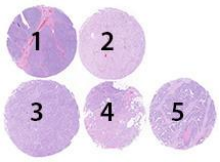
Assessment Run 54 2018
GATA3

Material
The slide to be stained for GATA3 comprised:



1. Tonsil 2. Kidney, 3. Urothelial carcinoma, 4. Breast ductal carcinoma,
5. Colon adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a GATA3 staining as optimal included:

- An at least moderate, distinct nuclear staining reaction of virtually all epithelial cells in collecting ducts and podocytes in renal glomeruli
- An at least moderate nuclear staining reaction of the majority of T helper cells (particularly Th2 cells) in the T-zones in the tonsil and dispersed T-cells in all tissues.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells in the breast ductal carcinoma.
- An at least weak to moderate nuclear staining reaction of the majority of neoplastic cells in the urothelial carcinoma.
- No staining reaction of neoplastic cells of the colon adenocarcinoma.

Participation

Number of laboratories registered for GATA3, run 54	261
Number of laboratories returning slides	245 (94%)

Results
245 laboratories participated in this assessment. 187 (76 %) laboratories achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Less successful primary antibodies
- Use of less sensitive detection systems
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval (HIER)(too short efficient heating time or use of a citrate-based buffer)

Performance history
This was the second NordiQC assessment of GATA3. A pass rate of 76% was observed, which was a small increase compared to the previous run.

Table 2. **Proportion of sufficient results for GATA3 in the two NordiQC runs performed**

	Run 44 2015	Run 54 2018
Participants, n=	124	245
Sufficient results	72%	76%

Conclusion
The mAb clone **L50-823** and the rmAb clone **EP368** could both be used for demonstration of GATA3. The vast majority of participants used the mAb clone L50-823 either within a laboratory developed (LD) assay or as a Ready-to-use (RTU) format. Used within a LD assay, optimal results could be obtained on all four main IHC systems (Dako Autostainer, Dako Omnis, Ventana BenchMark and Leica Bond). Efficient HIER, preferable in an alkaline buffer, careful calibration of the primary antibody and use of a sensitive detection system were the most important prerequisites for optimal staining results.
The corresponding RTU system 760-4897 (Ventana) of the mAb clone **L50-823** provided a high proportion of sufficient and optimal results, especially if OptiView (760-700) was used as detection system.
Normal kidney and tonsil are recommendable as positive and negative tissue controls for GATA3. Virtually all epithelial cells in collecting ducts and podocytes in renal glomeruli must show a moderate to strong nuclear staining reaction, while an at least moderate but distinct nuclear staining reaction of T helper cells (Th2) must be seen. No staining reaction of B-cells should be seen.

Table 1. **Antibodies and assessment marks for GATA3, run 54**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **L50-823**	38 77 4 2 1 1 1 1 1 1 2	Biocare Cell Marque BD Bioscience Zytomed Immunologic Zeta DBS DCS Lifespan Neomarkers Nordic Biosite Menapath	51	39	14	26	69%	80%
mAb clone **HG3-31**	2	Santa Cruz	0	0	0	2	-	-
rmAb clone **EP368**	3	Cell Marque	3	0	0	0	-	-
Unknown	2		1	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **L50-823 760-4897**	92	Ventana	65	19	6	2	91%	97%
mAb Clone **L50-823 390M-17,18,10**	9	Cell Marque	3	3	3	0	-	-
mAB Clone **L50-823 PM 405AA**	3	Biocare Medical	0	2	1	0	-	-
mAB clone **L50-823 PM199**	1	Path N Situ	0	0	0	1	-	-
mAB clone **L50-823 MAB-0695**	1	Maixin	0	1	0	0	-	-
mAB clone **L50-823 MAD-000632QD**	2	Master Diagnostica	0	0	1	1	-	-
Total	245		123	64	25	33	-	
Proportion			50%	26%	10%	14%	76%	

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

Detailed analysis of GATA3, Run 54
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **L50-823**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/18)*, Cell Conditioning 1 (CC1, Ventana) (32/46), Cell Conditioning 2 (CC2, Ventana) (1/2), Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (14/43) and Tris-EDTA/EGTA pH 9 (1/4) as retrieval buffer. The mAb was typically diluted in the range of 1:50-300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 65 of 81 (80%) laboratories produced a sufficient result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP368:** Protocols with optimal results were all stained on the Dako Omnis with HIER in TRS pH 9 (3-in-1). The rmAb was diluted 1:200 and EnVision FLEX+ (GV800/GV823+GV809) was used as the detection system. All protocols were assessed as optimal (3/3).

