Assessment Run B8 2009

Smooth Muscle Heavy Chain Myosin (SMH)

The slide to be stained for SMH comprised:
1. Tonsil, 2. Esophagus, 3. Breast hyperplasia, 4 – 5. Breast ductal carcinoma
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SMH staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining of the vascular smooth muscle cells in all the specimens and the esophageal lamina muscularis mucosae.
- An at least weak but distinct cytoplasmic staining of dendritic follicular cells of the germinal centres in the tonsil.
- A moderate to strong, distinct cytoplasmic staining of the glandular myoepithelial cells of the breast hyperplasia and in the remnants of the normal glands in the two breast carcinomas.

19 laboratories participated in this assessment. 79 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for SMH, run B8**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **SMMS-1**	14	Dako	5	5	3	1	71 %	75 %
mAb clone **S131**	1	Novocastra	1	0	0	0	-	-
Ready-To-Use Abs								
mAb clone **SMMS-1, IR066**	1	Dako	0	1	0	0	-	-
mAb clone **SMMS-1, 760-2704**	2 1	Ventana Cell Marque	2	1	0	0	-	-
Total	19		8	7	3	1	-	-
Proportion			42 %	37 %	16 %	5 %	79 %	-

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 **SMMS-1**: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (2/2)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako), (2/2) or Cell Conditioning 1 (BenchMark, Ventana) (1/4). The mAb was typically diluted in the range of 1:200– 1:1.500 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 8 (75 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **S131**: the protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Bond Epitope Retrieval Solution 2 (Bond, Leica) and a dilution of 1:25 of the mAb.

Ready-To-Use Abs
mAb clone **SMMS-1** (Ventana, prod. no 760-2704): One optimal protocol was based on HIER using standard Cell Conditioning 1 (Benchmark, Ventana), an incubation time of 32 min in the primary Ab and UltraView as the detection system. The other optimal protocol was based on a combined pre-treatment, using HIER in standard Cell Conditioning 1 followed by Protease 3 for 4 min. The primary Ab was incubated for 60 min and iView was used as the detection system.

The most frequent causes of insufficient stains were:
- Insufficient HIER – too short efficient heating time
- Use of Citrate pH 6 as HIER buffer.

In this assessment the prevalent feature of an insufficient staining was a generally too weak staining of the structures supposed to be demonstrated. This pattern was in particular observed in the myoepithelial cells of the glands in the breast hyperplasia, whereas virtually all laboratories could demonstrate SMH in the smooth muscle cells of lamina muscularis mucosae in the esophagus and in large vessels. It was seen, that both HIER and a combination of HIER followed by a mild proteolytic pre-treatment could be used to obtain an optimal result with the most commonly used mAb clone SMMS-1. When HIER was used alone as pre-treatment, it seemed to be mandatory to use an alkaline buffer, as none of the six laboratories using Citrate pH 6 obtained an optimal result (3 were assessed as good, 3 as insufficient) whereas 6 laboratories out of 11 using an alkaline buffer for HIER obtained an optimal result.

As control, the tonsil displayed the most informative reaction pattern as critical staining indicator for SMH. In the optimal protocols the dendritic follicular cells in the germinal centres showed a weak to moderate but distinct reaction, while the vascular smooth muscle cells showed a strong reaction. In the stains deemed too weak and insufficient the dendritic follicular cells typically were negative.

Conclusion
The mAb clones SMMS-1 and S131 are both recommendable Abs for the demonstration of SMH. HIER in an alkaline buffer is mandatory to obtain an optimal result. Tonsil is an appropriate control tissue: The dendritic follicular cells in the germinal centres must show at least a weak staining reaction without any staining of the lymphocytes.

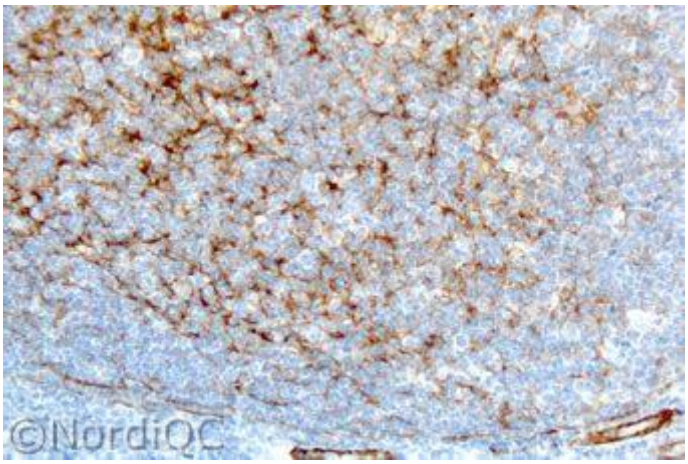

Fig. 1a
Optimal SMH staining of the tonsil using the mAb clone SMMS-1 correctly calibrated and with HIER in an alkaline buffer (Tris-EDTA pH 9). Both the smooth muscle cells of the vessels and dendritic follicular cells of the germinal centre show a moderate and distinct staining. No staining is seen in the lymphocytes.

