Assessment Run 73 2025

Alpha-smooth muscle actin (ASMA)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC assays for ASMA performed by the NordiQC participants, identifying smooth muscular origin of cancers of unknown origin and differentiation between leiomyoma and schwannoma. Relevant clinical tissues, both normal and neoplastic disorders, were selected to display a broad spectrum of antigen expression for ASMA (see below).

Material
The slide to be stained for ASMA comprised:

1. Appendix, 2. Tonsil, 3. Liver, 4. Leiomyoma, 5. Leiomyosarcoma, 6. Schwannoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an ASMA staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of all smooth muscle cells in the muscularis propria, lamina muscularis mucosae and myofibroblasts lining crypts and surface epithelium of the appendix.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the majority of perisinusoidal cells (hepatic stellate cells) in the liver.
- A strong, distinct cytoplasmic staining reaction of all neoplastic cells in the leiomyosarcoma and leiomyoma.
- A strong, distinct cytoplasmic staining reaction of smooth muscle cells throughout the specimens in the block (e.g. vessels).
- No more than a weak, focal staining reaction (<10%) in the neoplastic cells in the schwannoma.
- No staining reaction of other cells, including lymphocytes (all specimens), squamous epithelial cells of the tonsils, columnar epithelial cells of the appendix and hepatocytes in the liver.

KEY POINTS FOR ASMA IMMUNOASSAYS
- The mAb clone **1A4** was used by 81% of all participants.
- The performance of the mAb clone **1A4** was inferior on BenchMark platforms.
- RTUs developed for Autostainer, Omnis and BOND platforms, all obtained high pass rates, when applying vendor recommended protocol settings.
- Appendix, tonsil and liver are recommendable as positive and negative tissue controls.

Participation

Number of laboratories registered for ASMA, run 73	411
Number of laboratories returning slides	392 (95%)

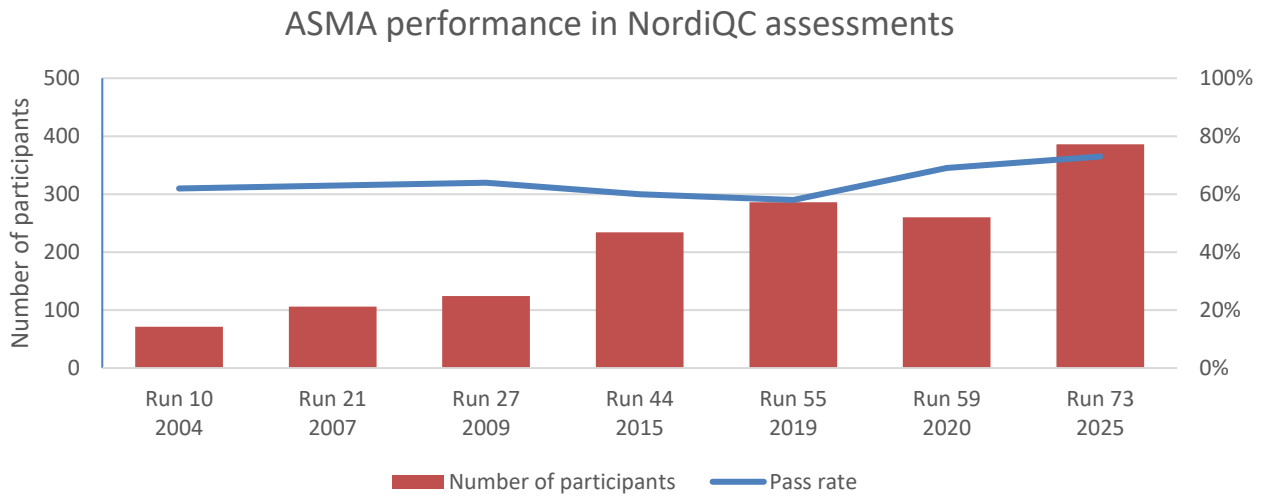
All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

Results
392 laboratories returned slides for this assessment. Six of these used an inappropriate Ab, the pan-muscle marker mAb clone HHF35. These are not included in the data below. Of the remaining 386 laboratories, 281 (73%) achieved a sufficient mark (optimal or good, see Table 1a (see page 3). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3-4).

The most frequent causes of insufficient staining reactions were:
- Poor performance of the mAb clone 1A4 on the BenchMark platforms (Ventana/Roche)
- Too high concentration of the primary Ab

Performance history
This was the seventh NordiQC assessment of ASMA. The pass rates have constantly been relatively low throughout all runs. A pass rate of 73% was seen, which is the highest level obtained in all NordiQC assessments of ASMA (see Graph 1).

