunostaining, scanner view

Provided sections in this run.

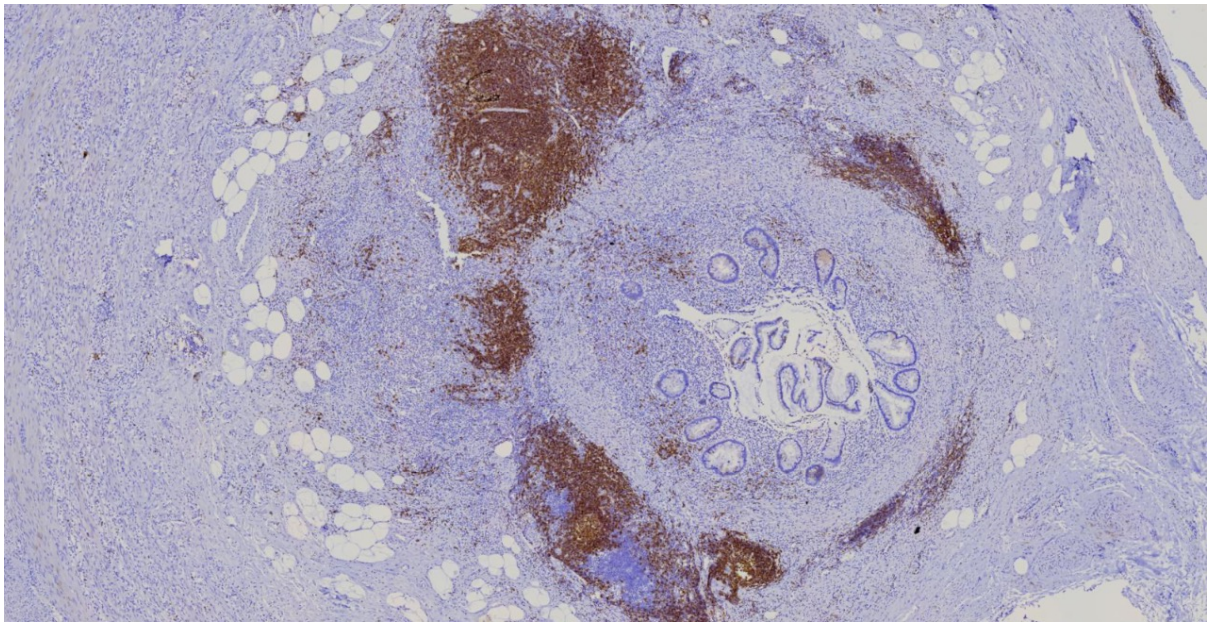
All the provided tissue contained CD20 positive lymphoid cells. The cells other than these positive cells, served as negative control. The sections have to be very crisply stained and no background staining is expected. The tonsillar and appendicial lymphoid tissue provided intense staining, DLBCL as moderate intensity and SLL as weaker intensity. We were surprised by rather intense staining of SLL by some of the participants.

Tonsil, CD20 staining, 20x



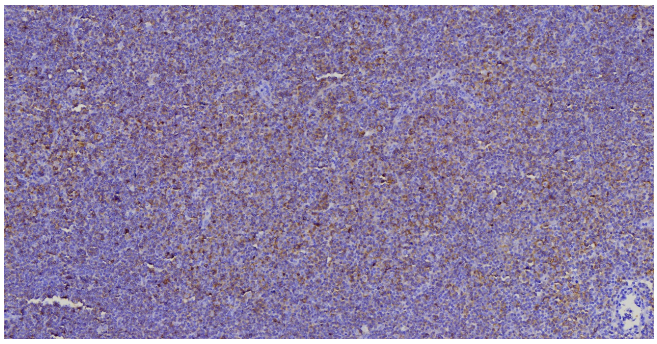
Intense membranous and cytoplasmic staining of majority of the cell in the lymphoid follicles and paracortex.

Appendix, CD20 staining, 2x



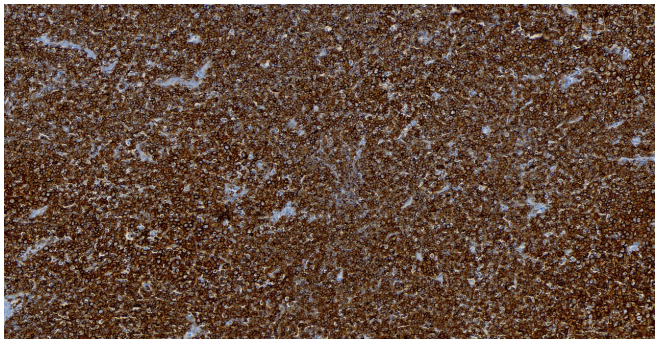
Intense staining of cells in the lymphoid follicles of the appendicular wall.

