Assessment Run 30 2010

Vimentin (VIM)

The slide to be stained for VIM comprised:
1. Tonsil, 2. Kidney, 3. Seminoma, 4. Melanoma, 5. Renal cell carcinoma
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a VIM staining as optimal included:

- 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 the tonsil.
- A moderate to strong cytoplasmic staining of virtually all the neoplastic cells of the melanoma.
  An at least weak to moderate cytoplasmic and membranous staining of the neoplastic cells of the renal cell carcinoma.
- No staining reaction in the squamous epithelial cells of the tonsil and the epithelial cells of the proximal tubules in the kidney.

164 laboratories participated in this assessment. 83 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for VIM, run 30**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **V9**	48 7 7 5 1 1 1 1 1	Dako BioGenex Novocastra/ Leica NeoMarkers Cell Marque BioCare Monosan Zymed Zytomed	45	17	6	4	86 %	91 %
mAb clone **Vim 3B4**	31 1 1	Dako APR Progen	8	16	9	0	73 %	94 %
rmAb clone **SP20**	4 1	NeoMarkers Master Diagnostica	2	0	3	0	20 %	100 %
Ready-To-Use Abs								
mAb clone **V9, 790-2917**	30	Ventana	11	19	0	0	100 %	100 %
mAb clone **V9, IR630**	13	Dako	12	1	0	0	100 %	100 %
mAb clone **V9, AM074-5M**	2	BioGenex	1	1	0	0	-	-
mAb clone **V9, PM048**	1	Biocare	0	1	0	0	-	-
mAb clone **V9, 347M-18**	3	Cell Marque	0	1	1	1	-	-
mAb clone **V9, N1521**	1	Dako	0	1	0	0	-	-
mAb clone **Vim 3B4, 760-2512**	3	Ventana	0	0	3	0	-	-
mAb clone **SRL, PA0033**	1	Novocastra/Leica	0	0	0	1	-	-

Total	164		79	57	22	6	-	-
Proportion			48 %	35 %	13 %	4 %	83 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **V9**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (16/21)*, TRS pH 9 (Dako) (5/6), TRS pH 9 (3-in-1,Dako) (7/8), Cell Conditioning 1 (BenchMark, Ventana) (5/13), Bond Epitope Retrieval Solution 2 (Bond, Leica) (5/6) Bond Epitope Retrieval Solution 1 (Bond, Leica) (3/4), Diva Decloaker (Biocare) (1/2) or Citrate pH 6 (3/7) as the retrieval buffer. The mAb was typically diluted in the range of 1:100– 1:8.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 60 out of 66 (91 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

mAb clone **Vim 3B4**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/4), TRS pH 9 (Dako) (1/4), TRS pH 9 (3-in-1,Dako) (1/7), Cell Conditioning 1 (BenchMark, Ventana) (2/9), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/2) or Diva Decloaker (Biocare) (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:150 – 1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 17 out of 18 (94 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **SP20**: The protocols giving an optimal result were based on heat induced epitope retrieval (HIER) using TRS pH 9 (Dako) (1/1) or EDTA/EGTA pH8 (1/1) as the retrieval buffer. The mAb was diluted in the range of 1:100– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **V9** (prod. no. 790-2917, Ventana): The protocols giving an optimal result were all based on HIER using mild or standard Cell Conditioning 1, an incubation time of 12-52 min in the primary Ab and UltraView (760-500) as the detection system. 1 lab used amplification. Using these protocol settings all of 23 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **V9** (prod. no. IR630, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9, TRS pH 9 (3-in-1) or TRS pH 6.1 and an incubation time of 20 min in the primary Ab and a 2-step polymer system, EnVision (K4007), EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 12 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **V9** (prod. no. AM074-5M, BioGenex): The protocol giving an optimal result was based on HIER using mild Cell Conditioning 1, an incubation time of 32 min in the primary Ab and iView (760-091) as the detection system.

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Omission of HIER
- Inappropriate epitope retrieval - i.e., proteolysis.