Graph 2. **Proportion of sufficient results for ASMA in the seven NordiQC runs performed**



Conclusion
The mAb clones **1A4**, **BS66, asm-1** and rmAb clone **EP188** could all be used to obtain an optimal staining result. The mAb clone 1A4 was used by the majority of laboratories.
The performance of assays based on the mAb clone 1A4, both as concentrated format and RTU systems, were challenged when applied on the fully automated IHC platform BenchMark (Ventana/Roche) giving only 6% optimal staining results (9 of 140). For the mAb clone 1A4, the majority of insufficient staining results were characterized by an aberrant false positive nuclear staining reaction, which was mostly seen when the clone was applied on the BenchMark (Ventana/Roche) platform with HIER as pre-treatment. Irrespective of the Ab, HIER in an alkaline buffer and careful calibration of the titre of the primary antibody were the main prerequisites for optimal results. The Ready-To-Use (RTU) systems from Dako/Agilent and Leica Biosystems based on mAb clone 1A4 and asm-1, respectively, were in this assessment most successful and provided high proportions of sufficient and optimal results.

Controls
Appendix, liver and tonsil are recommendable positive and negative tissue controls for ASMA. Virtually all smooth muscle cells in vessels, appendiceal muscularis mucosae and lamina propria must show a moderate to strong cytoplasmic staining reaction, while the vast majority of perisinusoidal cells (hepatic stellate cells) in the liver must show an at least weak to moderate, distinct staining reaction. No staining reaction should be seen in appendiceal columnar epithelial cells, lymphocytes, tonsillar squamous epithelial or liver cells.

Table 1a. **Overall results for ASMA, run 73**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	120	45	42	32	1	73%	38%
Ready-To-Use antibodies	266	105	89	69	3	73%	39%
Total	386	150	131	101	4		
Proportion		39%	34%	26%	1%	73%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for ASMA, run 73**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1A4**	1	Abcam	1	0	0	0	-	-
	2	Biocare Medical	0	1	1	0	-	-
	7	Cell Marque	3	2	2	0	71%	43%
	66	Agilent/Dako	26	21	19	0	71%	39%
	1	Epredia	0	0	1	0	-	-
	1	Master Diagnostica	1	0	0	0	-	-
	3	Sigma Aldrich	0	2	1	0	-	-
	1	Zeta Corporation	0	0	1	0	-	-
	3	Zytomed Systems	1	2	0	0	-	-
mAb clone **asm-1**	3	Leica Biosystems	0	2	1	0	-	-
mAb clone **BS66***	6	Nordic Biosite	3	3	0	0	100%	50%
mAb clone **BSB-15**	2	Bio SB	1	0	1	0	-	-
rmAb clone **EP188**	18	Cell Marque	6	7	4	1	72%	33%
rmAb clone **QR110**	5	Quartett	3	2	0	0	100%	60%
pAb **501-2464**	1	Zytomed Systems	0	0	1	0	-	-
Total	120		45	42	32	1		
Proportion			38%	35%	26%	1%	73%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
*Terminated by vendor

Table 1c. **Ready-To-Use antibodies and assessment marks for ASMA, run 73**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1A4 IR/IS611 (VRPS)**[3]	12	Agilent/Dako	7	5	0	0	100%	58%
mAb clone **1A4 IR/IS611 (LMPS)**[4]	16	Agilent/Dako	7	5	4	0	75%	44%
mAb clone **1A4 GA611 (VRPS)**[3]	54	Agilent/Dako	48	4	2	0	96%	89%
mAb clone **1A4 GA611 (LMPS)**[4]	32	Agilent/Dako	18	13	1	0	97%	56%
mAb clone **1A4 760-2833 (VRPS)**[3]	13	Ventana/Roche	0	8	5	0	62%	0%
mAb clone **1A4 760-2833 (LMPS)**[4]	76	Ventana/Roche	3	29	43	1	42%	4%
mAb clone **1A4 202M-9x**	11	Cell Marque	1	3	7	0	36%	9%
mAb clone **1A4 8292-C010**	3	Sakura Finetek	3	0	0	0	-	-
mAb clone **1A4 MAD-001195QD**	2	Master Diagnostica	1	0	1	0	-	-
mAb **1A4 AM28-10M**	1	Biogenex	0	0	1	0	-	-
mAb clone **1A4 BFM-0221**	1	Bioin Biotechnology	0	1	0	0	-	-
mAb clone **1A4 BMS001**	1	Zytomed Systems	0	1	0	0	-	-
mAb clone **1A4 GM085101**	1	Gene Tech	1	0	0	0	-	-
mAb clone **1A4 I11362E-05**	1	Biolynx Biotechnology	0	0	1	0	-	-
mAb clone **1A4 PDM003**	1	Diagnostic BioSystems	0	0	0	1	-	-
mAb clone **1A4 PM001**	1	Biocare Medical	0	0	1	0	-	-
mAb clone **asm-1 PA0943 (VRPS)**[3]	24	Leica Biosystems	12	12	0	0	100%	50%
mAb clone **asm-1 PA0943 (LMPS)**[4]	11	Leica Biosystems	3	5	2	1	73%	27%
mAb clone **BSB-15 BSB 5030**	1	BioSB	0	1	0	0	-	-
mAb clone **C1C1 CAM-0191**	1	Celnovte	0	1	0	0	-	-
mAb clone **MX097 MAB-0890**	1	Maixin	0	1	0	0	-	-
Ab clone **763F9A3 PA141**	1	Abcarta	1	0	0	0	-	-
Ab clone **DY49005 4920522**	1	Dakewe	0	0	1	0	-	-
Total	266		105	89	69	3		
Proportion			39%	34%	26%	1%	73%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).

