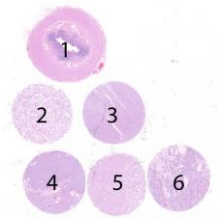
Assessment Run 43 2015

Sal-like protein 4 (SALL4)

The slide to be stained for SALL4 comprised:



1. Appendix, 2. Testis, 3. Renal clear cell carcinoma, 4. Seminoma, 5. Intratubular germ cell neoplasia (IGCN), 6. Embryonal carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SALL4 staining as optimal included:

- An at least weak to moderate nuclear staining reaction of the majority of spermatogonia lining the basement membrane in seminiferous tubules of the normal testis.
- A moderate to strong nuclear staining reaction of virtually all neoplastic cells in the IGCN, seminoma and embryonal carcinoma.
- No staining of neoplastic cells in the renal clear cell carcinoma.
- No staining of any cells in the appendix.

Participation

Number of laboratories registered for SYP, run 43	53
Number of laboratories returning slides	51 (96%)

51 laboratories participated in this assessment. 50 (98%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The cause of insufficient staining was:
- Too high concentration of the primary antibody

Performance history
This was the first NordiQC assessment of SALL4. Table 2 shows the proportion of sufficient results. It is very encouraging that the proportion of sufficient results was so high despite SALL4 is a relatively new marker.

Table 2. **Proportion of sufficient results for SALL4 in the first NordiQC run**

	Run 43 2015
Participants, n=	51
Sufficient results	98%

Conclusion
The mAb clone **6E3** was found to be a very robust and recommendable antibody for demonstration of SALL4. In concentrated format, optimal results could be obtained on all three main IHC systems and in general a high pass rate was observed. HIER and careful calibration of the primary antibody were the most important prerequisites for optimal staining results.
Correponding RTU systems of the mAb clone **6E3** (Ventana/Cell Marque and BioCare) also provided a high pass rate and proportion of optimal results. Normal testis is recommendable as positive tissue control for SALL4. The majority of spermatogonia must show an at least weak to moderate nuclear staining reaction. Appendix can be used as negative tissue control. No staining reaction should be seen.

Table 1. **Antibodies and assessment marks for SALL4, run 43**

Concentrated antibodies:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **6E3**	4 6 2 10 1 2 3 1 1 1	Abnova Biocare Biosite Cell Marque Master Diagnostica Novus Biological Sigma Aldrich Abcam Beijingzhongsan Novus Biologicals	22	8	1	0	98%	97%
Ready-To-Use Abs:								
mAb clone **6E3**	8	Ventana/Cell Marque	5	3	0	0	100%	100%
mAb clone **6E3 CM385**	7	Cell Marque	5	2	0	0	100%	100%
mAb clone **6E3 MAD-000572QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **6E3 MAB-0691**	2	Maixin	2	0	0	0	-	-
mAb clone **6E3 PM384**	1	Biocare	1	0	0	0	-	-
Total	51		36	14	1	0	-	
Proportion			71%	27%	2%	0	98%	

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

Detailed analysis of SALL4, Run 43

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies

mAb clone **6E3**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/2)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (4/5), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/3), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/2) or Cell Conditioning 1 (BenchMark, Ventana)(14/17) as retrieval buffer. The mAb was typically diluted in the range of 1:30–1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 29 out of 30 (97%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Table 3. **Proportion of optimal results for SALL4, mAb clone 6E3 as conc. on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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 clone **6E3**	3/3**	0/1	14/18 (78%)	0/1	2/3	1/2

* 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 **6E3,** product no. **760-4864**, Ventana, BenchMark XT, ULTRA:
Protocols with optimal result were based on HIER using Cell Conditioning 1 (efficient heating time 32-64 min.), 16-32 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 8 of 8 (100%) laboratories produced a sufficient staining result.

mAb clone **6E3**, product no. **PM384**, Biocare, intelliPATH:
One protocol with an optimal result was based on HIER using Citrate pH 6.0 (Biocare) in a pressure cooker, 45 min. incubation of the primary Ab and MACH4 (M4U534) as detection system.