SLL, CD20, 10x



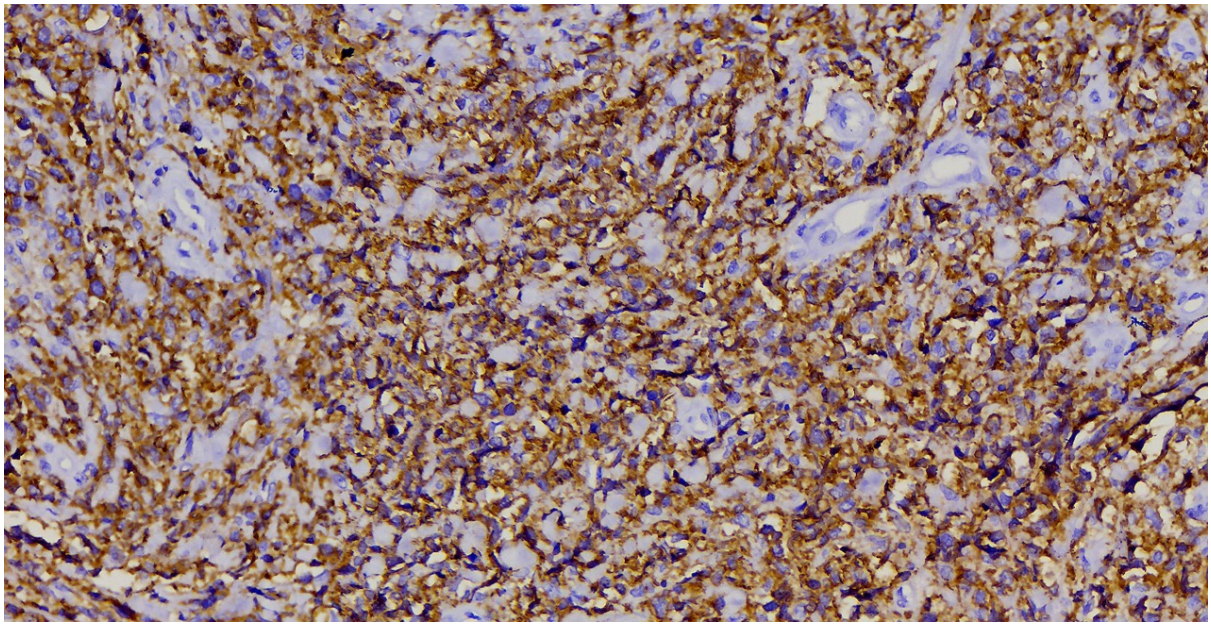
The peer group and some of the participants showed weak staining of SLL case.

SLL, CD20, 10x



Many participants showed intense staining of SLL cells.

DLBCL, CD20, 20x



Intense membranous and cytoplasmic staining of all the atypical lymphoid cells.

Protocol used by the top 3 in this run

Variable	1	2	3
Technique	MANUAL	Automated, Ventana XT- Benchmark, Fully automated. Slide preparation system- Roche company	AUTOMATED, VENTANA, BENCHMARK , GX
Clone	L26	L26	L26
Vendor	PATH N SITU CAT#PM080	CELL MARQUE and 120M-85	VENTANA, 760-2531
Format	RTU	Concentrated	RTU
Batch/Year	R08080EA	118658	H25081, 10.09.2021
Expiry	08/2022	31.08.2023	09.09.2023
Dewaxing temperature	60 DEGREE CENTIGRADE	74°C in company system	76 DEGREES
Retrieval	HIER	HIER	HIER
Enzyme	NO	Not Applicable	NA
HIER	PRESSURE COOKER	Company system- Ventana XT	VENTANA
Peak T and Time	121 DEGREE CENTIGRADE;2 MINUTES	Temperature: 94°C AND Time: 60 minutes	100 DEGREES ,4 MINUTES
Peak Pressure and Time	15 PSI;2 MINUTES	Not Applicable	NA
Retrieval Buffer	INHOUSE MADE	Company provided, Cell conditioning (CC1), Ventana XT	READY MADE CELL CONDITIONER 1, EDTA BASED
pH	6.5	pH 8.4	8.4
Blocking	H2O2;10 MINUTES	Ultra view Universal DAB inhibitor, Time: 4 Minutes	NA
Wash sol	TRIS BUFFERED SALINE	Reaction buffer	RTU, COMPANY BASED
Dilution of RTU	NO	No	NA
Dilution of conc	NA	1:200	NA
Diluent	NA	Antibody Dilution buffer, Ventana, ADB250	NA
Inc time of Primary	1 HR	44 minutes	32 MINUTES