Table 3. **Proportion of optimal results for GATA3 for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark 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 clone **L50-823**	3/21** (14%)	-	9/16 (56%)	0/3	28/41 (68%)	1/1	2/12 (17%)	0/2

* 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 **L50-823**, product no. **760-4897**, Ventana, BenchMark XT, ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min.), 16-40 min. incubation of the primary Ab and iView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 67 of 69 (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 strictly 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 GATA3 for the most commonly used RTU IHC system**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana RTU mAb clone **L50-823 760-4897**	100% (35/35)	77% (27/35)	81% (34/42)	67% (28/42)

* 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 were included.

Comments
In this second NordiQC assessment for GATA3, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 93% of the insufficient results (54 of 58 laboratories). Too weak staining result was characterized by a reduced staining reaction both in regard to the intensity and proportion of cells expected to be demonstrated. The remaining insufficient results were caused by a poor signal-to-noise ratio or extensive background compromising interpretation.
Virtually all laboratories were able to demonstrate GATA3 in high-level antigen expressing cells, such as neoplastic cells of the breast ductal carcinoma and epithelial cells of the renal collecting ducts. However, demonstration of GATA3 in low-level antigen expressing cells as normal T helper cells (Th2) (all specimens) or neoplastic cells of urothelial carcinoma was more challenging and required optimally calibrated protocols.

Optimal staining results could only be obtained with the mAb clone L50-823 and rmAb clone EP368. Used as concentrated format within a laboratory developed (LD) assay, optimal results for the mAb clone L50-823 could be obtained on all four main IHC platforms (Dako autostainer, Dako Omnis, Leica Bond and Ventana BenchMark) (see Table 3).
Used within a LD-assay, the performance of the mAb L50-823 was significantly influenced by the company/distributor of the primary Ab. In this assessment, 25% (33 of 130) and 59% (77 of 130) of the laboratories purchased the Ab from Biocare or Cell Marque, respectively. Using similar protocol settings, and applying the mAb L50-823 from Biocare, the overall pass rate was only 55% (21 of 38) of which 21% (8 of 38) were assessed as optimal, whereas the proportion of sufficient results were 78% (60 of 77) of which 49% (38 of 77) gave an optimal mark if laboratories used the primary Ab from Cell Marque. From internal studies in a NordiQC reference laboratory, it is known that the mAb L50-823 from Biocare can be difficult to optimize on the Omnis platform (Dako) and that the Ab benefit from dilution in low pH diluent for optimal performance (e.g. Renoir Red pH 6.2 or Van Gogh pH 6.0, Biocare). Indeed, and according to the data sheet from Biocare, Renoir Red is recommended as antibody diluent. Although vaguely, data from this run support this observation, as the clone L50-823 from Biocare in general provided a lower pass rate of 37% (3 of 8) on the Dako Omnis platform compared to 82% (9 of 11) if the primary Ab was based as

concentrated format from Cell Marque. Also, two of the three protocols, assessed as sufficient, used a low pH diluent (Renoir Red pH 6.2, Biocare) and one were assessed as optimal (diluted 1:200). The remaining protocol, assessed as optimal, used the standard diluent pH 7.3 from Dako but applied the primary Ab in an 8-fold higher concentration (1:25 versus 1:200).
As mentioned in the previous report run 44 (2015), parameters as efficient HIER in an alkaline buffer in combination with a careful calibration of the primary Ab was critical for optimal performance of mAb clone L50-823. In addition, the choice of detection system also impacted the overall performance of the assays in this run. Using optimal protocol settings as described above, the pass rate for 2-step multimer/polymer detection systems (e.g. UltraView or EnvFlex) was 38% (8 of 21) of which 10% (2 of 21) were assessed as optimal. In comparison, the pass rate was 83% (64 of 77) of which 56% (43 of 77) were optimal if a 3-step multimer/polymer detection system was applied (e.g. OptiView or EnvFlex+). Thus, substituting 2-step with a 3-step detection system may be advantageous and could improve the analytical sensitivity of the assay.

