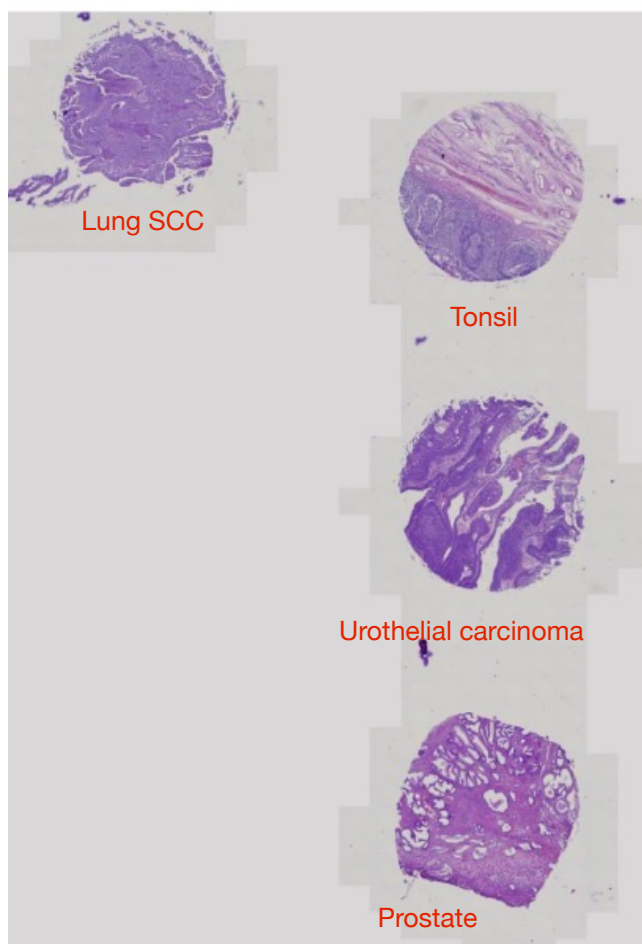
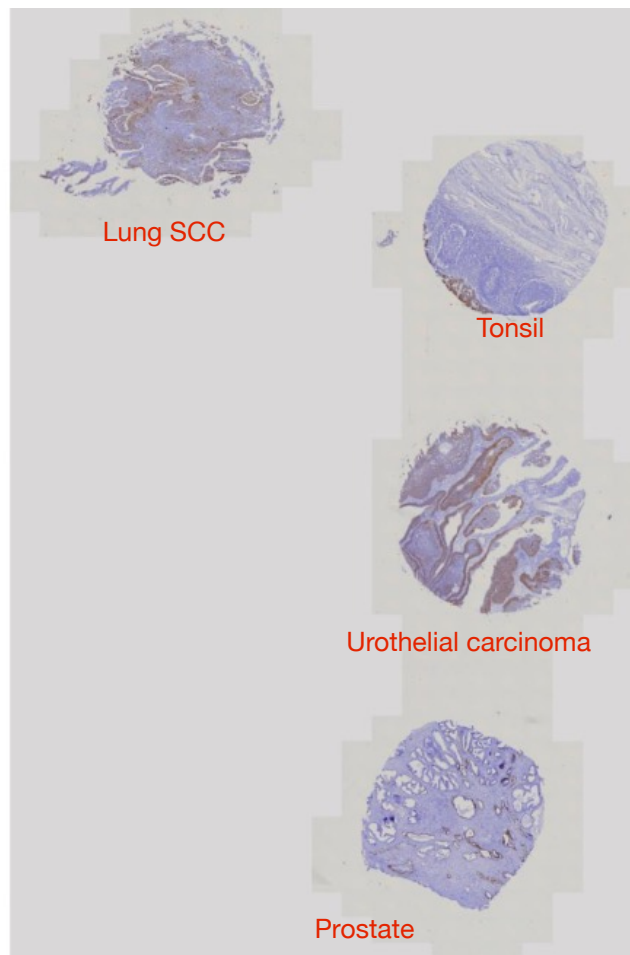


High molecular weight cytokeratin (HMWCK)



