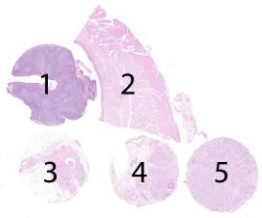
Assessment Run 50 2017

SMH (Myosin, smooth muscle heavy chain)



Material
The slide to be stained for SMH comprised:

1.Tonsil, 2. Esophagus, 3. Breast hyperplasia, 4. Breast ductal carcinoma in situ (DCIS), 5. Breast ductal carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing SMH staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining reaction of virtually all vascular smooth muscle cells in all specimens and smooth muscle cells in esophageal lamina muscularis mucosae.
- An at least weak but distinct cytoplasmic staining of the vast majority of follicular dendritic cells in the tonsillar germinal centers.
- A moderate to strong, distinct cytoplasmic staining reaction of myoepithelial cells lining ductal glands in the breast hyperplasia and in remnants of normal glands in the breast DCIS and breast ductal carcinoma.
- A moderate to strong, distinct cytoplasmic staining reaction of myoepithelial cells lining DCIS components in the breast DCIS and breast ductal carcinoma.
- No staining of epithelial cells in esophagus or breast specimens.

Participation

Number of laboratories registered for SMH, run 50	123
Number of laboratories returning slides	114 (93%)

Results
114 laboratories participated in this assessment. 4 participants used an inappropriate antibody and one performed IHC for SMH on a wrong slide. These participants were not included in the analysis. Of the remaining 109 laboratories, 78% achieved a sufficient mark (optimal or good).
Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less sensitive detection systems
- Insufficient HIER (too short efficient HIER time and/or use of non-alkaline HIER buffers)
- Off-label use of RTU formats

Performance history
This was the second NordiQC assessment of SMH.
Virtually the same pass rate was seen in the two runs performed (see Table 2).

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

	Run B08 2009	Run 50 2017
Participants, n=	19	114
Sufficient results	79%	78%

Conclusion
The mAb clones **SMMS-1** and **S131** were the two most successful antibodies for SMH. mAb clone SMMS-1 was used by the majority of laboratories and optimal results were obtained both within a laboratory developed (LD) assay on the main IHC platforms and as a Ready-To-Use system (Dako and Ventana). Within a LD assay, efficient HIER in an alkaline buffer and use of a sensitive and specific 3-step polymer / multimer based detection system gave the highest proportion of sufficient and optimal results.
Tonsil was found to be the preferred positive tissue control for SMH. Smooth muscle cells in vessels must show a moderate to strong cytoplasmic staining reaction, while follicular dendritic cells in the germinal centres must show an at least weak to moderate but distinct staining reaction. No staining must be seen in lymphocytes and epithelial cells.

Table 1. **Antibodies and assessment marks for SMH, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **SMMS-1**	48 5 2 1 1	Agilent/Dako Cell Marque Thermo/Neomarkers Biocare Zeta Corporation	24	19	11	3	75%	76%
rmAb clone **EP166**	1	Cell Marque	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **S131 PA0493**	2	Leica/Novocastra	2	0	0	0	-	-
mAb clone **SMMS-1 760-2704**	26	Roche/Ventana	13	9	4	0	85%	85%
mAb clone **SMMS-1 IR066/IS066**	17	Agilent/Dako	6	5	3	3	65%	100%
mAb clones **SMMS-1 pm420aa**	1	Biocare	0	1	0	0	-	-
mAb clone **SMMS-1 PDM175**	1	Diagnostic Biosystems	0	1	0	0	-	-
mAb clone **SMMS-1 MAB-0121**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP166 MAD-000718QB**	2	Master Diagnostica	0	2	0	0	-	-
rmAb clone **EP166 298R-18**	1	Cell Marque	0	1	0	0	-	-
Total	109		46	39	18	6	-	
Proportion			42%	36%	17%	5%	67%	

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

Detailed analysis of SMH, Run 50

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **SMMS-1**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) High pH (Dako) (4/14)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/5), Cell Conditioning 1 (CC1, Ventana) (12/22), Tris-EDTA pH 9 (3/5) or EDTA pH 8 (1/2) as retrieval buffer. Two protocols were based on a combined pre-treatment with HIER in CC1 (Ventana) followed by proteolysis in Protease 3 (Ventana). The mAb was typically diluted in the range of 1:50-1:1,000. Using these protocol settings, 38 of 50 (76%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