The rmAb clone EP368 was only used on the Dako Omnis and provided optimal results using the method describe above.

The mAb clone HG3-31 (Santa Cruz) showed a less successful performance due to false negative staining reaction. The Ab was used by 2 laboratories as a concentrate and despite using similar protocol settings, e.g. HIER, detection systems etc., as for the mAb clone L50-823, both protocols produced poor staining results as shown in Table 1. The same pattern was seen in the previous run 44, 2015 and in total 8 protocols has been assessed as insufficient (poor). Therefore, laboratories should substitute this clone with one of the more robust Abs providing optimal results (mAb L50-823 or rmAB EP368).

The Ready-To-Use system 760-4897 (Ventana) based on the mAb clone L50-823, provided higher pass rates and proportion of optimal results compared to LD assays. The pass rate was 91% and could be obtained both using the official protocol recommendations given by Ventana and by laboratory modified protocol settings, typically adjusting HIER time, incubation time of the primary Ab and/or choice of detection system. Applying vendor recommended protocol settings, 100% (35 of 35) of the protocols provided a sufficient result and 77% were assessed as optimal (see Table 4). The vendor recommended protocol settings are based on two fundamentally different detection systems: UltraView and OptiView. All protocols based on the 3-step multimer detection system OptiView were assessed as optimal (20 of 20). Therefore, laboratories (Ventana users) are encouraged to use this true plug and play system, as it is very robust and in this assessment superior compared to all other assays including LD systems.

The Ready-to-use from Cell Marque (mAb clone L50-823, product no. 390M-17, -18, -10) did only score optimal results on the Ventana Benchmark Ultra even though it was used on multiple platforms (4/9). Protocols with optimal results were based on HIER using CC1 (efficient heating time 36-64 min.), 24-32 min. incubation of the primary Ab and OptiView (760-700) as detection system (4/6).

This was the second assessment of GATA3 in NordiQC (see Table 2). A minor increase in pass rate was seen in this run 54, 2018 compared to the latest run 44, 2015. Several elements influenced the final outcome: 1) Within LD-assays, the mAb L50-823 from Biocare should be used in high concentration (dilution range 1:25-1:50) or diluted in low pH diluent (e.g. Renoir Red, dilution range 1:100-1:300), 2) Laboratories should perform efficient HIER in an alkaline buffer and use a 3-step multimer/polymer detection system providing the highest proportion of sufficient and optimal results, 3) The RTU system 760-4897 (Ventana) based on the mAb L50-823 was superior compared to all other systems especially if OptiView was applied as detection system, 4) The use of the mAb HG3-31 should be avoided due to poor performance.
Importantly, laboratories should apply an Ab that will work on the in-house IHC platform, calibrate the protocols correctly and stain according to the expected antigen level of the recommended control material (see below).

Controls
Kidney and tonsil are recommended as positive and negative tissue controls for GATA3. In kidney, moderate to strong nuclear staining reaction in virtually all epithelial cells lining the collecting ducts and podocytes in glomeruli must be seen. In the tonsil the vast majority of T helper cells (Th2) in the T-zones must show an at least moderate but distinct nuclear staining reaction. No staining of B-cells should be seen.

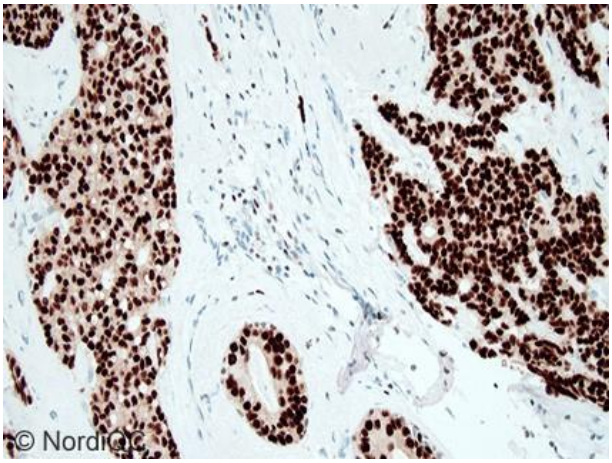


Fig. 1a (x200)
Optimal GATA3 staining of the ductal breast carcinoma (high-level GATA3 expressor) using the mAb clone L50-823 (Biocare) diluted 1:200 in Renoir Red pH 6.2 (Biocare), HIER in TRS pH 9 (Dako) and EnvFlex+ (Dako, Omnis) as the detection system.
A strong, but distinct nuclear staining reaction of all neoplastic cells is seen. T-cells in the surrounding connective tissue displays a moderate nuclear staining intensity - same protocol used in Figs. 2a-5a.

