Assessment Run 35 20125

Desmin (DES)

Material
The slide to be stained for DES comprised:

1. Appendix, 2. Placenta, 3. Leiomyoma, 4. Rhabdomyosarcoma, 5. Leiomyosarcoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a DES staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all the smooth muscle cells in the lamina muscularis mucosae and muscularis propria of the appendix
- An at least weak to moderate cytoplasmic staining reaction in most smooth muscle cells of the vessels in all the specimens tested.
- A moderate to strong, distinct cytoplasmic staining reaction of virtually all the neoplastic cells of the leiomyoma and the leiomyosarcoma.
- An at least weak to moderate cytoplasmic staining reaction of the majority of both the round and spindle shaped neoplastic cells of the rhabdomyosarcoma.
- No staining reaction of the appendiceal epithelial cells and the cytotrophoblastic and syncytiotrophoblastic cells in the placenta

194 laboratories participated in this assessment. 85 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1.**Abs and assessment marks for DES, run 35**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **D33**	88 7 2 1 1 1 1	Dako Thermo/NeoMarkers BioGenex Biocare Master Diagnostica Monosan Zytomed	45	34	20	2	78 %	79 %
mAb clone **DE-R-11**	15 1	Leica/Novocastra Dako	11	4	1	0	94 %	94 %
mAb clone **ZC18**	2	Invitrogen/Zymed	0	1	1	0	-	-
pAb **RB-9014-P**	1	Thermo/NeoMarkers	1	0	0	0	-	-
Ready-To-Use Abs:								
mAb clone **D33 IR/IS606**	29	Dako	16	11	2	0	93 %	93 %
mAb clone **D33 E057**	2	Linaris	1	0	1	0	-	-
mAb clone **D33 PM036**	1	Biocare	1	0	0	0	-	-
mAb clone **D33 243M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **D33 N1526**	1	Dako	0	1	0	0	-	-
mAb clone **DE-R-11 760-2513**	38	Ventana	24	13	1	0	97 %	100 %
mAb clone **DE-R-11 PA0032**	1	Leica/Novocastra	1	0	0	0	-	-
pAb **RB-9014-R7**	1	Thermo/NeoMarkers	0	0	0	1	-	-

Total	194	100	65	26	3	-	
Proportion		52 %	33 %	13 %	2%	85 %	

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 **D33**: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (13/17)*, TRS pH 9 (Dako) (1/9), Cell Conditioning 1 (CC1; BenchMark, Ventana) (12/35), Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (8/8), BERS 1 (Bond, Leica) (2/2), Diva Decloaker pH 6.2 (Biocare) (2/3), Tris-EDTA/EGTA pH 9 (5/17) or Citrate pH 6 (2/5) as the retrieval buffer.
The mAb was typically diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 74 out of 94 (79 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this reagent)

mAb clone **DE-R-11**: the protocols giving an optimal result were based on either HIER or enzymatic pre-treatment. In total 11 laboratories obtained an optimal staining result using one of these two pre-treatment procedures.
9 out of the 11 laboratories used HIER in either TRS pH 9 (3-in-1) (Dako) (1/1), CC1 (BenchMark, Ventana) (2/3), BERS 2 (Bond, Leica) (5/5) or BERS 1 (Bond, Leica) (1/2) as the retrieval buffer.
The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 out of 11 (100 %) laboratories produced a sufficient staining (optimal or good).
2 laboratories used enzymatic pre-treatment in either Protease 1 (Benchmark, Ventana) (1/2) or Bond Enzyme 1 (Leica) (1/3).
The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 4 (75 %) laboratories produced a sufficient staining (optimal or good).

pAb **RB-9014-P**: The protocol giving an optimal result was based on HIER using Citrate pH 6 (1/1) as the retrieval buffer. The pAb was diluted 1:800.