HE staining in scanner view



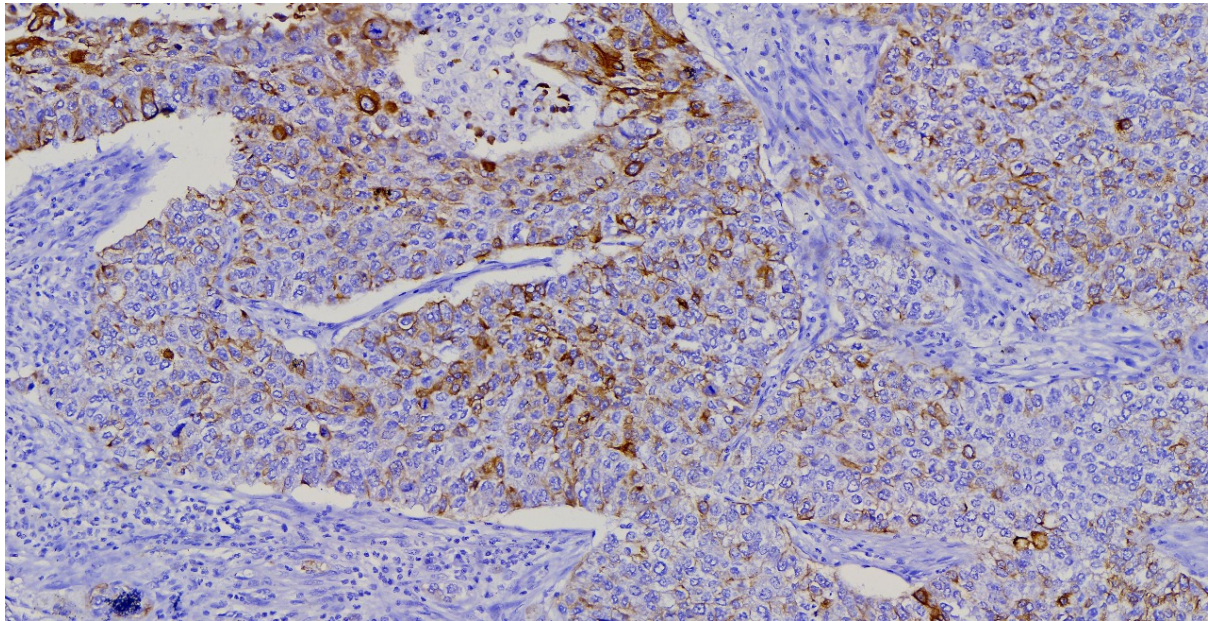
HMWCK immunostaining, scanner view

Provided sections in this run.

Acinar and ductal cells of prostatic tissue are the negative stains for this marker while squamous carcinoma of lung, tonsil, urothelial carcinoma and basal layer of the prostatic acini are positive stains for this marker.

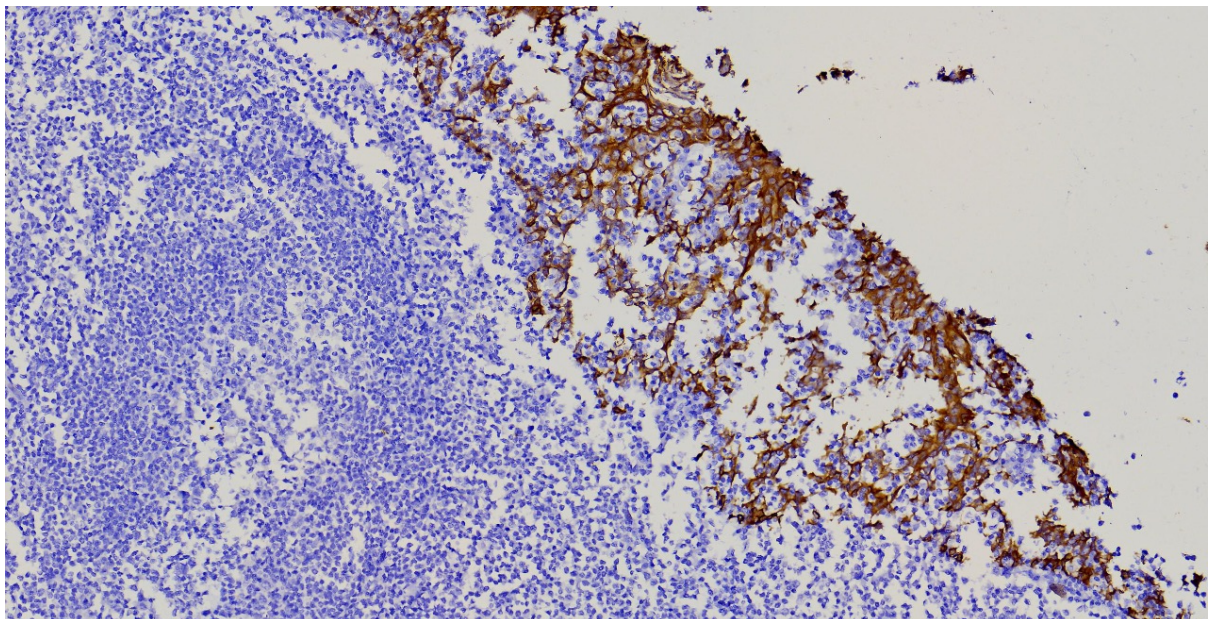
All the sections were of good quality and peeling or folding is not expected as majority of the participants have been able to retain the sections well.