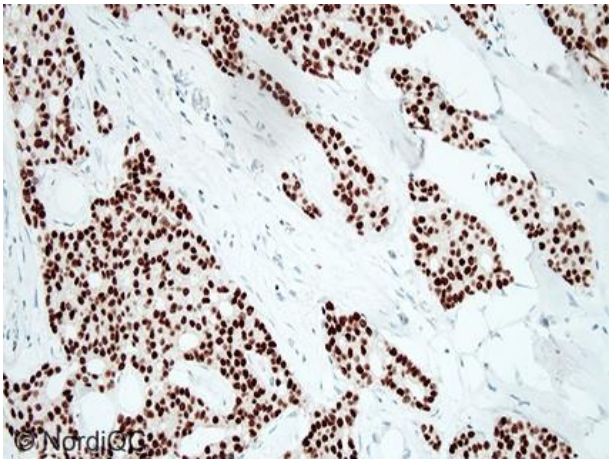


Fig. 1b (x200)
Insufficient GATA3 staining of the ductal breast carcinoma (high-level GATA3 expressor) using exactly the same protocol settings as in Fig 1a, except for dilution of the primary Ab in Dako antibody diluent pH 7.3. Although the neoplastic cells show a relative strong nuclear staining reaction, the protocol provided too low analytical sensitivity and T-cells in the surrounding connective tissue are false negative or only faintly demonstrated - same protocol used in Figs. 2b-5b. The Ab must be carefully calibrated (should be used in higher concentration) or the Ab must be diluted in appropriate low pH antibody diluent (e.g. Renoir Red) for optimal performance - compare optimal versus insufficient staining results in Figs. 1a-5b.

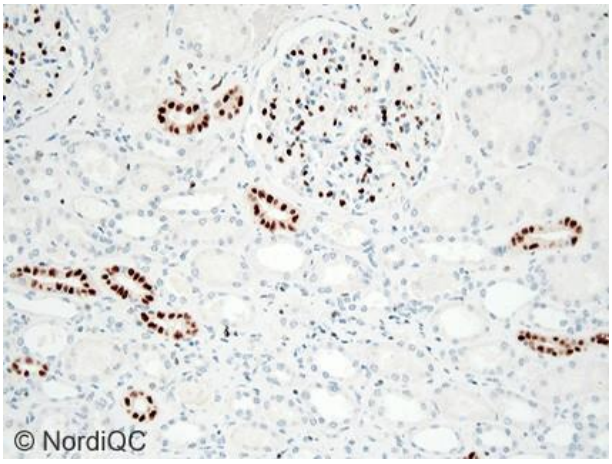


Fig. 2a (x200)
Optimal Gata3 staining of the kidney using the same protocol as in Fig. 1a. All epithelial cells in the collecting ducts and podocytes in glomeruli show a moderate to strong and distinct nuclear staining reaction.

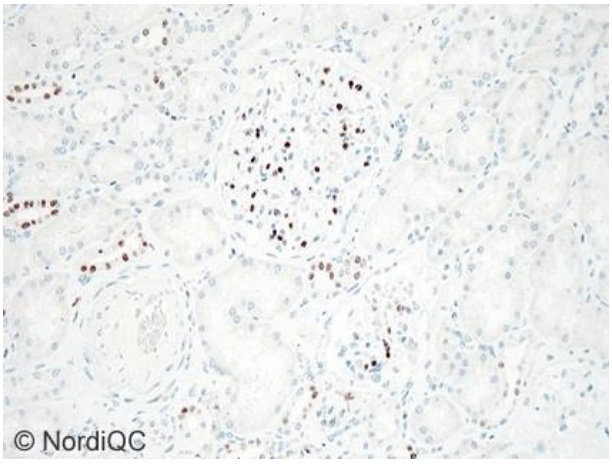


Fig. 2b (x200)
Insufficient GATA3 staining of the kidney using the same protocol as in Fig. 1b. Both staining intensity and proportion of positive nuclei in epithelial cells of the collecting ducts and of podocytes in glomeruli is significantly reduced – compare with Fig. 2a.