Ready-To-Use Abs
mAb clone **D33** (prod. no. IS/IR606, Dako): The protocols giving an optimal result were typically based on HIER in PT-Link (heating time for 10-20 min at 95-97°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20 min in the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as the detection system. Using these protocol settings 25 out of 27 (93 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **D33** (prod.no. PM036, BioCare): The protocol giving an optimal result was based on HIER in a Pressure Cooker using Diva Decloaker pH 6.2 as HIER buffer, an incubation time of 30 min in the primary Ab and MACH4 Universal HRP Polymer as the detection system.

mAb clone **DE-R-11** (prod. no. 760-2513, Ventana):
21 out of 24 optimal protocols were based on HIER using short, mild or standard CC1, an incubation time of 8-56 min in the primary Ab and iView (760-091), UltraView (760-500) or OptiView (760-700) as the detection system. Using these protocol settings 23 out of 23 (100 %) laboratories produced a sufficient staining (optimal or good).
1 lab used Protease 1 (8 min) as pre-treatment, an incubation time of 32 min in the primary Ab, and UltraView (760-500) + amplification kit as the detection system. Using this protocol setting 1 out of 1 (100 %) laboratories produced an optimal staining.
2 labs used a combined pre-treatment with Protease 3 (4 - 8 min.) and HIER in CC1 for 20 min., an incubation time of 28-32 min in the primary Ab and UltraView (760-500) as the detection system.
Using these protocol settings 2 out of 2 (100 %) laboratories produced an optimal staining.

mAb clone **DE-R-11** (product. no. PA0032, Leica/Novocastra): The protocol giving an optimal result was based on HIER using BERS 2 (Bond, Leica), an incubation time of 15 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system.

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary Ab
- Insufficient HIER (too short HIER time)

In this assessment and in concordance with the previous NordiQC assessments for DES, the prevalent feature of an insufficient staining was a too weak or false negative staining reaction of the cells expected to be demonstrated. A too weak or false negative staining reaction was thus seen in 97 % of the insufficient results (28 out of 29).
Virtually all the laboratories were able to demonstrate DES in high antigen expressing structures such as the smooth muscle cells in muscularis propria in the appendix and the neoplastic cells of the leiomyoma and the leiomyosarcoma, whereas the demonstration of DES in the neoplastic cells and in particular the spindle shaped cells of the rhabdomyosarcoma expressing less DES was much more challenging and required an optimally calibrated protocol.
Both the mAb clones D33, DE-R-11 and a polyclonal Ab RB-9014-P could be used to obtain an optimal staining result.
Applying the most widely used mAb clone D33 as a concentrate an optimal staining could only be obtained by using HIER as pre-treatment. It was observed that the pass rate was highly influenced by the sensitivity of the detections systems used. If a 2-step polymer or multimer based detection system e.g., EnVision Flex, Dako or UltraView, Ventana was used, 41 out of 60 laboratories obtained a sufficient staining result (69%) out of which 18 (30%) were assessed as optimal. If a more sensitive 3-step polymer or multimer based detection system e.g., EnVision Flex+, Bond Refine (Leica) or UltraView + amplification was used, 37 out of 39 laboratories produced a sufficient staining result (95%) of which 25 (64%) were optimal.
The mAb clone DE-R-11 was the 2' most widely used Ab for DES and an optimal staining result could both be obtained by using HIER, enzymatic pre-treatment and a combination of these two retrieval procedures. HIER as single pre-treatment or in combination with enzymatic pre-treatment was found to give a higher proportion of optimal staining results and in general raised the sensitivity compared to the use of enzymatic pre-treatment. If the mAb clone DE-R-11 was used with HIER a pass-rate of 100% was seen for the 33 laboratories using this pre-treatment procedure- irrespective of all other protocol parameters applied including the choice of detection system, titre selected of the primary Ab as a concentrate or use of a Ready-To-Use format. 31 of these 33 laboratories obtained an optimal score (94 %). If enzymatic pre-treatment was applied as single pre-treatment an overall pass-rate of 86 % was seen for the 14 laboratories using this retrieval setting. The proportion of optimal results was significantly reduced as on only 21 % obtained an optimal score. In this context it has to be mentioned that the main provider of the Ready-To-Use format prod. no 760-2513 of the mAb clone DE-R-11, Ventana, recommends enzymatic pre-treatment as epitope retrieval for the product in the data sheet.

