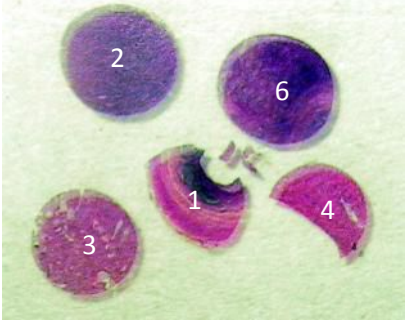
The slides to be stained for vimentin comprised :

1. Appendix, 2. Renal cell carcinoma, 3. Kidney, 4. Melanoma and 5. Dysgerminoma



All tissues sent were fixed in 10% neutral buffered formalin.

Criteria for assessing staining as optimal were:

- A moderate to strong cytoplasmic staining of virtually all the endothelial cells and fibroblasts in all the specimens.
- A moderate to strong cytoplasmic staining of all the peripheral B- and T-cells, the germinal centre macrophages and the follicular dendritic network in appendicular lymphoid follicles. No staining in the epithelial cells
- An at least weak to moderate cytoplasmic and membranous staining of the neoplastic cells of the renal cell carcinoma
- No staining reaction in the epithelial cells of the proximal tubules in the kidney
- A moderate to strong cytoplasmic staining of virtually all the neoplastic cells of the melanoma
- Heterogenous and often dot-like staining of neoplastic cells in dysgerminoma.

Participants' overall performance was assessed based on following criteria:

Selection of proper antibody, label, integrity of sections, artefacts affecting overall staining in all tissues, intensity of staining reaction in each tissue in the set, proportion of staining reaction in each tissue in the set, non-specific staining reaction in and background staining.

Participants' performance specific for the antibody related parameters was assessed separately based on following criteria:

Intensity of staining reaction in each tissue in the set and proportion of staining reaction in each tissue in the set.

Participation:

8 laboratories participated

Results:

The results were assessed based on following cut offs:

	Cut off	No. of labs (total 8)
Optimal	>90%	2
Good	>80%	2
Borderline	>70%	2
Poor	</=70%	2

Antibody details and performance analysis:

Clone	RTU	Conc	Vendor	N	Optimal	Good	Borderline	Poor
mAb clone V9	5		Dako	2			1	1
			Ventana	1		1		
			Cellmarque	1				1
			Biogenex	1		1		
		3	Dako	1	1			
			Biocare	2	1			1

The cause of insufficient staining was:

- Issues with concentration of antibody. RTUs in general had very differing behavior across the board (some having weak stain, some brighter and some having immense background)

Conclusions:

- Clone V9 is a good clone
- Concentrated antibody had distinctly better performance than RTU.
- Retrieval buffer at ph 9.0 and low pH were used in this run. Two out of three who used low pH had borderline scores. More alkaline buffer has some advantage.
- Appendix with well-developed germinal centre seems to be a recommendable and reliable positive control, in which virtually all the peripheral B- and T-cells must show at least a moderate and distinct cytoplasmic staining reaction. Tonsil with similar germinal centre could be an alternate. If only endothelial cells and germinal centre macrophages are demonstrated, the protocol most likely is too insensitive to detect VIM in low expressor neoplasia.

Critical parameter analysis:

Critical parameters were assessed based purely on the antibody performance and protocol setting (i.e staining intensity and proportion only while excluding integrity of sections, artefacts, background etc.)

Total 4 laboratories had optimal or good staining performance in critical parameter analysis. Following parameters were central to optimal staining performance:

RTU antibodies (2/4):

1 used manual method while the other used automation.

Optimal staining results obtained used HIER as retrieval method (2/2) in Tris-buffered saline at pH 9.0 (1/2) or Cell Conditioning Ventana (1/2).

The efficient heating time has been pressure cooker upto 1 whistle in 1/2 and 100 degree for 60 minutes conditioning in 1/2. Microwave was not used by any of them.

Primary antibody incubation time was 22 min and 40 min.

Secondary were all polymer based (Labvision and Ventana) with incubation time of 16 minutes.

No lab used expired primary antibody.

Concentrated antibodies (2/4):

Both used manual method.

HIER as retrieval method in Tris-buffered saline at pH 9.0

The heating time has been 95 degrees C for 30 minutes. Microwave was used by one. The other used Biocare decloaking chamber.

Primary antibody incubation time was 30 min with Dako and it was 60 min with Biocare.

Both used 'expired' antibodies in year 2011 and Dec. 2014.

Both used antibody in dilution of 1:300

Secondary antibody was Dako Envision, polymer based with incubation time of 30 minutes. The other lab used MACH1 Biocare with incubation time of 30 min.

Recommended protocol for vimentin for RTU

Obtained in General Module, run 1

Primary antibody

Clone	V9
Producer	Venatana, 790-2917
Product no. (Lot no.)	D08595
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	22 min./RT

Epitope retrieval, HIER

Device	Company system, Benchmark Ventana
Buffer, pH	Cell conditioner EDTA based buffer, pH 8.4
Warm-up / heating max / resting time	100 degrees standard 60 minute conditioning

Visualization system

Method	Polymer conjugate
Producer, product no.	UV HRP UNIV MULT, Ventana, 760-500, 28/7/2016
Incubation time / temperature	16 min./RT