Table 3. **Proportion of optimal results for SMH for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb **SMMS-1**	3/10** (30%)	-	1/4	0/1	13/23 (57%)	0/1	2/5 (40%)	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **S131** product no. **PA0493**, Leica/Novocastra, BOND III / BOND MAX:
Protocols with optimal results were based on HIER using Bond Epitope Retrieval Solution 2 (BERS2) (efficient heating time 20 min. at 99-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **SMMS-1**, product no. **760-2704** Roche/ Ventana, BenchMark XT / Benchmark Ultra:
Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1) (efficient heating time 24-98 min.), and 8-32 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 22 of 25 (85%) laboratories produced a sufficient staining result.

mAb clone **SMMS-1** product no. **IR066/IS066**, Dako, Autostainer+ / Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using Target Retrieval Solution (TRS) pH 9 (3-in-1) (efficient heating time 20 min. at 95-99°C), 20 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) as detection systems. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining result.

mAb clone **SMMS-1** product no. **MAB-0121**, Manual, Maixin
One protocol with an optimal result was based on HIER in Waterbath using Tris-EDTA pH9 (efficient heating time 20 min.), 60 min. incubation of primary Ab and Kit-0038 as detection system.

Table 4. **Proportion of sufficient and optimal results for SMH for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb SMMS-1 **IR/IS066**	100% (9/9)	67% (6/9)	2/3	0/3
Leica BOND mAb S131 **PA0493**	2/2	2/2	-	-
VMS Ultra/XT mAb SMMS-1 **760-2704**	1/3	0/3	91% (21/23)	57% (13/23)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In concordance with the previous NordiQC assessment for SMH (run B8, 2009), the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 67% of the insufficient results (18 of 24 laboratories). The remaining insufficient results were characterized by a generally poor signal-to-noise ratio, excessive background reaction, and/or aberrant cytoplasmic staining reaction in e.g. neoplastic cells of the DCIS component and breast carcinoma. A too weak staining reaction was typically characterized by a reduced intensity and proportion of cells expected to be demonstrated. This was in particular observed in the tonsillar follicular dendritic cells and the myoepithelial cells of both normal breast glands and lining the DCIS components. Virtually all laboratories successfully demonstrated SMH in vascular smooth muscle cells and smooth muscle cells in the esophageal lamina muscularis mucosae.

53% (58 of 109) of the laboratories used concentrated Ab formats within laboratory developed (LD) assays for SMH. The mAb clone SMMS-1 was the most widely used Ab and could be used to obtain optimal staining results on all main IHC platforms as shown in Table 3. Used within a LD assay, the mAb clone SMMS-1 gave an overall pass rate of 75% (43 of 57) and 42% (24 of 57) were optimal.
Efficient HIER in an alkaline buffer in combination with a 3-step polymer/multimer based detection system provided the highest proportion of optimal results. As an example, on the Ventana BenchMark platform, 75% of the protocols (8 of 12) provided an optimal result if mAb clone SMMS-1 was used as a concentrate in the range of 1:100-1,000, HIER performed in CC1 for 32-64 min. and the 3-step multimer based OptiView (760-700) was applied as detection system. Using same settings, but with the 2-step multimer based detection system UltraView (760-500) only 38% of the protocols (6 of 16) provided an optimal result.

HIER in an alkaline buffer either as single retrieval method or in combination with mild proteolytic pre-treatment was found essential for optimal performance. HIER in low pH buffers or proteolysis as single retrieval method was found to be less successful.

47% (51 of 109) of the laboratories used Abs in Ready-To-Use (RTU) formats. The most frequently used RTU systems for SMH were the Ventana 760-2704 for BenchMark and the Dako IR066/IS066 system for Autostainer, both based on mAb clone SMMS-1.
The Ventana RTU system was the most widely used RTU system applied by 26 laboratories. An overall pass rate of 85% was seen and 50% were optimal. Optimal results could only be achieved by laboratory modified protocol settings. Ventana recommends HIER in CC1 for 32 min. at 95-100°C and 16 min. incubation of the primary antibody (760-2704) using UltraView (2-step multimer) as detection system. Only 3 of the 26 laboratories followed the Ventana recommendations, of which one protocol provided a result assessed as Good, whereas the remaining two protocols gave an insufficient result (too weak).
In contrast, the 23 laboratories that used laboratory modified protocol settings had a pass rate of 91% and 57% optimal. Typically the laboratories changed from 2-step multimer (UltraView) to a 3-step multimer (OptiView) detection system and/or prolonged the incubation of the primary antibody to 24-32 min. HIER was performed for 24-64 min. in CC1. These data clearly indicate that Ventana should change the recommended protocol settings in order to improve the performance of the RTU system for SMH.