Variable	1	2	3
Detection	POLYMER BASED SYSTEM	Polymer based system	POLYMER BASED
Cat No	POLY EXCEL HRP/DAB DETECTION SYSTEM PATH N SITU BIOTECHNOLIES PVT.LTD. CAT#OSH001 D08001OA4 EXPIRY DATE : 04/2023	Ultra view universal DAB detection system, Ventana XT, 760-500, H19915, 2021/10/13 & 2023/04/14	UV HRP, UNIV MULT, VENATANA, 760-500, H21101, 14.07.2023
Inc time of Sec	30 MINUTES	8 Minutes	8 MINUTES
Chro-substrate	5 MINUTES	8 minutes	8 MINUTES
Post-treatment	NA	Yes, Ultra view Universal DAB copper-enhancer-8 minutes	UV COPPER, 4 MINUTES
Counterstain	HARRIS HAEMATOXYLIN	Hematoxylin	HEAMATOXYLIN

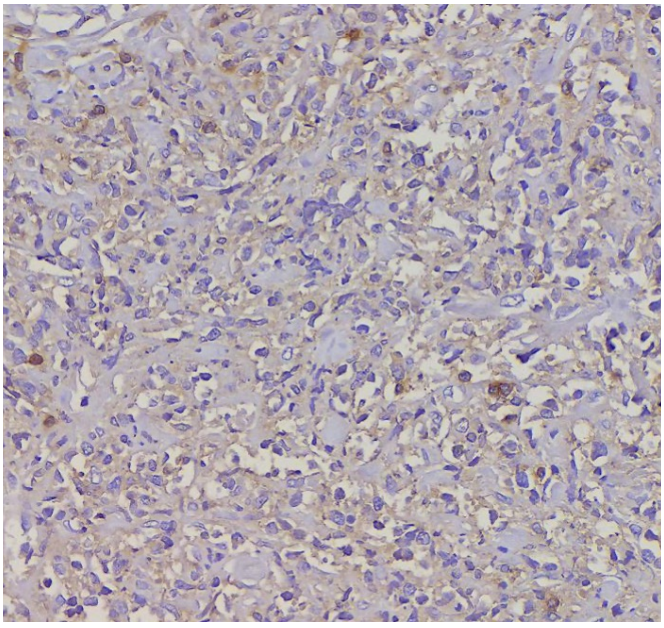


Fig.1 Weak intensity staining in a participant who uses expired antibody with dilution of 1:300. May need to tweak the dilution and validate. This case also showed drying.

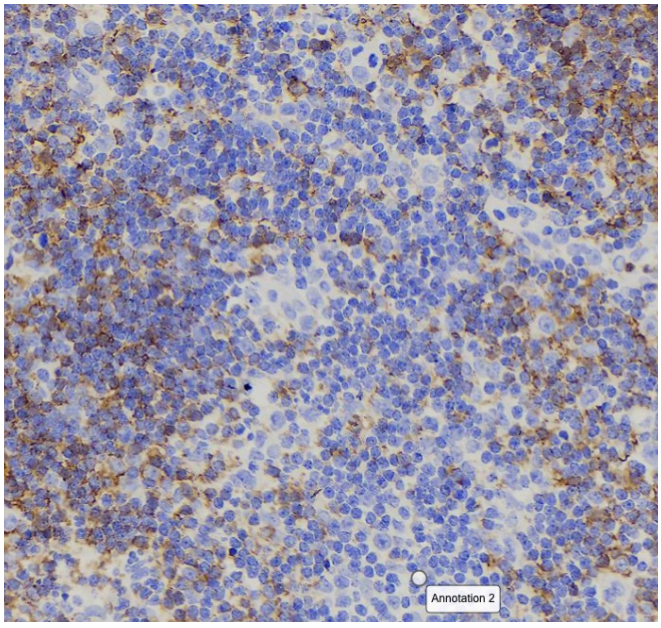


Fig. 2 Patchy and weak staining, usually is result of inadequate epitope retrieval.