Chromogen

Type	
Enhancement, type	Copper suphate

Immunostainer

Type	Automated. Benchmark-Ventana

Abbreviations HIER: Heat induced epitope retrieval, RT: Room temperature

Recommended protocol for Vimentin for concentrated antibody

Obtained in General Module, run 1

Primary antibody

Clone	V9
Producer	Biocare
Product no. (Lot no.)	061413
Dilution	1:300
Diluent buffer and additive(s)	NA
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Biocare, Decloaking chamber
Buffer, pH	Tris-EDTA buffer, pH 9
Warm-up / heating max / resting time	95 Degree 30 mins. 0.1 psi

Visualization system

Method	Polymer conjugate
Producer, product no.	MACH1, BIOCARE MEDICAL, CAT No: M1U539L10, Batch No: 080514
Incubation time / temperature	30 min./RT

Chromogen

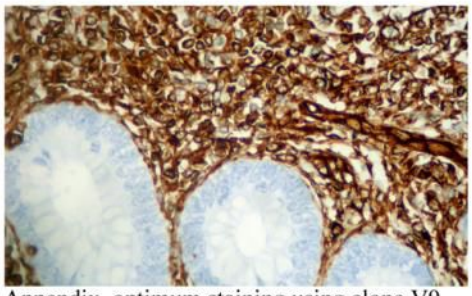
Type	DAB
Enhancement, type	-

Immunostainer

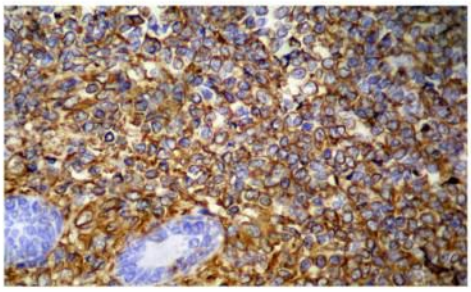
Type	Manual

Abbreviations HIER: Heat induced epitope retrieval, RT: Room temperature

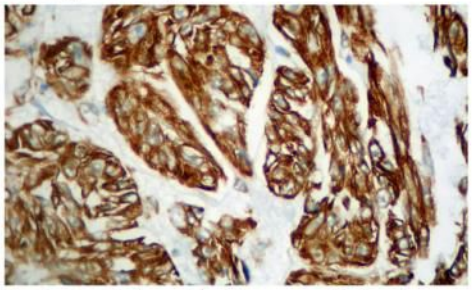
Steps in brief: 1)Fixation 2) Deparffinization 3) rehydration 15 mins(5x3) 4) Wash under running water 10 mins 5) Antigen retrieval 30 mins 6) Bring to RT 10 mins 7) Rinse in deionized water 8)Rinse with TBS 9) 3% H2O2 10 mins 10) Rinse twice in TBS 11) Background quencher 10 mins (12) Rinse in TBS (13) Primary antibody incubation 60 mins (14) Rinse with TBS twice 15) Probe incubation 10 mins 16) Rinse with TBS(17)HRP 30 mins 18)Rinse with TBS thrice 19) DAB chromogen 5 mins (20) Wash with deionized water (21)Counterstain with haematoxylin 5 mins 22)Clear with Xylene and mount



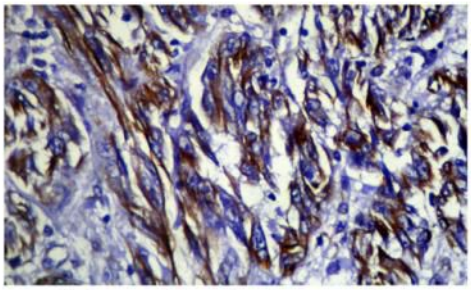
Appendix, optimum staining using clone V9 in 1:300 dilution with HIER at 95 C for 30 min. Note the crisp staining of lymphocytes and no staining of the crypts.



Appendix, sub-optimal staining using clone V9, RTU. HIER time was 90 C for 20 min.

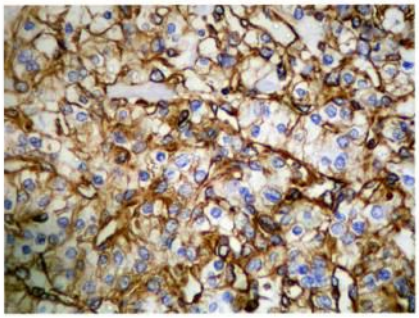


Optimal staining of melanoma cells by the same participant as above in optimal appendix staining.

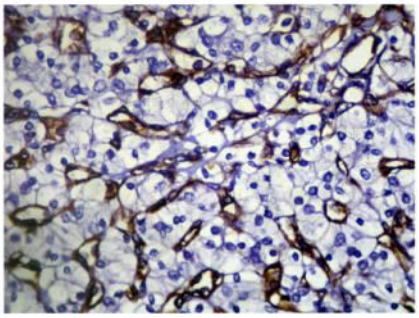


Insufficient staining of melanoma cells. Note too intense counterstain also.

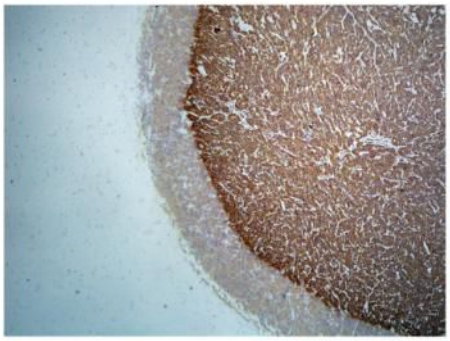
P.T.O.



Optimum staining of RCC case. Note the faint but distinct staining of tumor cells.



Suboptimal staining of RCC case. The faint staining is not appreciable, partly contributed by too bright counterstain.



Drying artefact at periphery. Use of humidified chamber with closed lid could have avoided that.