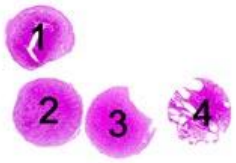
Assessment Run 21 2007

Desmin (DES)



The slide to be stained for desmin (DES) comprised:
1. Appendix, 2. Embryonal rhabdomyosarcoma, 3. Leiomyosarcoma,
4. Mesothelioma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a DES staining as optimal included:

- A strong, distinct cytoplasmic staining of all the smooth muscle cells in the lamina muscularis mucosae and muscularis propria, in most smooth muscle cells of the submucosal vessels in the appendix and scattered smooth muscle cells of large vessels..
- A strong, distinct cytoplasmic staining in the normal mesothelial cells, while the malignant mesothelioma should be negative.
- A strong, distinct cytoplasmic staining of virtually all the neoplastic cells of the leiomyosarcoma and the majority of both the round and spindle shaped neoplastic cells of the rhabdomyosarcoma.

119 laboratories submitted stains. At the assessment 57 achieved optimal marks (48 %), 38 good (32 %), 18 borderline (15 %) and 6 poor marks (5 %).

The following Abs were used:
mAb clone **D33** (Dako n=78, NeoMarkers n=5, Monosan n=3, BioCare n=1, Linaris n=1)
mAb clone **DE-R-11** (Ventana n=11, Dako n=11, Novocastra n=4)
mAb clone **33** (BioGenex n=3, Biomeda n=1)
mAb clone **ZC18** (Zymed n=1)

Optimal staining for DES in this assessment was obtained with the mAb clone **D33** (41 out of 88), clone **DE-R-11** (15 out of 26) and clone **ZC18** (1 out of 1).

D33: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (31/56)*, Citrate pH 6 (3/6), Cell Conditioning 1 (BenchMark, Ventana) (3/11), Bond Epitope Retrieval Solution 2 (Bond, Leica-Microsystems) (2/3) or EDTA/EGTA pH 8 (2/3) as heating buffer. The mAb was typically diluted in the range of 1:20 – 1:300 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) antibody. Using these protocol settings 64 out of 74 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

DE-R-11: Both HIER, proteolysis and a combination of HIER and proteolysis could be used to produce an optimal staining. Using HIER both Tris-EDTA/EGTA pH 9 (4/6)*, Cell Conditioning 1 (BenchMark, Ventana) (5/6), Citrate pH 6 (1/2), EDTA/EGTA pH 8 (1/1), and Target Retrieval Solution pH 6,1 (TRS, Dako) (1/1) could be used as heating buffer.
Using proteolytic pre-treatment Protease 1 (BenchMark, Ventana) (1/6) and Bond Enzyme Pretreatment Kit (Bond, Leica-Microsystems) (1/1) could be used.
Also the combination of HIER in Cell Conditioning 1 and Protease 3 (BenchMark, Ventana) (1/1) could be used.
The Ab was diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed or as a RTU antibody. Using these protocol settings 20 out of 23 (87 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

It seemed that HIER was slightly more robust than proteolytic pre-treatment when using the clone DE-R-11, as 89 % (16 out of 18 laboratories) using HIER as pre-treatment produced an sufficient staining marked optimal or good compared to 57 % (4 out of 7 laboratories) using proteolytic pre-treatment.

ZC18: The protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 and a dilution of 1:100.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Omission of epitope retrieval

In the assessment and in concordance with the observations in the previous DES assessment (run 5 2002) almost all laboratories were able to demonstrate DES in the smooth muscle cells in the muscle layers in the appendix, whereas the prevalent feature of the insufficient staining in this run was a too weak or false negative staining of the rhabdomyosarcoma of especially the spindle shaped neoplastic cells, which seemed to express a lower concentration of DES than the round myoblastic cells.
A too weak or false negative staining was seen in 92 % of the insufficient results (22 out of 24), while in 8 % (2 out of 24) a poor signal/noise ratio was observed.
It was difficult to identify a robust and easy interpretable stain quality indicator in the material distributed for this assessment. However it was clear, that normal muscle cells in the muscle layers in the appendix could not be recommended, as these cells were demonstrated in virtually all protocols. The best choice seemed to be the smooth muscle cells in the submucosal vessels in the appendix, which should show an as strong as possible reaction without any reaction in cells not expected to stain.