The Dako IR066/IS066 system provided sufficient and optimal results both by recommended and laboratory modified protocol settings. Used according to the recommended protocol settings, the IR066/IS066 system had a pass rate of 100%, 67% optimal (see Table 4). Lacking a RTU alternative tailored for the Dako Omnis platform, 5 laboratories used the IR066/IS066 system on the Dako Omnis. All 5 laboratories produced a result evaluated as insufficient. However significantly modified protocol settings were applied, such as reduced incubation of the primary Ab and/or use of HIER in TRS low pH.

The Leica RTU system PA0493 based on mAb clone S131 was used by two participants and both produced an optimal result.
Both laboratories applied the protocol settings given by Leica, using HIER in BERS2 for 20 min., 15 min. incubation of the primary Ab and Refine as detection system.

Controls
Tonsil was found to be the preferred positive and negative tissue control for SMH. Virtually all smooth muscle cells in vessels must show a moderate to strong cytoplasmic staining reaction, while the follicular dendritic cells in the germinal centres must show an at least weak to moderate but distinct staining reaction.
No staining should be seen in lymphocytes and epithelial cells.

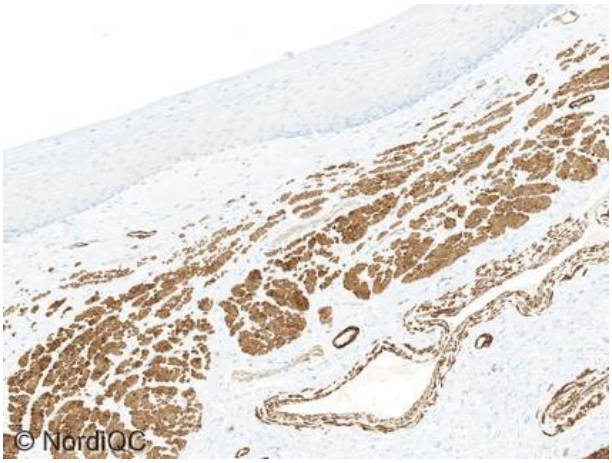


Fig. 1a
Optimal staining for SMH of the esophagus using the mAb clone SMMS-1 within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step multimer based detection system.
Virtually all smooth muscle cells in vessels and lamina muscularis mucosae show a moderate to strong cytoplasmic staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

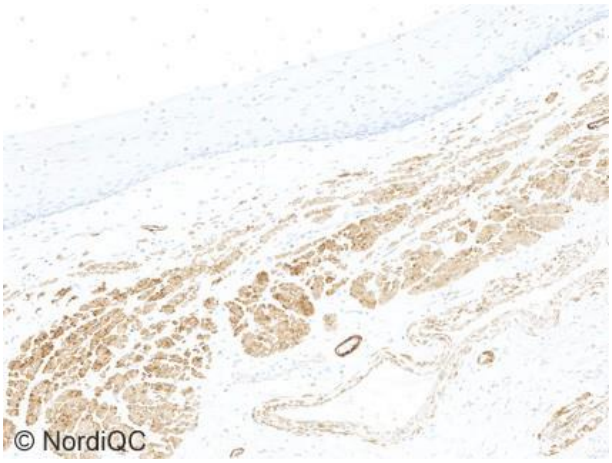


Fig. 1b
SMH staining of the esophagus using an insufficient protocol based on the mAb clone SMMS-1 within a laboratory developed assay providing a too low analytical sensitivity. A too low titre of the primary Antibody and the use of 2-step multimer system, UltraView Ventana, were the main causes for the insufficient result, which especially is seen in Figs. 2b - 5b – same protocol.
In esophagus – same field as Fig. 1a, a moderate staining reaction is seen in virtually all smooth muscle cells.
As described in the assessment report, smooth muscle cells cannot be recommended as positive tissue control for SMH due to the high level of SMH expression.