Lung SCC, HMWCK staining, 10x



Moderate intensity of staining in more than 60% of the tumour cells.

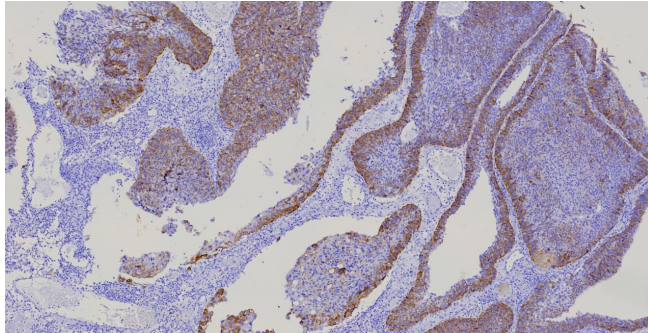
Tonsil, HMWCK staining, 10x



Intense cytoplasmic staining of surface squamous epithelial cells.

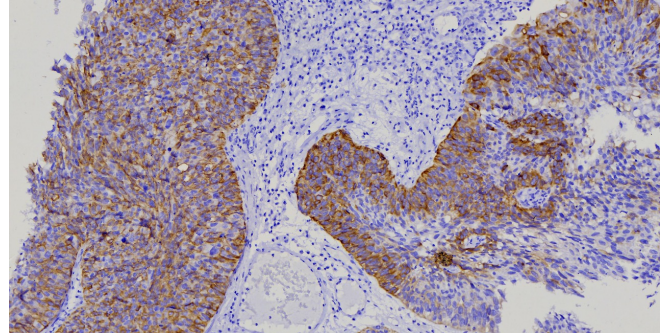


Urothelial carcinoma, HMWCK, 4x



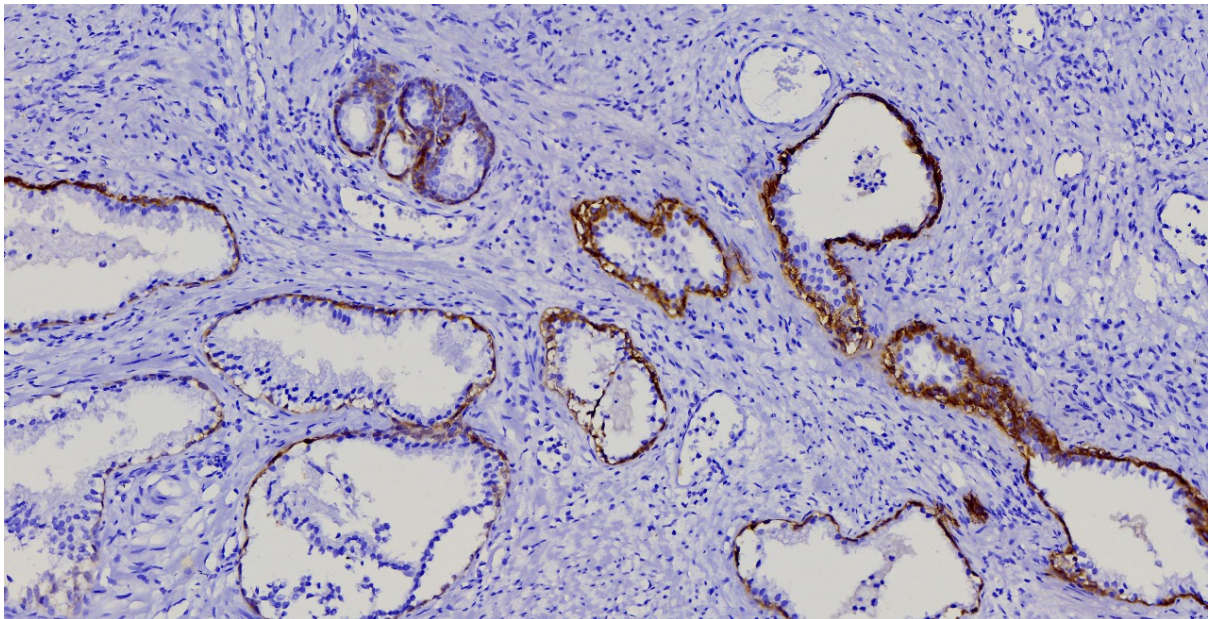
Staining in majority areas, while it lacks in certain foci.

Urothelial carcinoma, HMWCK, 10x



Moderate to intense cytoplasmic staining of tumour cells.

Prostate, HMWCK, 10x



Intense cytoplasmic staining of majority of basal cells of prostatic acini. No staining of acing lining epithelial cells and stromal cells.



Protocol used by the top 3 in this run

Variable	1	2	3
Technique	Manual technique	MANUAL	AUTOMATED VENTANA (BMXT)
Clone	34BE12	34BE12	34BE12
Vendor	DAKO IS051	PATH N SITU CAT#PM092	VENTANA, 790-4373
Format	RTU	RTU	RTU
Batch/Year	10064617	R07092LA	G05888, 13.04.2020
Expiry	10/2013; Validation done and found satisfactory.	01/2022	23.03.2022
Dewaxing temperature	65 degree	60 DEGREE CENTIGRADE	76 DEGREE CELSIUS
Retrieval	HEIR EDTA BUFFER pH 9.0	HIER	HIER
Enzyme	--	NO	NOT APPLICABLE
HIER	MICROWAVE	PRESSURE COOKER	COMPANY SYSTEM VENTANA,BMXT