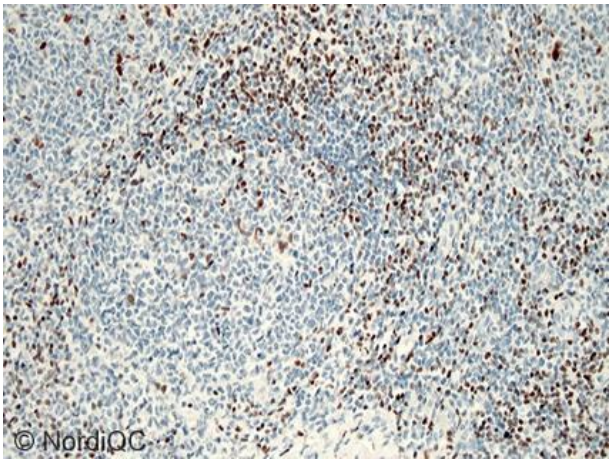


Fig. 3a (x200)
Optimal GATA3 staining of the tonsil using the same protocol as in Figs. 1a-2a. The vast majority of T helper cells (Th2) displays a moderate to strong nuclear staining reaction.

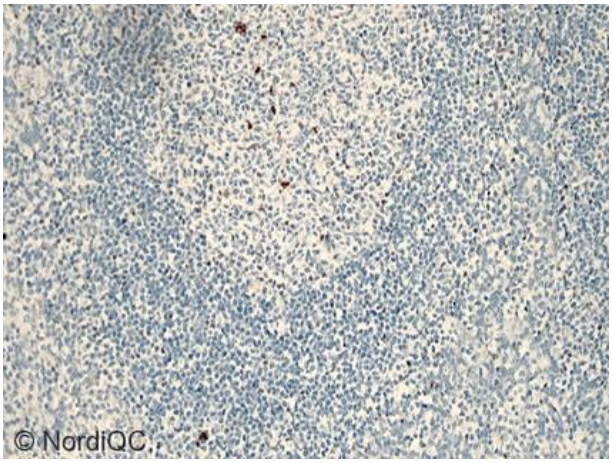


Fig. 3b (x200)
Insufficient GATA3 staining of the tonsil using the same protocol as in Figs. 1b-2b. The vast majority of T helper Cells (Th2) are false negative and only a fraction of germinal centre T-cells are weakly demonstrated – compare with Fig. 3a.

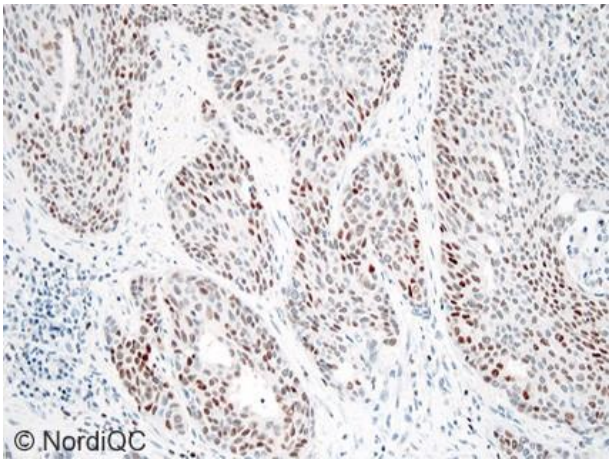


Fig. 4a (x200)
Optimal GATA3 staining of the urothelial carcinoma using the same protocol as in Figs. 1a-3a. The vast majority of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