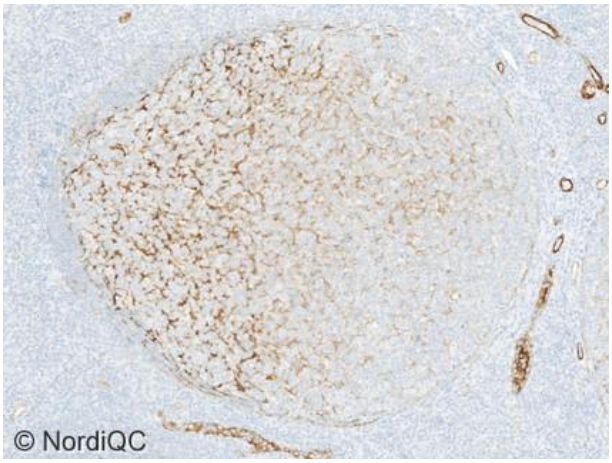


Fig. 2a
Optimal SMH staining of the tonsil using same protocol as in Fig. 1a.
A weak to moderate staining reaction is seen in the follicular dendritic network in the germinal center.
A high signal-to-noise ratio is observed.

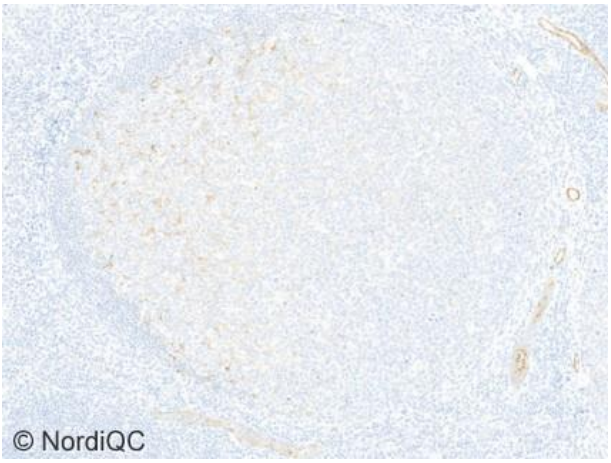


Fig. 2a
Insufficient SMH staining of the tonsil using same protocol as in Fig. 1b.
The follicular dendritic network in the germinal center is virtually negative and only vascular smooth muscle cells are demonstrated.

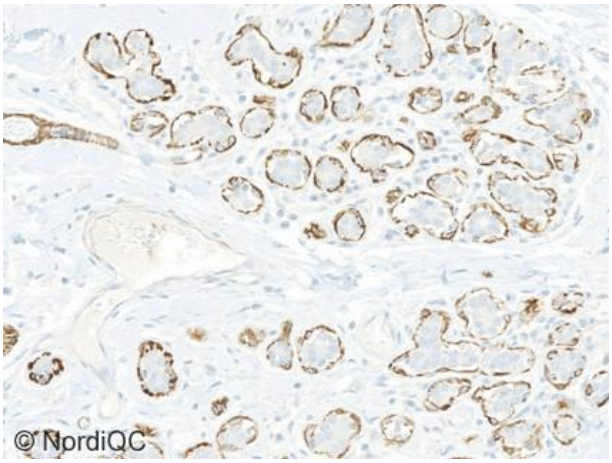


Fig. 3a
Optimal SMH staining of the breast hyperplasia using same protocol as in Figs. 1a and 2a.
A moderate and distinct staining reaction is seen in virtually all myoepithelial cells lining the breast glands.
A high signal-to-noise ratio is observed.

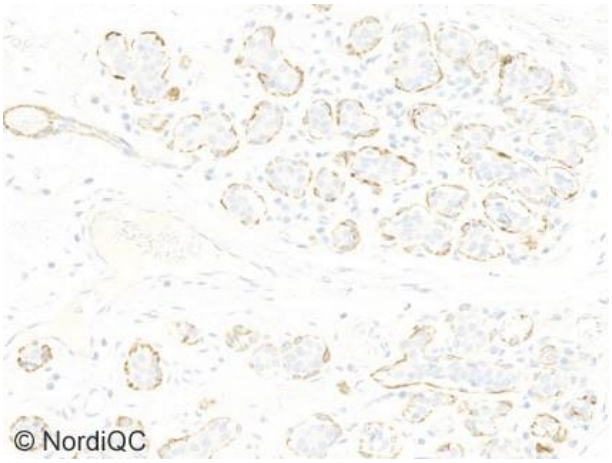


Fig. 3b
Insufficient SMH staining of the breast hyperplasia using same protocol as in Figs. 1b and 2b.
Only a weak and patchy staining reaction is seen in the myoepithelial cells lining the breast glands.