Peak T and Time	98 DEGREE 20 MINUTES	121 DEGREE CENTIGRADE;2 MINUTES	100 DEGREE CELSIUS, 60 MINUTES
Peak Pressure and Time	---	15 PSI;2 MINUTES	NOT APPLICABLE
Retrieval Buffer	IN HOUSE MADE	INHOUSE MADE	COMPANY ,CC1
pH	EDTA BUFFER pH 9.0	6.5	8.5
Blocking	3 % HYDROGEN PEROXIDE WITH METHANOL	H2O2;10 MINUTES	3% H2O2 SOLUTION,ULTRAVIEW UNIVERSAL DAB INHIBITOR,04 MINUTES
Wash sol	TBST BUFFER pH 7.4	TRIS BUFFERED SALINE	REACTION BUFFER ,TRIS BASED BUFFER
Dilution of RTU	--	NO	NO
Dilution of conc	--	NA	NOT APPLICABLE
Diluent	---	NA	NOT APPLICABLE
Inc time of Primary	30 MINUTES	1 HR	20 MINUTES

Variable	1	2	3
Detection	POLYMER BASED SYSTEM (MASTER POLYMER PLUS DETECTION SYSTEM)	POLYMER BASED SYSTEM	POLYMER BASED
Cat No	MASTER POLYMER PLUS DETECTION SYSTEM MAD-000237QK-R 237QK00039 04/2023	POLY EXCEL HRP/DAB DETECTION SYSTEM PATH N SITU BIOTECHNOLIES PVT.LTD. CAT#OSH001 D08001OA4 EXPIRY DATE : 04/2023	ULTRAVIEW DAB DETECTION VENTANA KIT,760-500, H19915,13.10.2021, 14.4.2023
Inc time of Sec	30 MINUTES	30 MINUTES	8 MINUTES
Chro-substrate	4-6 MINUTES	5 MINUTES	8 MINUTES
Post-treatment	--	NA	YES, ULTRAVIEW UNIVERSAL COPPER (CuSO4 VIA ACETATE BUFFER,04 MINUTES)
Counterstain	HEMATOXYLIN	HARRIS HAEMATOXYLIN	HEMATOXYLINE