Detailed analysis of ASMA, Run 73
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **1A4**: 32 of 85 (38%) protocols were assessed as optimal. Optimal results could be obtained both with Heat Induced Epitope Retrieval (HIER) as pretreatment and by omission of HIER.
Protocols with optimal results based on HIER used Target Retrieval Solution (TRS) High pH 9 (Dako/Agilent) (15/22)*, Cell Conditioning 1 (CC1, Ventana/Roche) (1/20), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (8/16), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/4) or Tris-EDTA pH 9 (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:2,000**. Using these protocol settings, 45 of 63 (71%) laboratories produced a sufficient staining result, 27 (43%) optimal. Protocols without HIER, but same dilution range as above, provided a sufficient staining result in 72% (13 of 18), but only 28% (5 of 18) optimal.
* (number of optimal results/number of laboratories using this HIER buffer)
**Concentrate from Sigma Aldrich was diluted 1:8000-32,000, however, no optimal results.

mAb clone **BS66**: 3 of 6 (50%) protocols were assessed as optimal.
Two protocols with optimal results based on HIER as single pretreatment using CC1 (Ventana/Roche) (2/4) as retrieval buffer. The mAb was diluted in the range of 1:500-1:2,000 and OptiView as detection system. Using these protocol settings, 3 of 3 laboratories produced a sufficient staining result.
One protocol with an optimal result was based on a combined pretreatment using HIER in CC1 followed by Protease 3 (Ventana/Roche) (1/2). The mAb was diluted 1:200 with OptiView as detection system.

rmAb clone **EP188**: 6 of 18 (33%) protocols were assessed as optimal.
Protocols with optimal results were based on combined pre-treatment using proteolysis (Protease 2 or 3 (Ventana/Roche) for 4 min.) followed by HIER in CC1 (Ventana/Roche) for 32 min. The rmAb was diluted 1:100-200. Using these protocol settings, 9 of 11 (82%) laboratories produced a sufficient staining result.

rmAb clone **QR110**: 3 of 5 (60%) protocols were assessed as optimal.
Protocols with optimal results were based on HIER as pretreatment using CC1 (Ventana/Roche) (3/4) as retrieval buffer. The rmAb was diluted in the range of 1:100-300 and OptiView as detection system. Using there protocol settings, 4 of 4 laboratories produced a sufficient staining result.

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

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**			**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **1A4**	3/8 (38%)	-	12/14 (86%)	-	0/18 (0%)	0/1	-	8/15 (53%)	1/4
mAb clone **BS66**	-	-	-	-	2/3	1/2	-	-	-
rmAb clone **EP188**	-	-	-	-	0/3	6/12 (50%)	-	-	-
rmAb clone **QR110**	-	-	-	-	3/4	-	-	0/1	-

* 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)
1) Autostainer Classic, Link 48+.
2) BenchMark XT, GX Ultra, Ultra Plus.
3) Bond Max, III, Prime