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative reaction of the cells expected to be demonstrated. The majority of the laboratories were able to demonstrate VIM in high antigen expressing cells such as endothelial cells, germinal centre macrophages in the tonsil and the neoplastic cells of the melanoma, whereas low antigen expressing cells like lymphocytes and the neoplastic cells of the renal cell carcinoma and in particular the seminoma was more challenging and required an optimally calibrated protocol.
In this assessment HIER was mandatory to obtain an optimal staining result. Both alkaline HIER buffers and Citrate pH 6 could be used. The most widely used Abs were the mAb clones V9 and Vim 3B4. Both could be used to give an optimal staining. However a significantly higher proportion of optimal staining results were seen for the mAb clone V9, as 63 % of the protocols based on this clone as concentrate were assessed as optimal, compared to 24 % for the mAb clone Vim 3B4.
For the mAb clone V9, the pass rates were 100 % of the Ready-To-Use (RTU) formats/systems for VIM from

Ventana and Dako.
Omission of HIER or enzymatic pre-treatment could not be used to obtain an optimal result irrespective of the mAb clone applied - typically the morphology was impaired due to excessive digestion of the membranes of the lymphocytes and the sparse cytoplasm of the neoplastic cells of the seminoma.
6 out of 7 laboratories using the mAb clone Vim 3B4 with proteolysis obtained an insufficient result (and none was optimal). Thus, the vendors' data sheets for the mAb clone Vim 3B4 gives misleading information concerning the epitope retrieval: Ventana recommends proteolysis as pre-treatment whereas Dako indicates that either proteolysis or HIER can be used.

Tonsil 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. If only endothelial cells and germinal centre macrophages are demonstrated, the protocol most likely is too insensitive to detect VIM in low expressor neoplasias.
This was the 2nd assessment of VIM in NordiQC, as VIM also was assessed in run 12, 2004 (table 2). The proportion of sufficient results slightly decreased from 94 % to 83 %, which probably is due to a combination of new material circulated and a significant increase in the number of new participants.

Table 2. **Proportion of sufficient results for VIM in the two NordiQC runs performed**

	Run 12 2004	Run 30 2010
Participants, n=	79	164
Sufficient results	94 %	83 %

Conclusion

The mAbs clones Vim 3B4 and V9 and the rmAb clone SP20 can all be used to obtain an optimal staining for VIM. The mAb clone V9 both as concentrate and RTU format give the highest proportion of optimal results. HIER, irrespective of the clone applied, is mandatory to obtain an optimal staining. Proteolysis should not be used. Tonsil is a recommended positive control: Virtually all the peripheral B- and T-cells must show at least a moderate cytoplasmic staining reaction, while no staining reaction should be seen in the squamous epithelial cells.

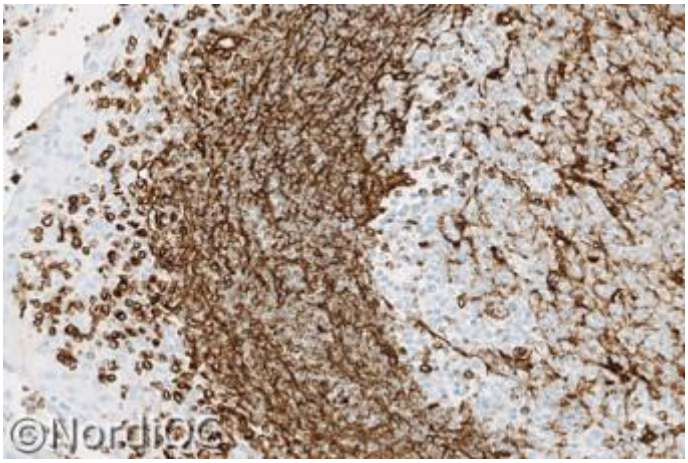

Fig. 1a
Optimal VIM staining of the tonsil using the mAb clone V9 carefully calibrated after HIER. The intraepithelial lymphocytes, the mantle zone B-cells and the germinal centre macrophages show a strong and distinct staining. No staining is is seen in the squamous epithelial cells.

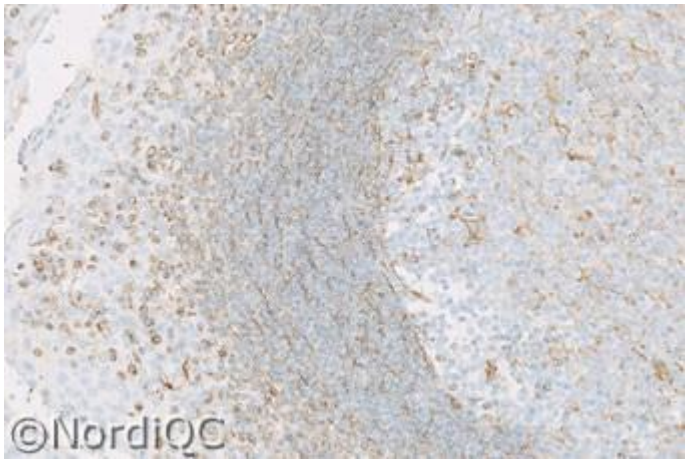

Fig. 1b
Insufficient VIM staining of the tonsil, using the mAb clone V9 in a too low concentration – same field as in Fig. 1a. Both the proportion and intensity of the demonstrated cells is significantly reduced. Also compare with Figs. 2b & 3b – same protocol.