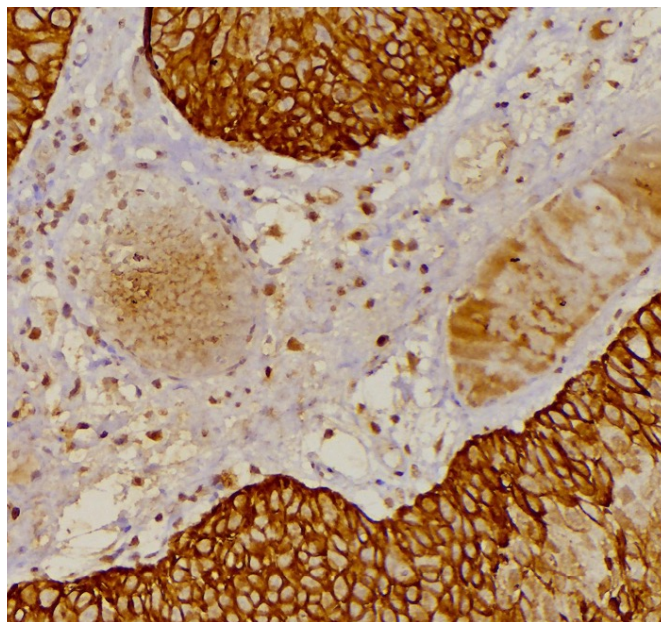


Fig.1 Non-specific staining of leukocytes. The participant has faced problem of background staining also in spite of diluting the RTU 20x. One may need to titrate the antibody dilution through multiple validations.

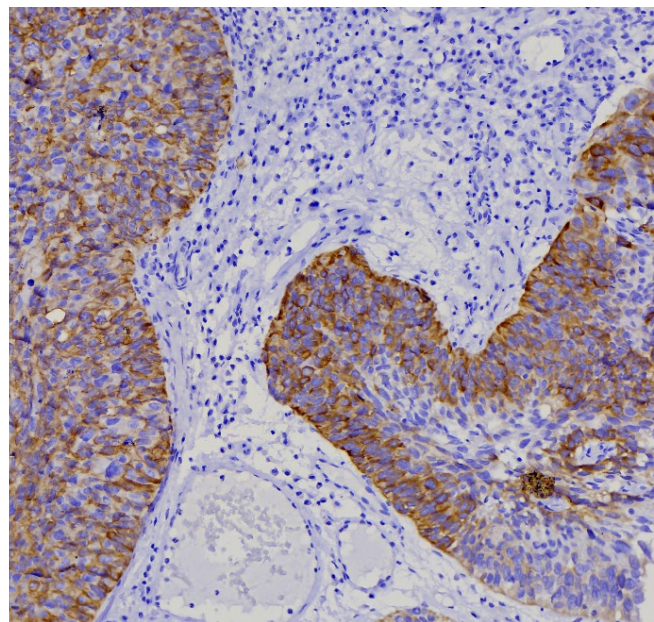


Fig. 2 Crisp staining of tumour and no staining of leukocytes in participant using optimum protocol