Examples of drying artefact in this run

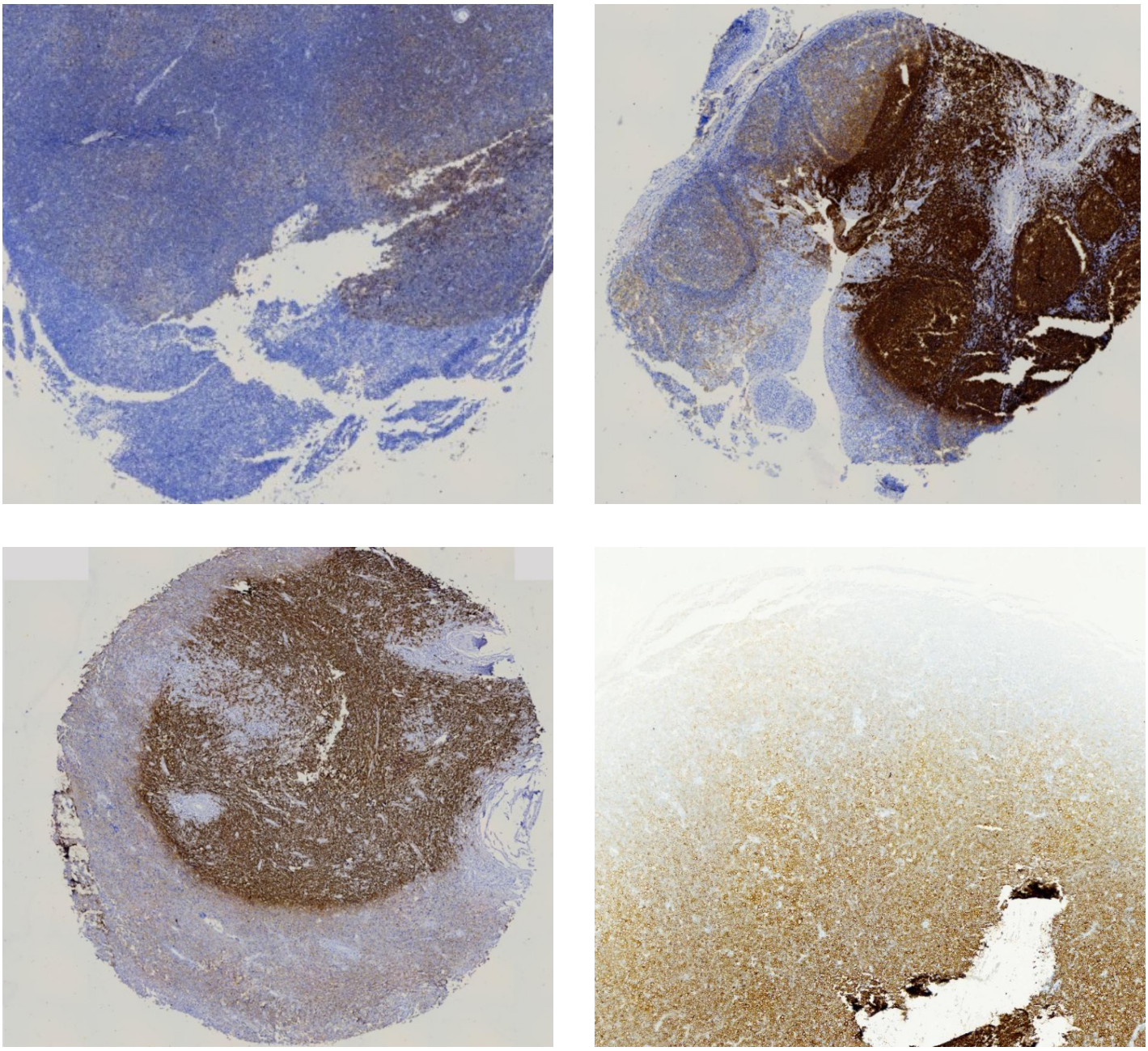


Fig.3: ***Drying artefact*** *is a peculiar issue that sometimes can become little concerning. When a small biopsy bit of a needle core is completely under the influence of drying, one may wrongly interpret the case as negative, though an astute observer can make out the issue in such situation. The issue is commonly observed in manual staining. This phenomenon is observed when the incubation chamber has not been covered with lid or lacks humidity or directly exposed to the air flow.*