Ready-To-Use antibodies and corresponding systems
mAb clone **1A4**, product no. **IS611/IR611**, Dako/Agilent, Autostainer/Autostainer Link 48+:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20-40 min. at 97°C), 15-30 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings, 16 of 16 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **1A4**, product no. **GA611,** Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (efficient heating time 10-30 min. at 97°C), 10-30 min. incubation of the primary Ab and EnVision (GV800) or EnVision Flex+ (GV800/GV021) as detection system. Using these protocol settings, 81 of 84 (96%) laboratories produced a sufficient staining result.

mAb clone **1A4**, product no. **760-2833,** Ventana/Roche, BenchMark Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 16-32 min. at 100°C), 32-104 min. incubation of the primary Ab and OptiView (760-700) with or without OptiView Amplification

(760-099) as detection system. Using these protocol settings, 6 of 10 (60%) laboratories produced a sufficient staining result.

mAb clone **asm-1**, product no. **PA0943**, Leica Biosystems, Bond III/Prime:
Protocols with optimal results were typically based on HIER using BERS1 or BERS2 (efficient heating time 10-20 min. at 100°C) or no pretreatment, 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.
Using these protocol settings, 31 of 32 (97%) laboratories produced a sufficient staining result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

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

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako Autostainer Link 48+ mAb 1A4 **IR611/IS611**	12/12 (100%)	7/12 (58%)	7/7 (100%)	4/7 (57%)
Dako Omnis mAb 1A4 **GA611**	52/54 (96%)	48/54 (89%)	29/30 (97%)	16/30 (53%)
Ventana BenchMark XT/Ultra/Ultra Plus mAb 1A4 **760-2833**	8/13 (62%)	0/13 (0%)	32/76 (42%)	3/76 (4%)
Leica Bond III/Prime mAb asm-1 **PA0943**	24/24 (100%)	12/24 (50%)	8/10 (80%)	3/10 (30%)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments
In this assessment, false positive, too weak or false negative staining reactions were the main features of insufficient results.

In 56% of the insufficient results (59 of 105), a false positive staining reaction was observed and primarily characterized as an aberrant nuclear staining reaction of e.g. lymphocytes in the tonsil. This observation was in concordance with previous NordiQC assessments for ASMA. 98% (58 of 59) protocols applied the mAb clone 1A4, typically on a Benchmark platform (Ventana/Roche) (n=47). The aberrant staining reaction was in particular prominent when protocols with high level of technical and analytical sensitivity was applied e.g. high titer of the primary Ab and/or efficient HIER. An aberrant nuclear reaction could also be observed on the Bond platform (Leica Biosystems), Omnis or Autostainer Link 48 (Dako/Agilent) but to a lesser degree.

A weak or false negative staining reaction was seen in 30% (32 of 105) of the insufficient results and was typically caused by protocols with too low technical and analytical sensitivity. The majority of the laboratories were able to demonstrate ASMA in cells with high-level antigen expression as smooth muscle cells in appendiceal muscularis mucosae, smooth muscle cells in large vessels and neoplastic cells of the leiomyosarcoma, whereas demonstration of ASMA in cells with low-level antigen expression as hepatic perisinusoidal cells could only be obtained with an optimally calibrated protocol. The ability and importance to demonstrate ASMA in the hepatic perisinusoidal cells serving as limit of demonstration of ASMA has been shown in previous ASMA assessments e.g. run 27. If these cells were negative or only faintly demonstrated, the diagnostic sensitivity in e.g. leiomyosarcomas with low-level ASMA expression levels was compromised.
14 laboratories (14%) obtained an insufficient staining result because of other technical issues.

31% (120 of 386) of the laboratories used a concentrated format within a laboratory developed (LD) assay for detection of ASMA.
The mAb clone **1A4** was the most widely used concentrated format within a LD assay. The mAb clone 1A4 provided a pass rate of 71% (60 of 85) but only 38% (32 of 85) were assessed as optimal (see Table 1b). The vast majority of protocols assessed as optimal were typically based on HIER (preferable in an alkaline buffer) in combination with a careful calibration of the primary Ab. Both 2- and 3-step polymer-based detection systems could be used to obtain an optimal result. As shown in Table 3, the best performance was obtained on the Dako Omnis (Dako/Agilent) where 86% (12 of 14) of the protocols produced an optimal result, whereas no optimal results (0 of 19) were seen on BenchMark (Ventana/Roche).

Although the number of participants using the rmAb clone **QR110** within a LD-assay was low, the Ab seems to be robust as all protocols (5 of 5) based on this Ab obtained a sufficient staining result (see Table 1b). In addition, this primary Ab provided optimal results on Benchmark (Ventana/Roche) and thus might be an alternative to the mAb clone 1A4 on especially this IHC platform series. mAb clone 1A4 has in all assessments for ASMA shown an inferior performance on the BenchMark IHC platform series. However, further validation is required on a larger tissue cohort, to secure the analytical sensitivity and specificity.