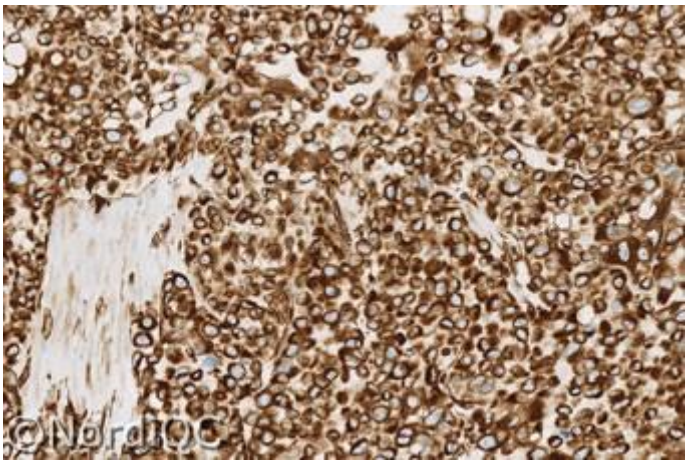

Fig. 2a
Optimal VIM staining of the melanoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining.

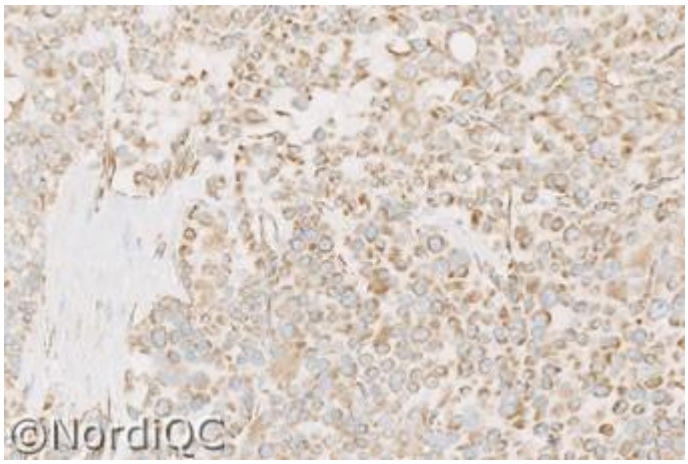

Fig. 2b
Insufficient VIM staining of the melanoma using same protocol as in Fig. 1b - same field as in Fig. 2a.
The majority of the neoplastic cells only show a weak or equivocal staining. Also compare with Fig. 3b - same protocol.

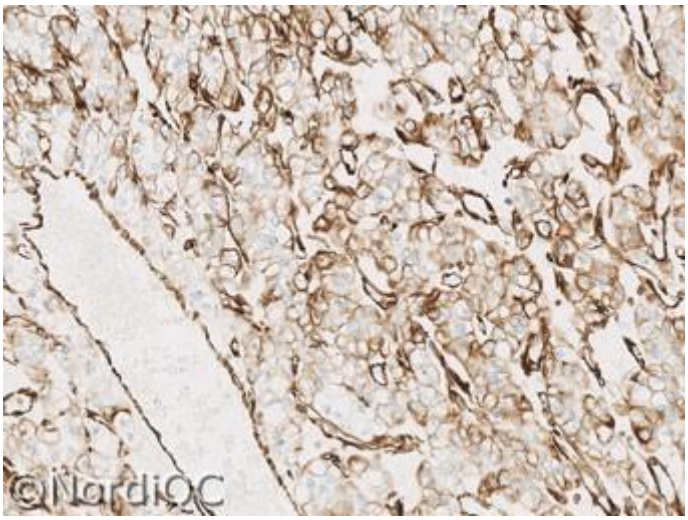

Fig. 3a
Optimal VIM staining of the renal cell carcinoma using same protocol as in Figs. 1a. & 2a. The majority of the neoplastic cells show a weak to moderate predominantly membranous staining.