As concluded in the previous assessments for DES it is difficult to identify a robust and easy interpretable critical stain quality indicator for DES. Normal muscle cells in the muscle layers in the appendix can not be recommended, as these cells were demonstrated in virtually all protocols and have a very high expression of DES and can not be used to identify a protocol with a too low sensitivity. The best choice seems to be the smooth muscle cells in the vessels in the appendix, which should show an as strong as possible staining reaction without any reaction in cells not expected to be demonstrated as e.g. the appendiceal epithelial cells. The staining pattern in these smooth muscle cells will typically be seen as a patchy intracytoplasmic staining reaction and not a consistent homogenous staining reaction as seen for alpha smooth muscle cell actin in these cells.

This was the 3rd assessment of DES in NordiQC (Table 2) and a nearly constant pass rate has been achieved in the 3 runs performed.

Table 2. **Proportion of sufficient results for DES in the three NordiQC runs performed**

	Run 5 2001	Run 21 2007	Run 35 2012
Participants, n=	42	119	194
Sufficient results	86 %	80 %	85 %

Conclusion
The mAb clones D33, DE-R-11 and the pAb RB-9014-P can all be recommended for the demonstration of DES. For all clones HIER was found to preferable as epitope retrieval giving a higher sensitivity compared to the use of enzymatic pre-treatment.
Appendix is the recommended control for DES. An at least weak to moderate staining reaction must be seen in dispersed smooth muscle cells in the vessels. No staining shall be seen in the appendiceal epithelial cells.

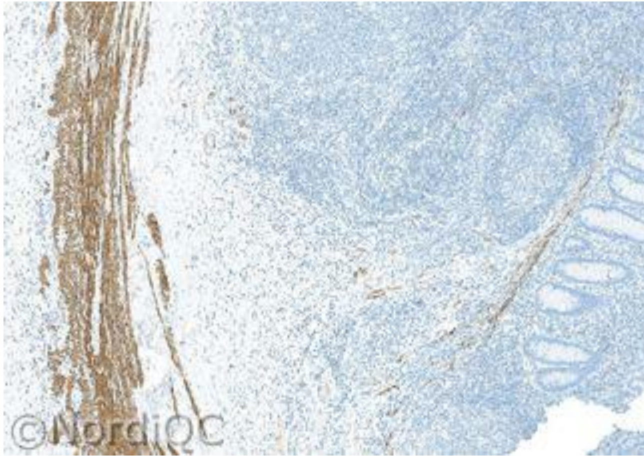

Fig. 1a
Optimal staining for DES of the appendix using the mAb clone DE-R-11 optimally calibrated and with HIER - low magnification x50.
The smooth muscle cells of lamina muscularis mucosae and muscularis propria show a strong staining reaction, which can be identidfied even at low magnification.
No background staining is seen.
Also compare with Figs. 2a - 4a – same protocol.

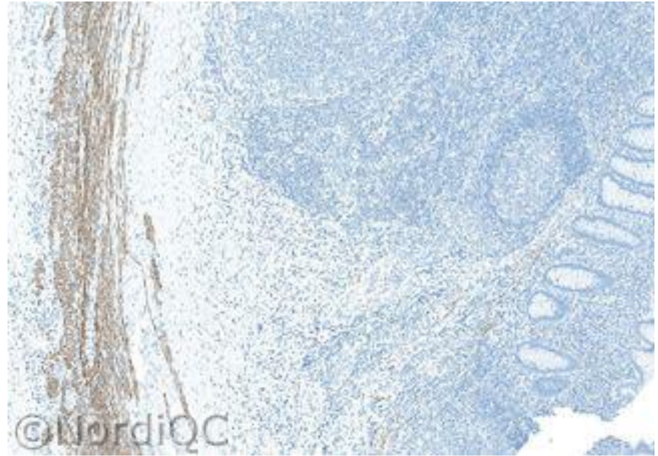

Fig. 1b
Insufficient staining for DES of the appendix using the mAb clone D33 too diluted and/or using insufficient HIER – same field as in Fig. 1a.
The smooth muscle cells of lamina muscularis mucosae and muscularis propria show a reduced intensity and proportion of positive cells compared to the result in Fig. 1a.
Also compare with Figs. 2b - 4b – same protocol.