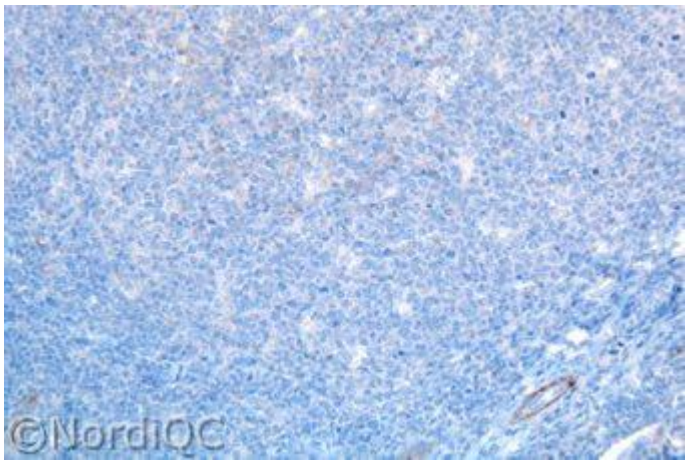

Fig. 1b
Insufficient SMH staining of the tonsil using the mAb clone SMMS-1 too diluted. Only the smooth muscle cells of the vessels are demonstrated, while the dendritic follicular cels of the germinal centre are virtually negative. Also compare with Fig. 2b - same protocol.

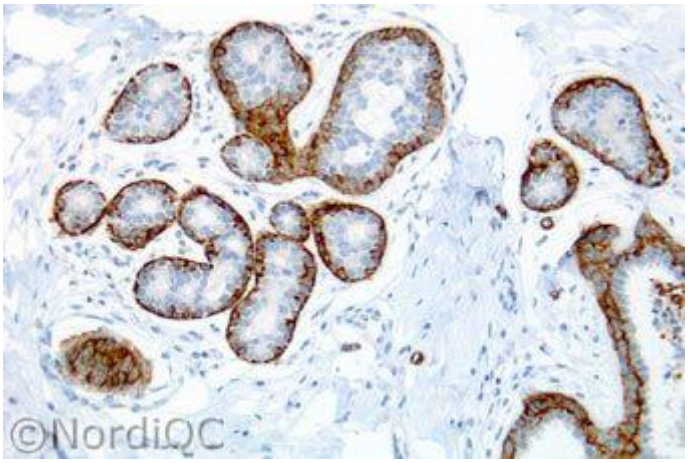

Fig. 2a
Optimal SMH staining of the breast hyperplasia using same protocol as in Fig. 1a. Virtually all the glandular myoepithelial cells show a strong and distinct reaction with no background reaction.

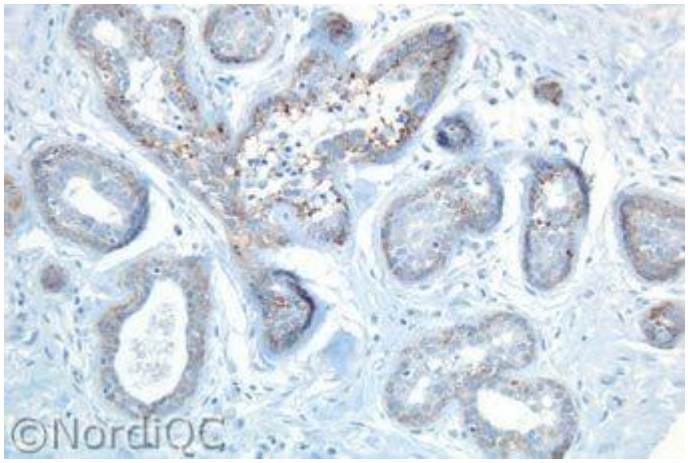

Fig. 2b
Insufficient SMH staining of the breast hyperplasia using same protocol as in Fig. 1b.
The myoepithelial cells only show a weak, ambiguous staining.

SN/HN/MV/LE 4-12-2009