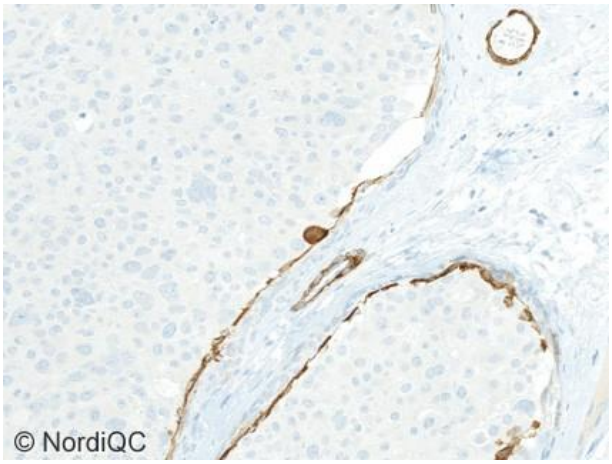


Fig. 4a
Optimal SMH staining of the breast DCIS using same protocol as in Figs. 1a - 3a.
A moderate, distinct and continuous staining reaction is seen in the myoepithelial cells lining the breast DCIS component.

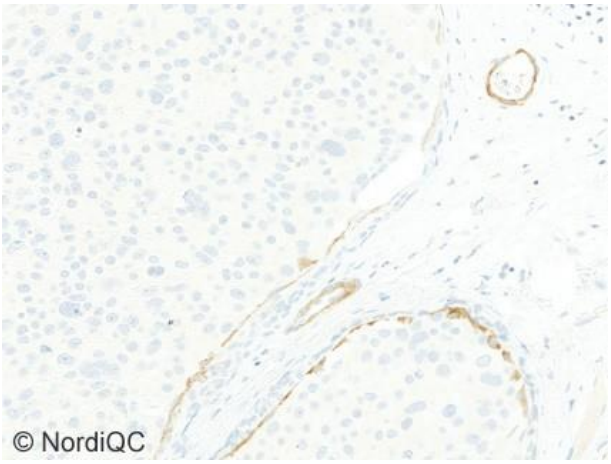


Fig. 4b
Insufficient SMH staining of the breast DCIS using same protocol as in Figs. 1b – 3b.
Only a weak and disrupted staining reaction is seen in the myoepithelial cells lining the breast DCIS component.

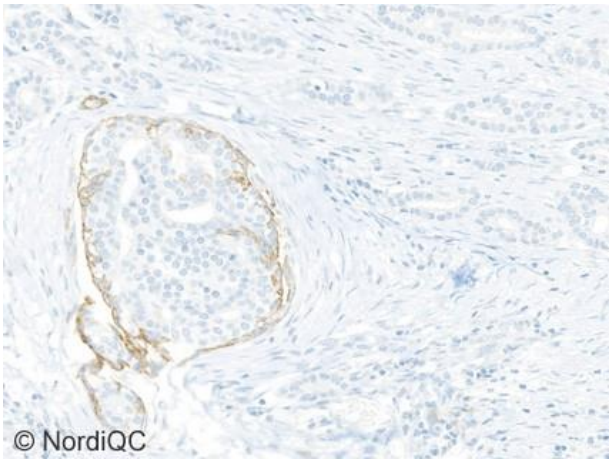


Fig. 5a
Optimal SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
A moderate, distinct and continuous staining reaction is seen in the myoepithelial cells lining the breast DCIS component, while the invasive components show no staining.

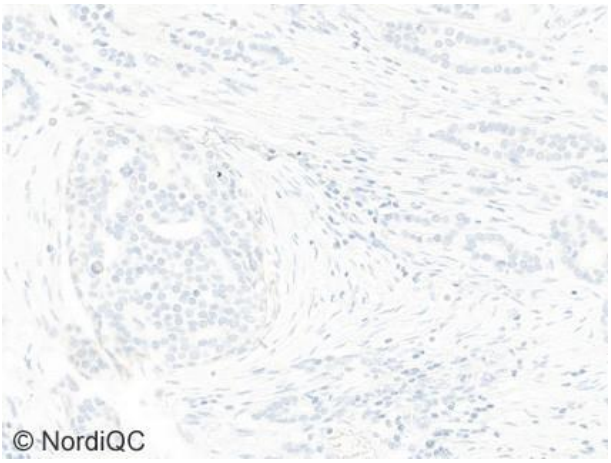


Fig. 5b
Insufficient SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
No staining is seen in neither the DCIS nor the invasive components and thus not possible to differentiate these two entities.

SN/LE/RR 03.07.2017