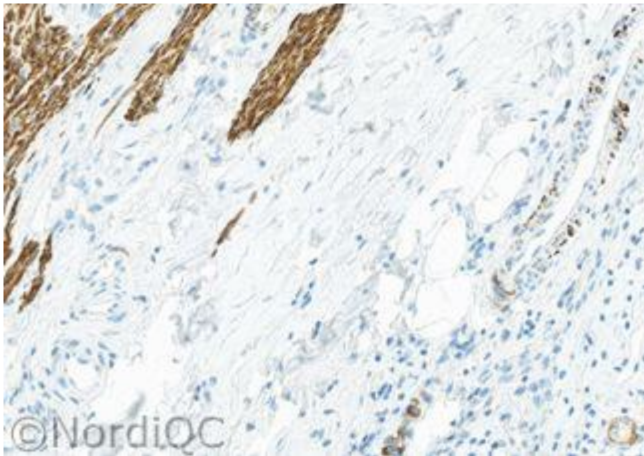

Fig. 2a
Optimal staining for DES of the appendix using the same protocol as in Fig. 1a. - high magnification x200
The smooth muscle cells of muscularis propria show a strong staining reaction (left top), but most important is the distinct staining reaction of scattered smooth muscle cells in large and small vessels (right).

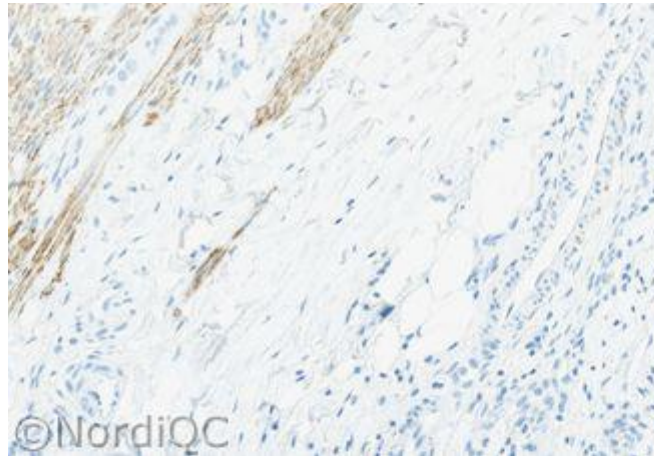

Fig. 2b
Insufficient staining for DES of the appendix using the same protocol as in Fig. 1b - same field as in Fig. 2a.
Only the smooth muscle cells of muscularis propria are demonstrated, while the smooth muscle cells of the vessels are false negative.

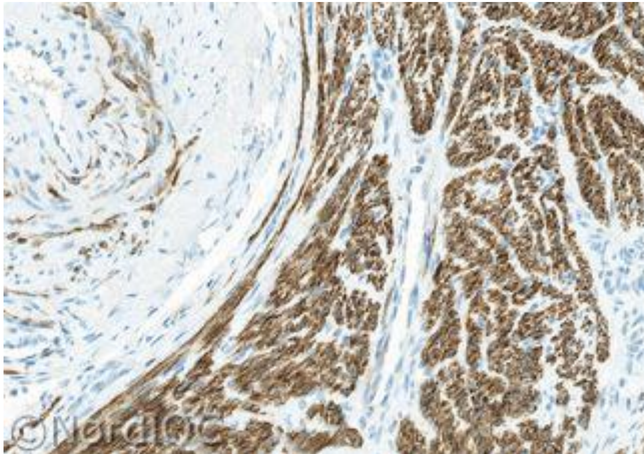

Fig. 3a
Optimal staining for DES of the leiomyoma using the same protocol as in Figs. 1a & 2a.
Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction. Also note the smooth muscle cells/myofibroblasts of the vessels (left top) show a distinct staining reaction.