The rmAb clone **EP188** could provide sufficient results on BenchMark Ultra (Ventana/Roche), applying a combined pre-treatment using proteolysis in P2 or P3 followed by HIER in CC1 with a pass rate of 92% (11 of 12), 50% optimal (see Table 3).

In runs 55 (2019) and 59 (2020) the number of participants using the promising mAb clone **BS66** within a LD-assay increased from 7 to 50. During the latest assessment run 59, the vendor has informed that the sale has been terminated *"due to decreased specificity and inconsistency between batches"*. This was in line with an inferior performance observed in run 59. In this run 73, 6 laboratories used the mAb clone BS66, despite the termination, with a 100% pass rate (see Table 1b).

69% (266 of 386) of the laboratories used a RTU format for the demonstration of ASMA. Ideally, a RTU format of a primary Ab should be used within a system that has been thoroughly validated, providing precise information on vendor recommended protocol settings, equipment, reagents and test performance characteristics (expected reaction patterns).

28 laboratories used the RTU format **IR/IS611** (Dako/Agilent) based on the mAb clone 1A4, of which 12 laboratories used Autostainer Link 48+ platform with recommended protocol settings, with a pass rate of 100% – see Table 4. Seven laboratories modified the protocol (minor changes in HIER and incubation times), with a 100% pass rate. 9 laboratories used the IR/IS611 on a different stainer platform.

Using the Dako/Agilent RTU for Omnis (**GA611**) with recommended protocol settings, the pass rate was 96% (52 of 54), 89% optimal. When modifying the protocol, a pass rate of 97% was observed, 53% optimal (see Table 4).

The Ventana/Roche RTU system **760-2833** based on mAb clone 1A4 was the most widely used RTU system with similar observations as for LD assays. 13 laboratories used the RTU as recommended by Ventana, which resulted in a pass rate of 62%, no optimal results (see Table 4). As shown in Table 4, the Ventana RTU system with modified protocol settings obtained a pass rate of 42% (32 of 76) but only 3 optimal results. When calibrating the protocol to increase the technical and analytical sensitivity, an aberrant nuclear staining result frequently was seen. If the protocol was calibrated to avoid the aberrant nuclear staining a reduced analytical sensitivity was seen. In last assessment (run 59, 2020), few laboratories added a blocking step after incubation of primary Ab, which seemed to increase the technical and analytical specificity. In this resent assessment run 73, three laboratories added a blocking step, but with limited success as only 1 of 3 protocols gave a sufficient result, indicating that further validation is needed.

The RTU system **PA0943** (Leica Biosystems) based on the mAb clone asm-1 and applied by recommended protocol settings a pass rate of 100%, 50% optimal (see Table 4) was obtained. When modifying the protocol settings, a pass rate of 80% was seen, 30% optimal. One laboratory used the RTU on a different platform, with an insufficient result.

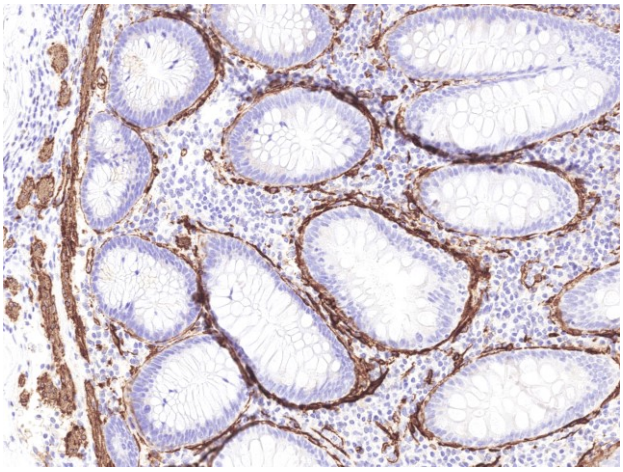


Fig. 1a
Optimal ASMA staining reaction of the appendix using the mAb clone 1A4 using the RTU product GA611 for Dako Omnis (Dako/Agilent) applying vendor recommended protocol settings. Smooth muscle cells of lamina muscularis mucosae and myofibroblasts lining the epithelial crypts show a distinct cytoplasmic staining reaction, while epithelial cells are negative.
Same protocol used in Figs. 2a-5a.