The results are comparable with those of run 5 2002, where 42 laboratories participated. Also in run 5 it was concluded that both the clones D33 and DE-R-11 were appropriate Abs for DES and that HIER was found to be the best pre-treatment.

Conclusion
The mAb clones D33, DE-R-11 and ZC18 are all useful for the demonstration of desmin. Efficient epitope retrieval, especially use of HIER, is important to obtain an optimal result. The concentration of the primary Ab should be carefully calibrated. Appendix is an appropriate control: The submucosal arterioles must show a strong staining reaction while no staining should be seen in the epithelial cells.

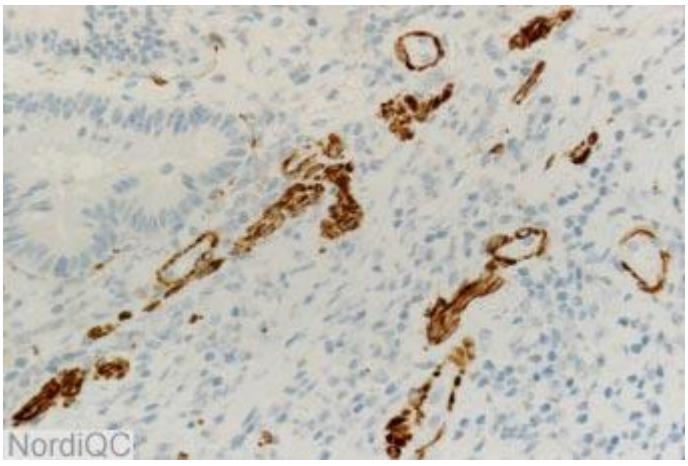


Fig. 1a
Optimal DES staining of the appendix using the mAb clone D33 with HIER. Both the smooth muscle cells of the appendiceal lamina propria and most of the smooth muscle cells of the submucosal vessels show a distinct staining.

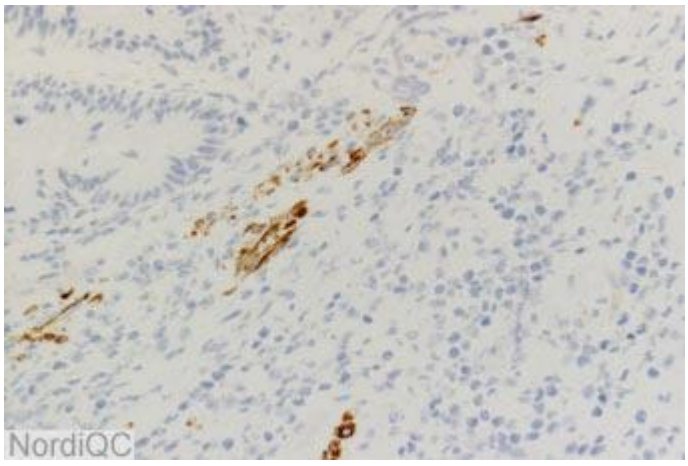


Fig. 1b
Insufficient DES staining of the appendix using the mAb clone D33 with HIER, but in a too low concentration – same field as in Fig. 1a. Only the smooth muscle cells of the appendiceal lamina propria show a distinct staining, while the smooth muscle cells of the submucosal vessels are unstained. Also compare with Fig. 2b.

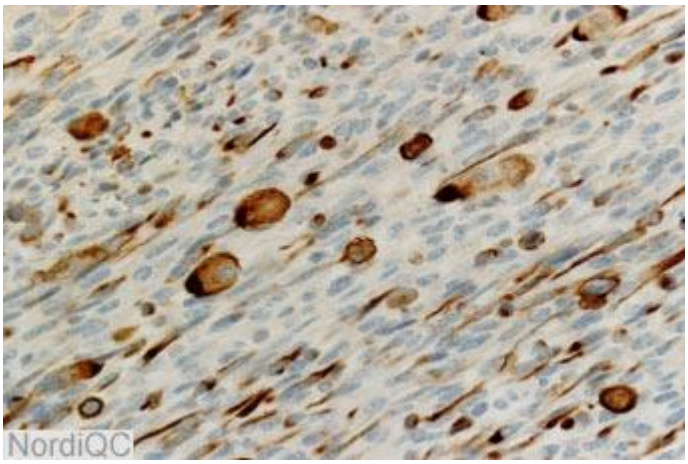


Fig. 2a
Optimal DES staining of the embryonal rhabdomyosarcoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a strong distinct reaction with no background reaction.