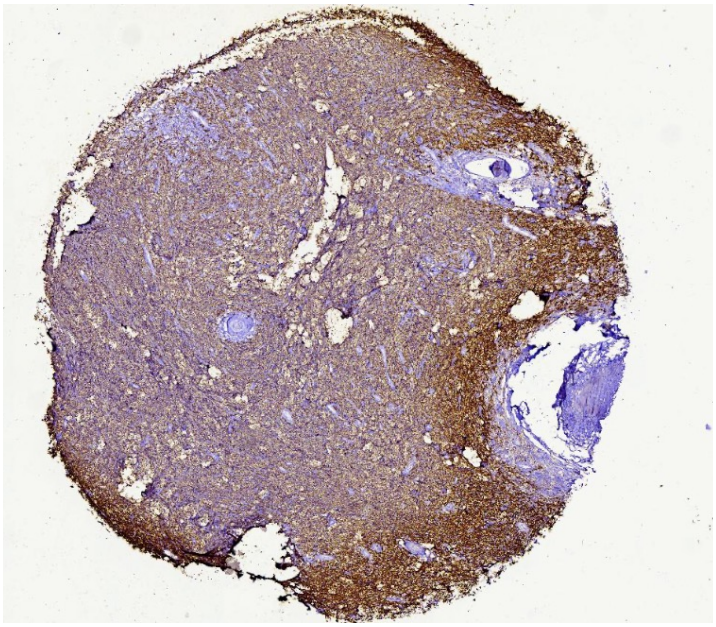


Fig.4: ***Antibody drift****: uneven section can make antibodies to concentrate in a particular part of the section. It may result in non-staining of the areas that otherwise would have stained with lesser intensity.*

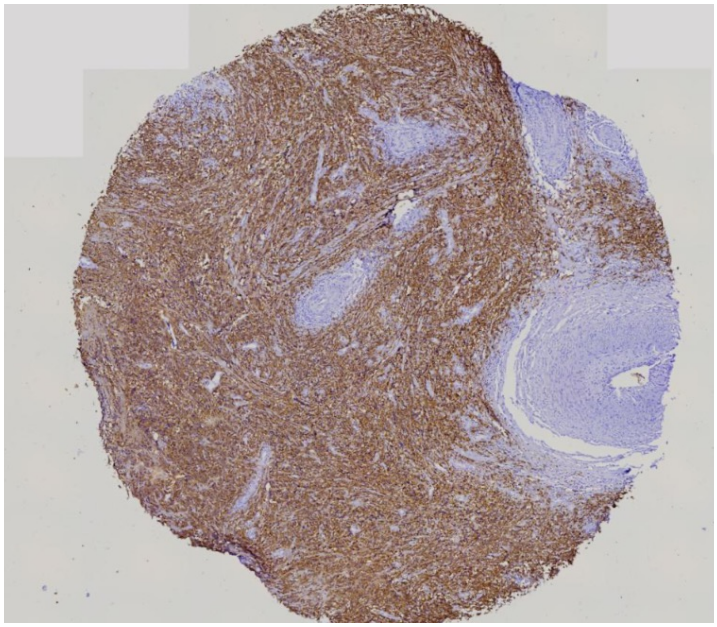
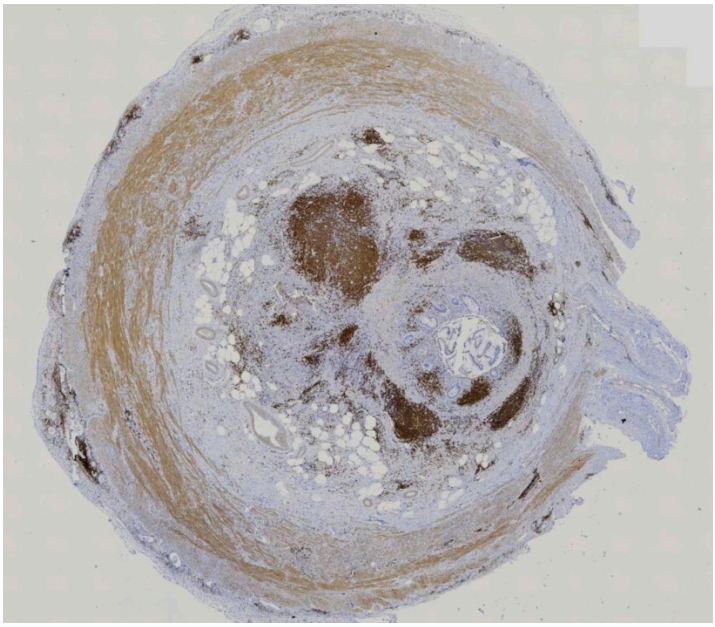


Fig.5: Compare the optimum staining where there is uniform staining of the tumour cells without any antibody drift.