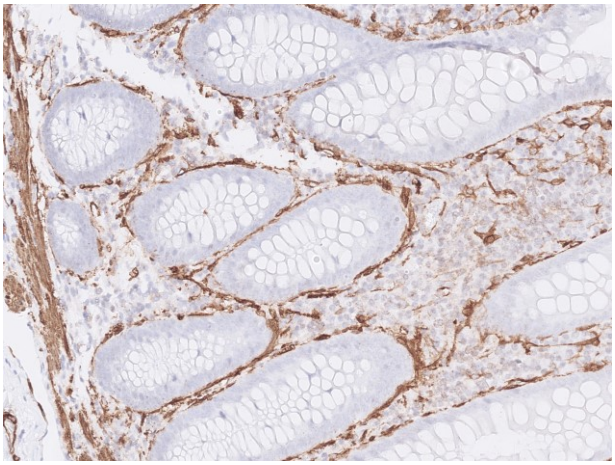


Fig. 1b
ASMA staining reaction of the appendix using the RTU product 760-2833 (Ventana/Roche) based on the mAb clone 1A4, using HIER in CC1 (not recommended by vendor). Same protocol used in Figs. 2b-5b.
Although the staining pattern is similar to the optimal result seen in Fig. 1a (same field), the assay provided a weaker staining reaction.

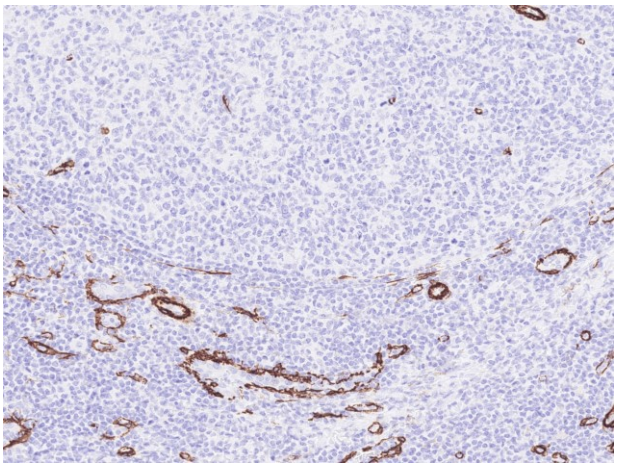


Fig. 2a
Optimal ASMA staining reaction of the tonsil using same protocol as in Fig. 1a. The vast majority of smooth muscle cells in both large and smaller vessels display the expected strong and distinct cytoplasmic staining reaction. No staining reation in lymphocytes is seen.

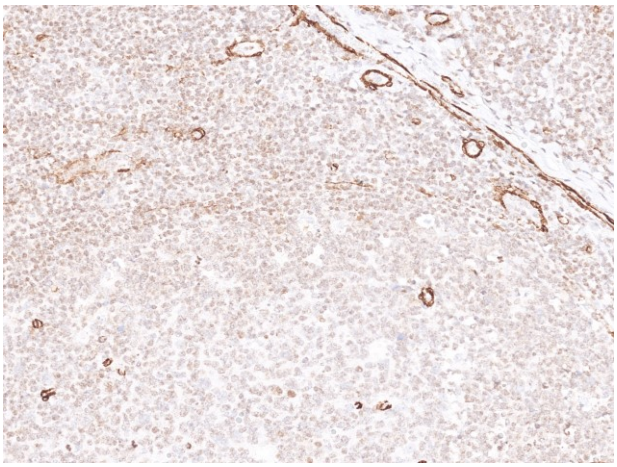


Fig. 2b
Insufficient ASMA staining reaction of the tonsil using same protocol as in Fig. 1b – same field as in Fig. 2a.
An aberrant nuclear staining reaction is seen in virtually all lymphocytes.
Also see Fig. 5b, same aberrant nuclear pattern in the Schwannoma.

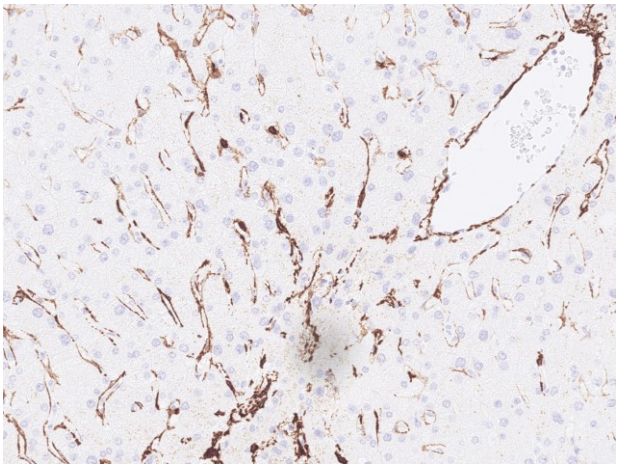


Fig. 3a
Optimal ASMA staining reaction of the liver using same protocol as in Figs. 1a and 2a. The smooth muscle cells of the portal vessels show a moderate to strong staining reaction. Importantly, the vast majority of hepatic stellate cells (perisinusoidal smooth muscle cells) show a distinct, weak to moderate staining reaction. The hepatocytes are negative.