Comments

In this first run of external quality assessment of SALL4, a pass rate of 98% was observed. Only 1 of 51 laboratories obtained an insufficient mark due to a poor signal-to-noise ratio. This was most likely caused

by a too high concentration of the primary Ab giving a diffuse background staining and aberrant cytoplasmic staining in cells not expressing SALL4. A weak cytoplasmic staining reaction in cells with high level SALL4 expression as neoplastic cells of the seminoma and embryonal carcinoma was accepted.

The mAb clone 6E3 was the only clone submitted in this run. Optimal results could be obtained both using the concentrated format within a laboratory developed (LD) assay and as ready-to-use (RTU) format. As a concentrate, this clone could be used to produce optimal staining results on all three main IHC systems, see table 3. No special requirements concerning HIER buffer and detection system were needed to provide optimal staining results provided that careful calibration of the primary Ab titer was performed. For example, the mAb clone 6E3, prod. no. CM385, Cell Marque thus provided an optimal result both by a highly sensitive protocol based on HIER 48 min. in CC1 (Ventana) and a 3-step multimer based detection system (OptiView 760-700, Ventana) but also by a less sensitive protocol based on HIER for 32 min. in CC1 and a 2-step multimer based system (UltraView 760-500, Ventana).

Corresponding RTU systems for the mAb clone 6E3 from Ventana and Biocare also provided optimal results. For the Ventana RTU system, optimal results could be obtained both by the official recommendations and by modified protocols adjusting HIER time, incubation time of the primary Ab and/or detection system.

Controls
Normal testis is recommended as positive tissue control for SALL4. The majority of spermatogonia lining the basement membrane in seminiferous tubules must show an at least weak to moderate but distinct nuclear staining reaction. A dot-like staining of nucleoli in dispersed spermatocytes may be seen.
No staining of stromal cells, including Leydig cells, should be seen. Appendix can be used as negative tissue control. No staining reaction should be seen.

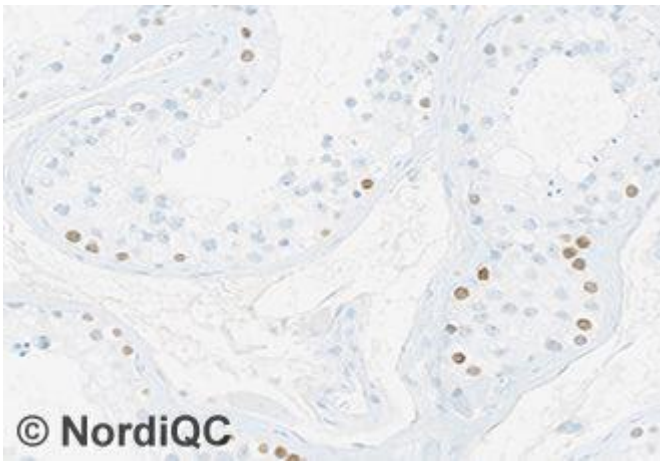


Fig. 1a
Optimal SALL4 staining of normal testis using the mAb clone 6E3 as a concentrate (CM384, Cell Marque) optimally calibrated at a titre of 1:100, HIER in CC1 FOR 48 min. and a 3-step multimer based detection system (OptiView 760-700, Ventana). Spermatogonia at the basement membrane of the tubules show a moderate distinct nuclear staining reaction and no background staining is seen. Also compare with Figs. 2a – 4a, same protocol.