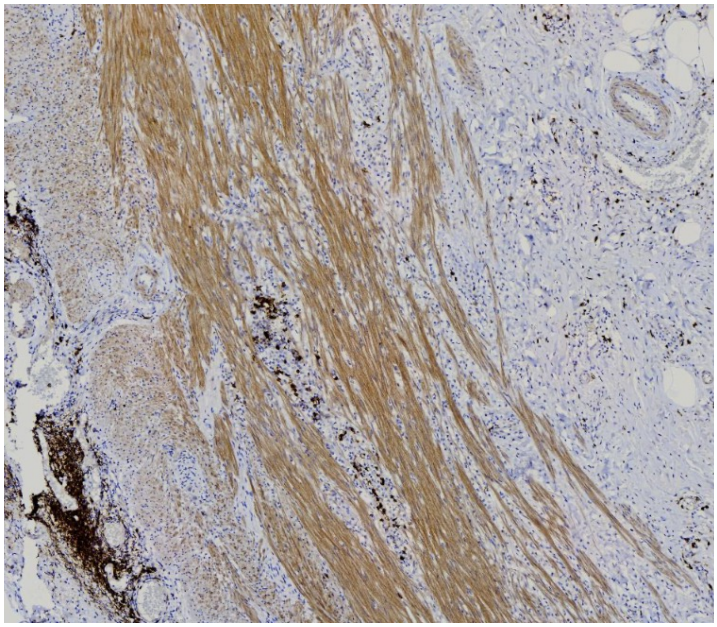
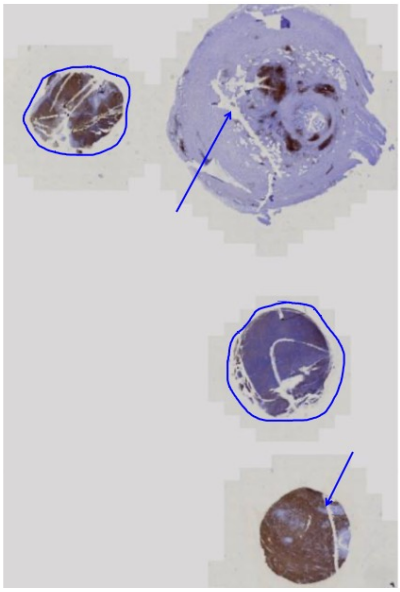
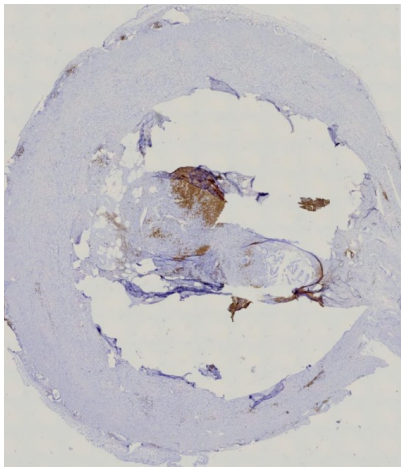


Fig.6: ***Non-specific staining*** *of smooth muscle cells. This was particularly observed in one participant using Dako, IS604, Lot No-20075400. Other participants using the same lot, however, did not show this degree of non-specific staining. We assume it is related to the properties of the buffers used by the participant.*

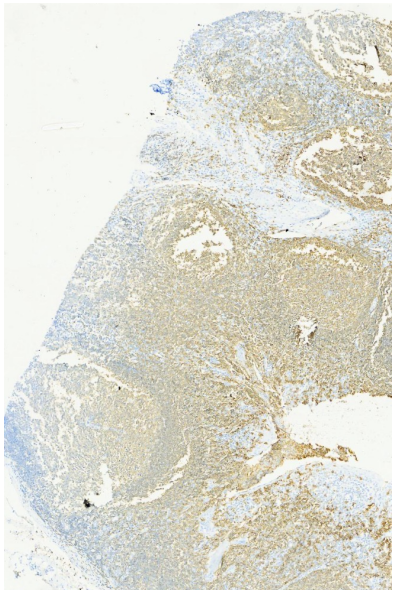
Some avoidable artefact



Wipe marks due to improper handling of the tissue section.



Poor tissue integrity



Patchy and uneven staining