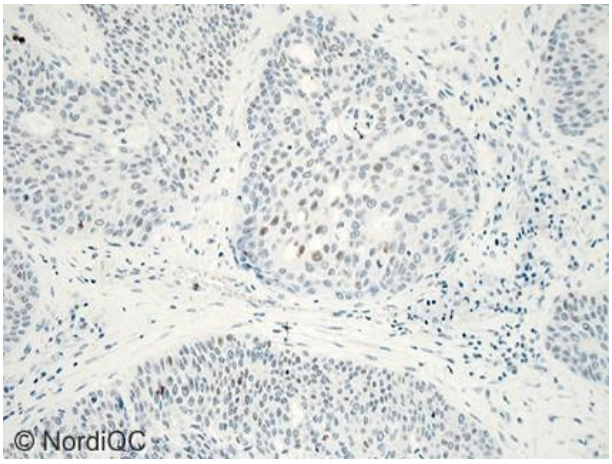


Fig. 4b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using the same protocol as in Figs. 1b-3b. The staining reaction of the nuclei is barely visible, and a significant proportion of the neoplastic cells are false negative which makes interpretation difficult and could lead to misdiagnosis – compare with Fig. 4a.

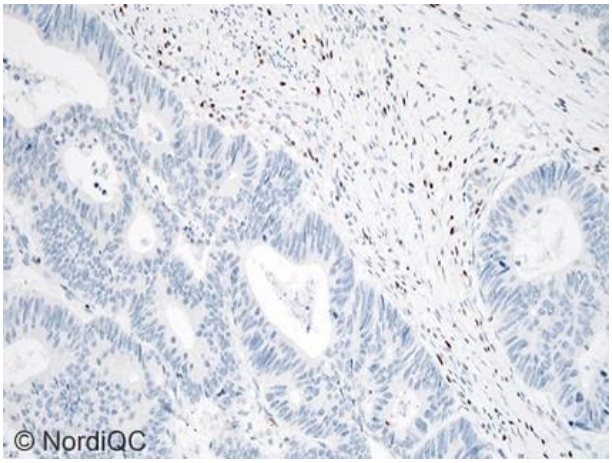


Fig. 5a (x200)
Optimal GATA3 staining of the colon adenocarcinoma using the same protocol as in Figs. 1a-4a. The neoplastic cells are negative and T-cells in the stromal compartment show a moderate to strong but distinct nuclear staining reaction.

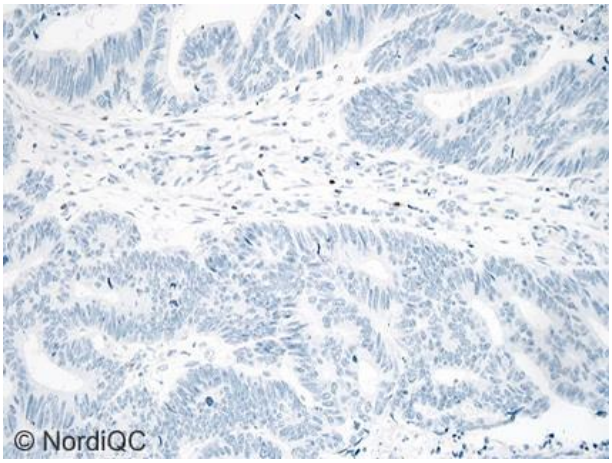


Fig. 5b (x200)
Insufficient GATA3 staining of the colon adenocarcinoma using the same protocol as in Figs. 1b-4b. As expected, the neoplastic cells are negative but staining intensity and proportion of positive T-cells in the stromal compartment is significantly reduced.

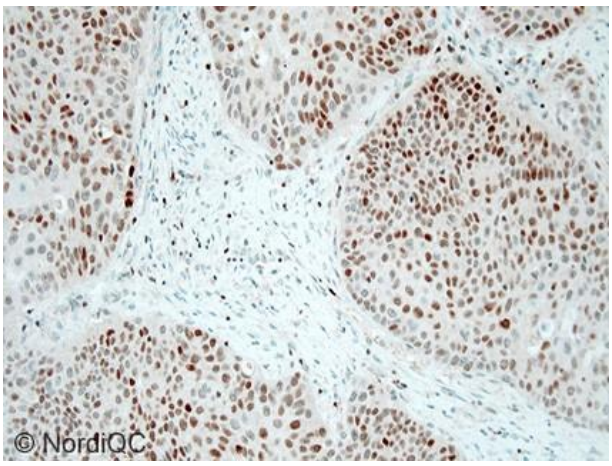


Fig. 6a (x200)
Optimal GATA3 staining of the urothelial carcinoma using the RTU product 760-4897 (Ventana) based on the mAb clone L50-823. The protocol settings were applied as recommended by the vendor: HIER in CC1 (32 min.), 32 min. incubation time in primary Ab and OptiView as detection system.
The vast majority of the neoplastic cells and T-cells displays the expected reaction pattern as seen in Fig. 4a.