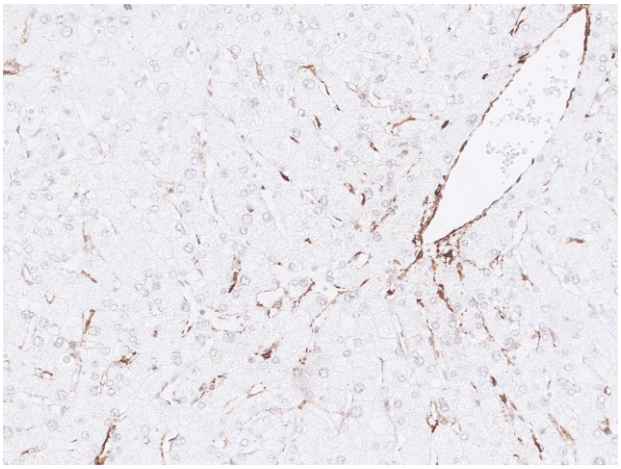


Fig. 3b
Insufficient ASMA staining reaction of the liver using same protocol as in Figs. 1b and 2b. The proportion of positive hepatic stellate cells is significantly reduced and only display a faint staining intensity - same field as in Fig. 3a.

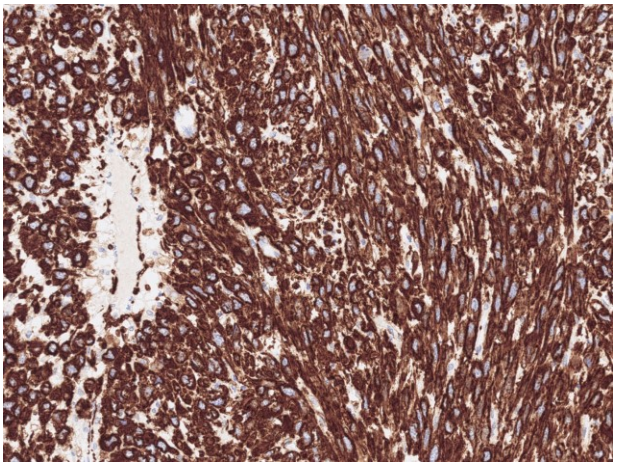


Fig. 4a
Optimal ASMA staining reaction of the leiomyosarcoma using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a strong distinct cytoplasmic staining reaction.

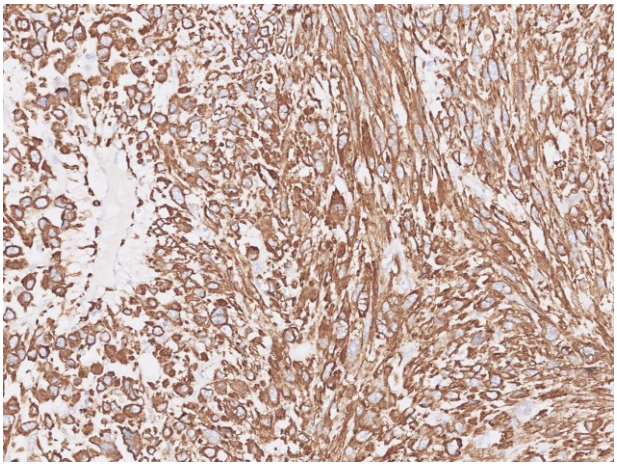


Fig. 4b
ASMA staining reaction of the leiomyosarcoma using same protocol as in Figs. 1b-3b. The neoplastic cells display a moderate staining reaction - same field as in Fig. 4a.