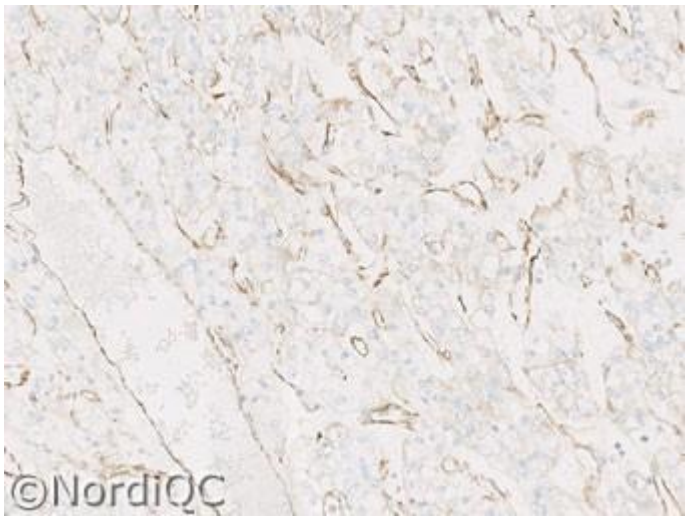

Fig. 3b
Insufficient VIM staining of the renal cell carcinoma using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a.
Virtually no staining reaction is seen in the neoplastic cells. Only the endothelial cells are demonstrated.

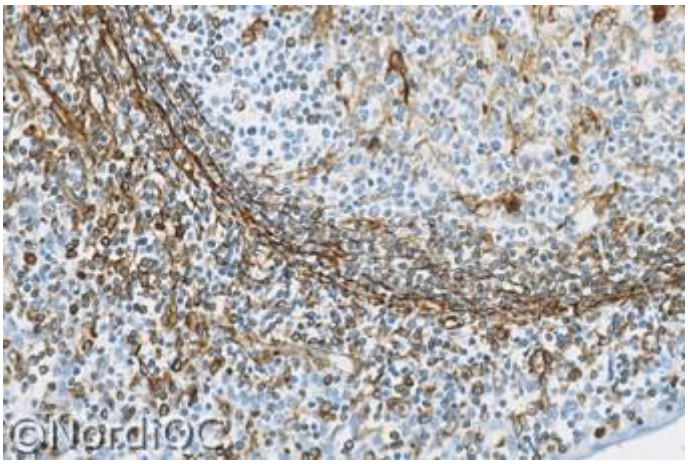

Fig. 4a
Insufficient VIM staining of the tonsil using the mAb clone 3B4 with proteolytic pre-treatment. The germinal centre macrophages and endothelial cells show a moderate staining reaction, whereas the lymphocytes virtually are negative due to excessive proteolysis and digestion of the fragile membranes. Also compare with Fig. 4b - same protocol.

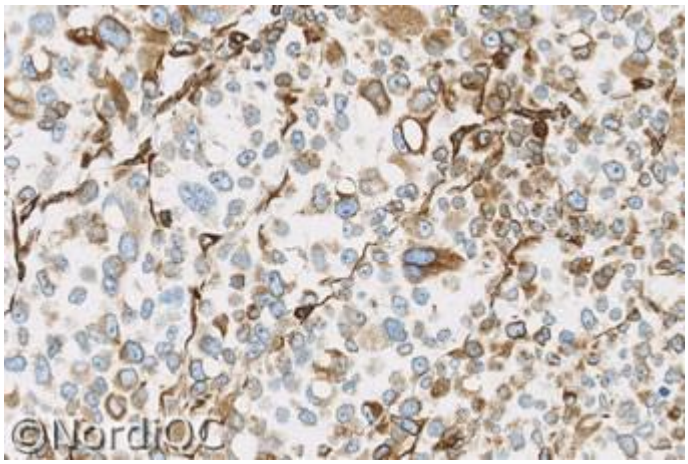

Fig. 4b
Insufficient VIM staining of the melanoma using same protocol as in Fig. 4a - mAb clone 3B4 with proteolytic pre-treatment. The neoplastic cells only show a weak and equivocal staining as the cytoplasmic compartment is digested and only the nuclei are left in the neoplastic cells. Also compare with Fig. 2a - same tissue.

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