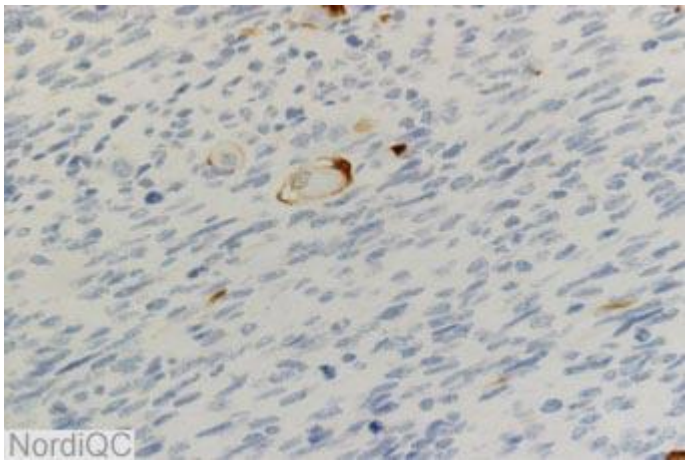


Fig. 2b
Insufficient DES staining of the embryonal rhabdomyosarcoma using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 2a.

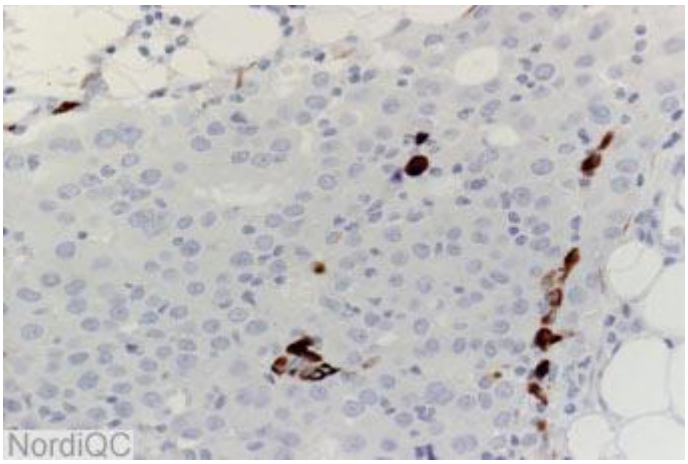


Fig. 3a
Optimal DES staining of the mesothelioma using the mAb clone DE-R-11 with HIER in an optimally calibrated protocol. The remnants of the normal reactive mesothelial cells are stained, while the neoplastic cells are negative.

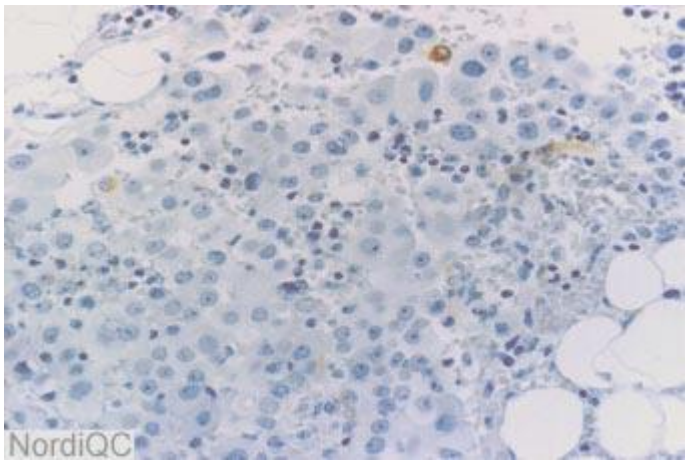


Fig. 3b
Insufficient DES staining of the mesothelioma using the mAb clone DE-R-11 with proteolytic pre-treatment and in a too low sensitive protocol. Virtually all cells are negative. Same field as in Fig. 3a.

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