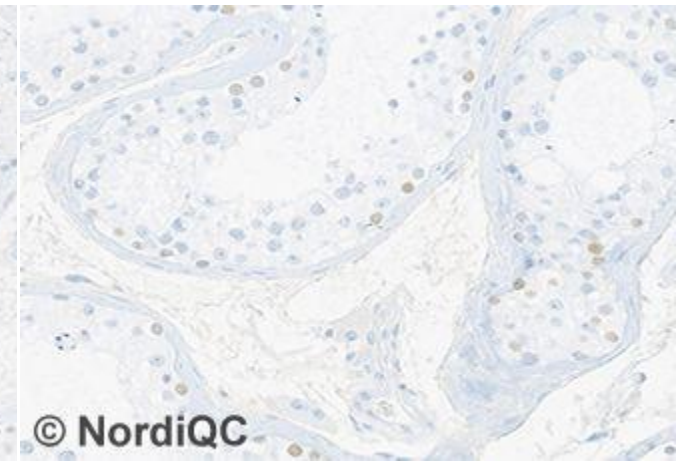


Fig. 1b
Staining for SALL4 of the normal testis assessed as "Good". The intensity of the nuclear staining reaction in the spermatogonia is reduced compared to the result obtained in Fig. 1a – same field. However also compare with Figs. 2b and 3b, same protocol. A fully diagnostic sufficient result overall is obtained. The protocol was based on the same mAb and titre as in Fig. 1a, but used with HIER for 32min. in CC1 and a 2-step multimer based system (UltraView 760-500, Ventana).

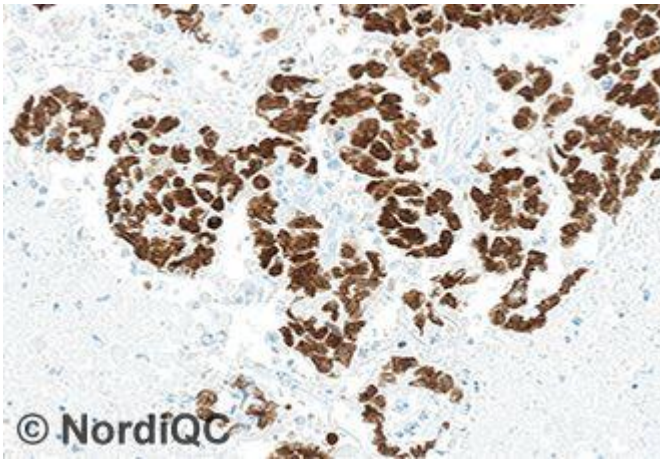


Fig. 2a
Optimal staining for SALL4 of the embryonal carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background staining is seen.

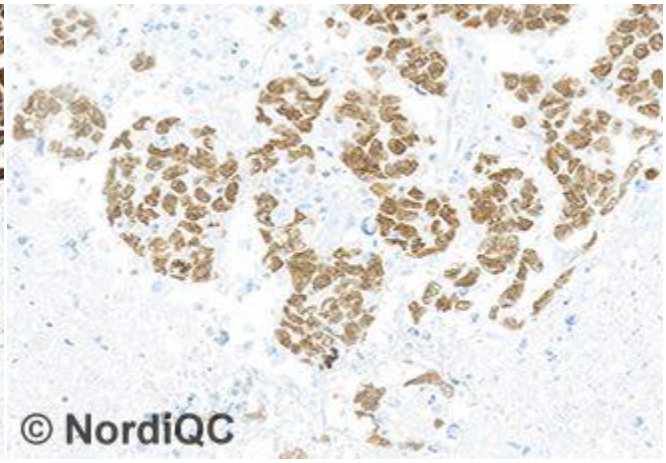


Fig. 2b
Staining for SALL4 of the embryonal carcinoma assessed as "Good" using same protocol as in Fig. 1b - same field as in Fig. 2a. The neoplastic cells are demonstrated, but the intensity is reduced.

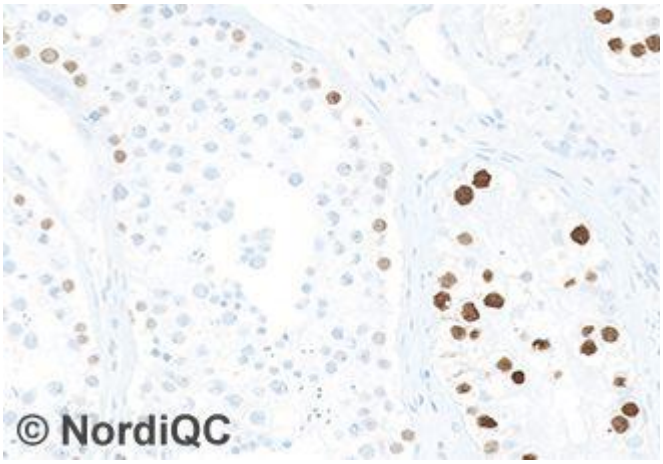


Fig. 3a
Optimal staining for SALL4 of the intratubular germ cell neoplasia using same protocol as in Figs. 1a and 2a.
At right virtually all the neoplastic cells show a strong and distinct nuclear staining reaction, whereas normal spermatogonia (left) show a weak to moderate nuclear staining reaction. No background staining is seen.