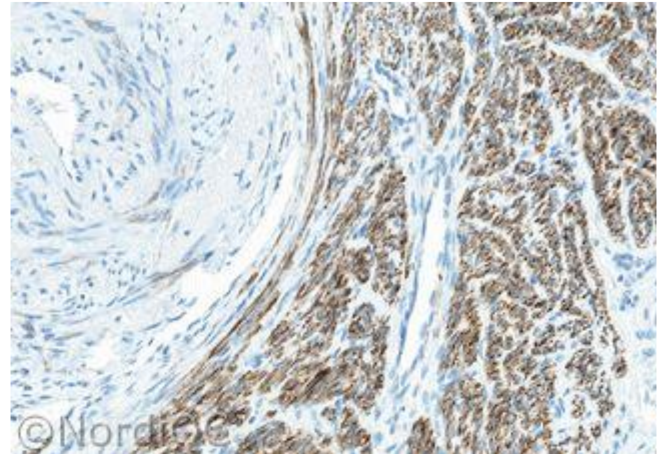

Fig. 3b
Insufficient staining for DES of the leiomyoma using the same protocol as in Figs. 1b & 2b - same field as Fig. 3a.
The neoplastic cells are demonstrated, whereas the smooth muscle cells/myofibroblasts of the vessels virtually are negative.

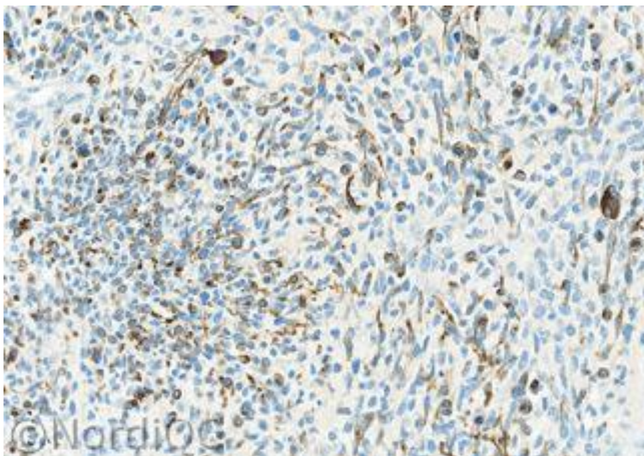

Fig. 4a
Optimal staining for DES of the rhabdomyosarcoma using same protocol as in Figs. 1a - 3a.
The majority of the neoplastic cells show a weak to strong and distinct cytoplasmic staining reaction. Both the round and spindle shaped neoplastic cells are demonstrated.

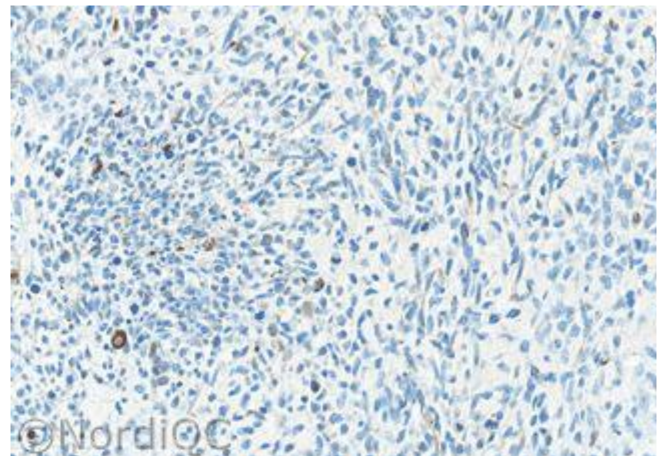

Fig. 4b
Insufficient staining for DES of the rhabdomyosarcoma using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a.
A significantly reduced intensity and proportion of positive cells is seen compared to the result in Fig. 4a. Especially the spindle shaped neoplastic cells only show an equivocal staining reaction.

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