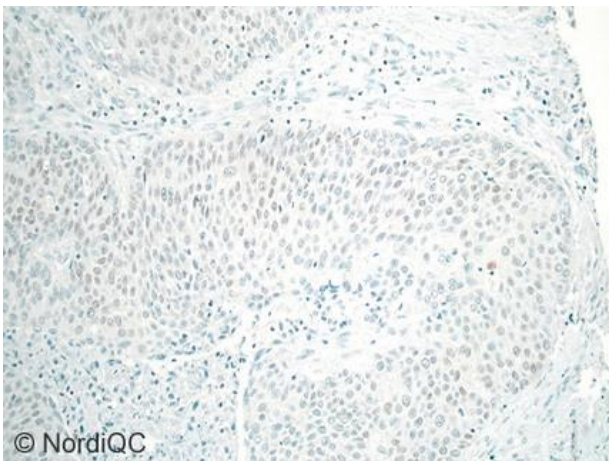


Fig. 6b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using a laboratory modified protocol of the RTU product described in Fig. 6a. Although applying a sensitive detection system (OptiView), the staining intensity and proportion of positive neoplastic cells is significantly reduced due to too short HIER time in CC1 (16 min.) and too short incubation time in primary Ab (16 min.). Using the protocol in Fig. 6a, all protocols were assessed as optimal (see description/comments above).

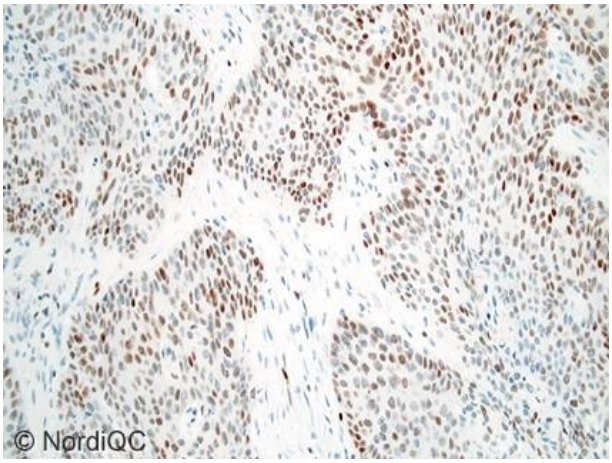


Fig. 7a (x200)
Optimal GATA 3 staining of the urothelial carcinoma using a protocol based on rmAb clone EP368**,** diluted 1:200 in Renoir Red pH 6.2, HIER in TRS pH 9 (Dako) and EnvFlex+ (Dako, Omnis) as detection system. Three Laboratories used the same system and all were assessed as optimal, providing the expected reaction pattern as seen in Fig. 4a and 6a.

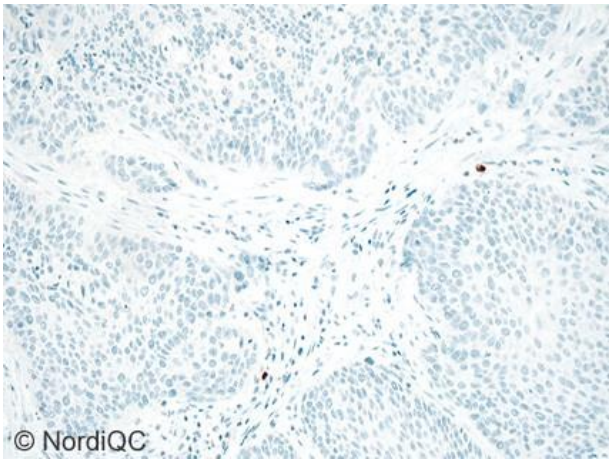


Fig 7b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using the mAb clone HG3-31 (Santa Cruz). Despite applying settings similar to optimal protocols for other clones, none of the protocols using this primary Ab have been assessed as sufficient in the two NordiQC runs performed. The primary Ab provides too low analytical sensitivity and should be substituted with either mAb L50-823 or rmAb EP368 – compare with Figs. 4a or 7a.

TJ/RR/LE/MV/MB 04.12.2018