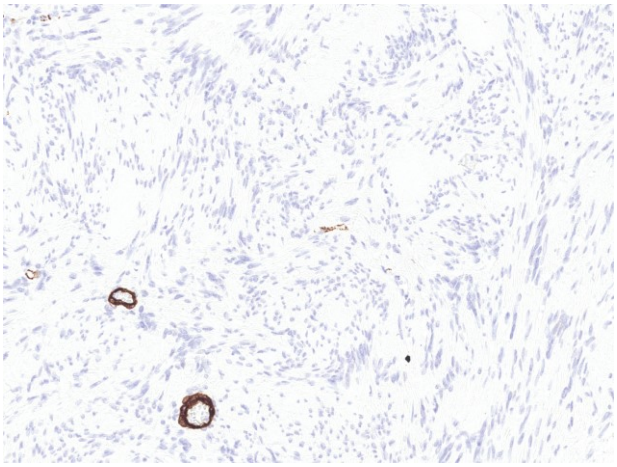


Fig. 5a
Optimal ASMA staining reaction of the schwannoma using the same protocol as in Figs. 1a-4a. All neoplastic cells are negative. Only scattered normal vascular smooth muscle cells display a strong staining intensity.

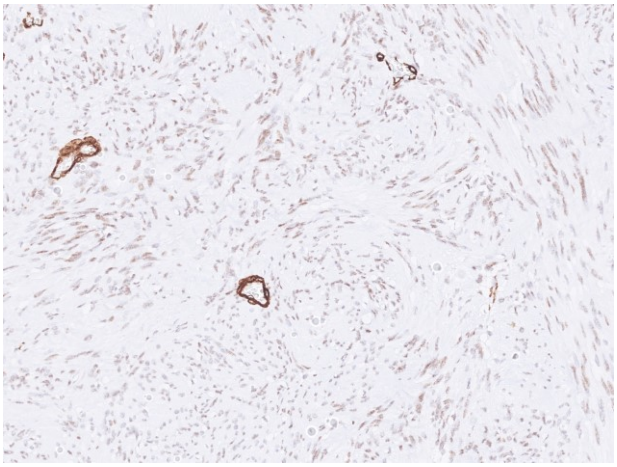


Fig. 5b
Insufficient ASMA staining reaction of the schwannoma using same protocol as in Figs. 1b-4b. An aberrant nuclear staining reaction is seen in the majority of cells - same field as in Fig. 5a.

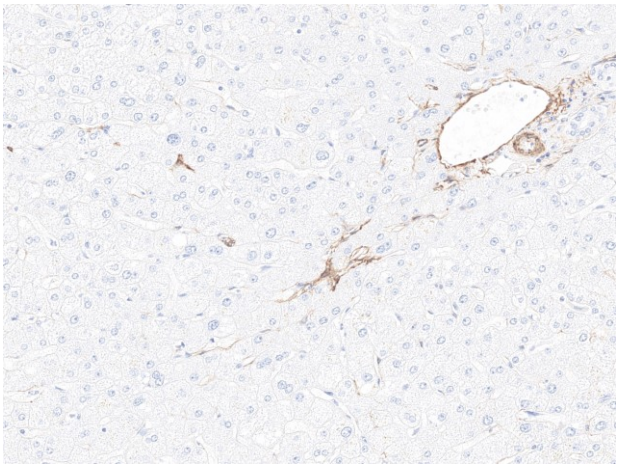


Fig. 6a
Insufficient ASMA staining of the liver using the mAb clone 1A4 as RTU (760-2833, Ventana/Roche) with recommended protocol settings (no pretreatment), giving a too weak staining reaction in cells expected to be optimal. Compare with Fig. 3a for optimal result.

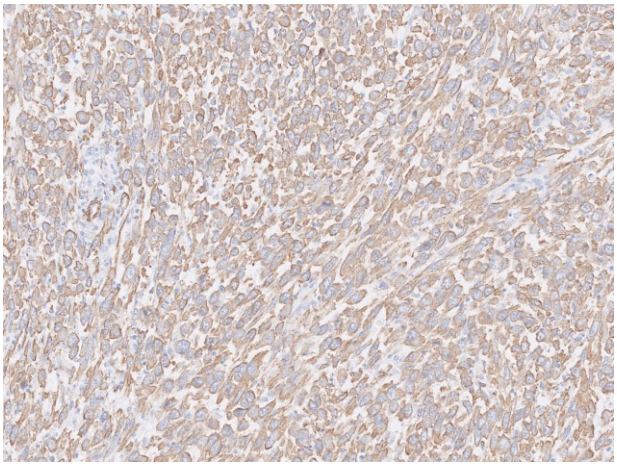


Fig. 6b
Insufficient ASMA staining of the leiomyosarcoma using the same protocol as in Fig. 6a. Only a weak staining reaction is seen in the neoplastic cells. Compare with Fig. 4a for optimal result.

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