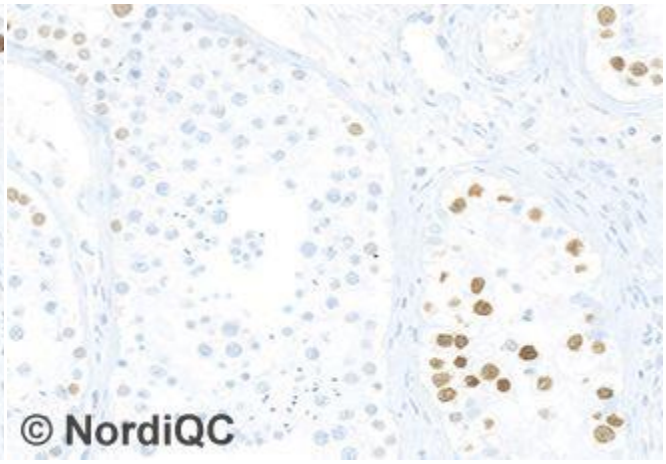


Fig. 3b
Staining for SALL4 of the intratubular germ cell neoplasia using same protocol as in Figs. 1b and 2b. – same field as in Fig. 3a. A reduced staining intensity is seen in both the neoplastic cells (right) and normal spermatogonia (left). The overall result was assessed as "Good".

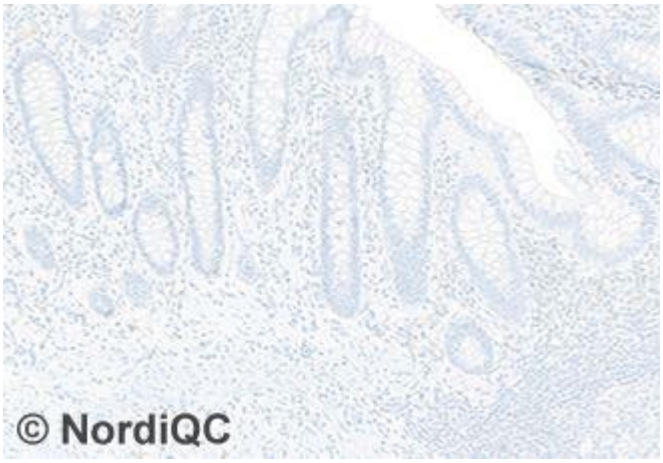


Fig. 4a
Optimal staining for SALL4 of the appendix using same protocol as in Figs. 1a - 3a. No staining reaction is seen.

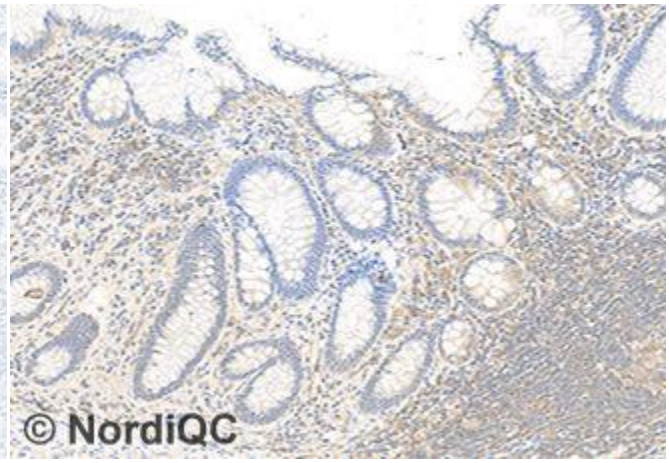


Fig. 4b
Insufficient staining reaction for SALL4 of the appendix. A diffuse background staining and aberrant cytoplasmic staining reaction of stromal cells, lymphocytes etc. complicates the interpretation. The result was assessed as "Borderline" and most likely caused by a too high concentration of the primary mAb clone 6E3.

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