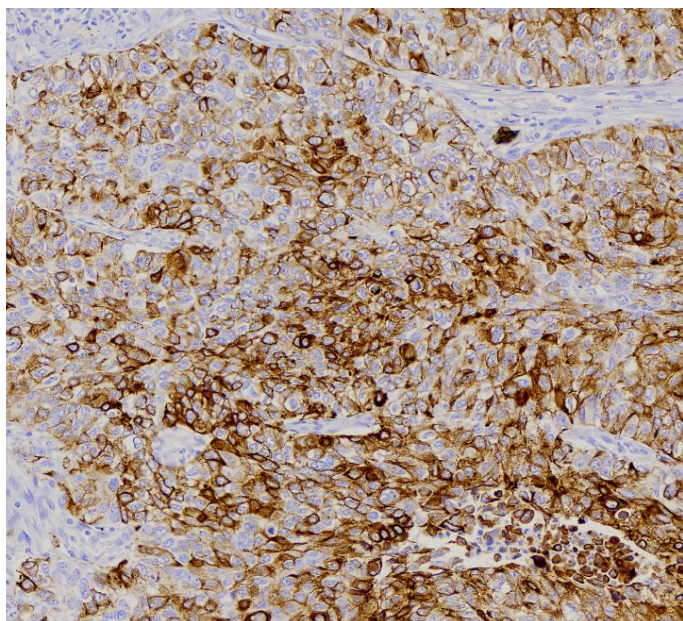


Fig.3 Crisp staining with expired antibody (2013) that is validated for use.

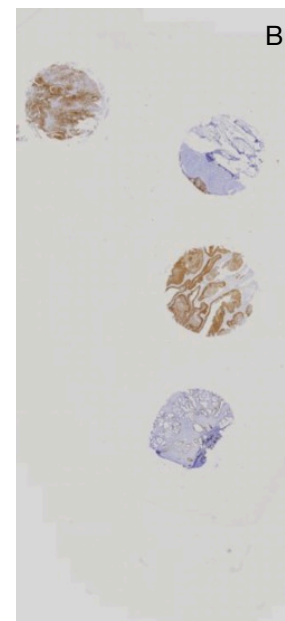


Fig.4 A). Use of expired antibody probably without validation, B). Expired but validated antibody.

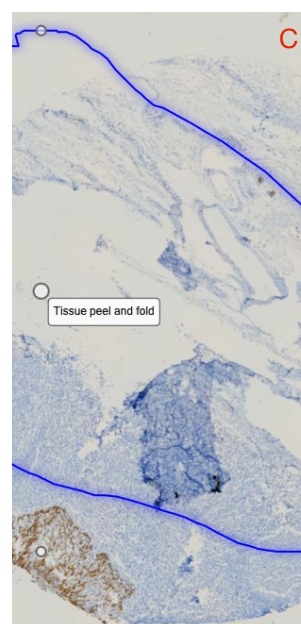
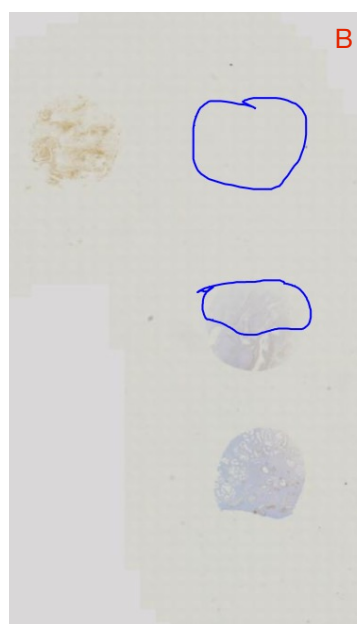
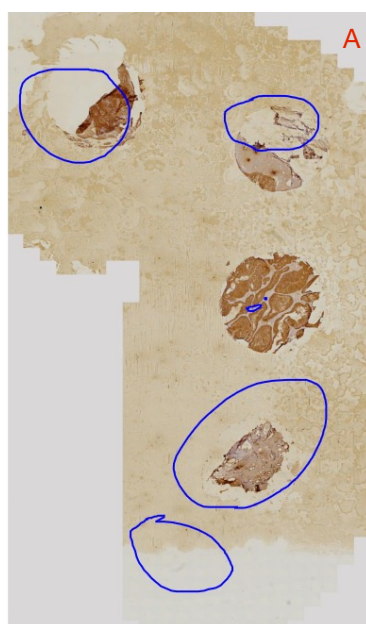


Fig.5 Various important practical issues noted in this run:

- A. Too concentrated antibody and poor wash cycles lead to heavier background and dirty background,
- B. Missing tissue and drying often results due to improper techniques, manual staining,
- C. Peeling of tissue due to improper handling of the tissue either during retrieval or during staining steps,
- D. Wiped off tissue due to poor handling while staining, manual staining.