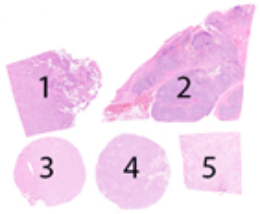
Assessment run 77 2026

Epithelial Membrane Antigen (EMA)

Purpose
Evaluation of the technical performance, analytical sensitivity and specificity of EMA IHC tests among the NordiQC participants, typically used in the diagnostic work-up of identifying carcinomas of epithelial origin in cancers of unknown primary (CUP) site.
Relevant clinical normal and neoplastic tissues were selected to include a broad spectrum of EMA antigen expression levels (see below).

Material
The slide to be stained for EMA comprised:



1. Colon adenocarcinoma, 2. Tonsil, 3. Kidney, 4. Lung adenocarcinoma, 5. Clear cell renal cell carcinoma (ccRCC).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing EMA staining as optimal included:

- A moderate to strong cytoplasmic staining reaction of the majority of the intermediate and superficial squamous epithelial cells and at least a weak predominantly membranous staining reaction of most plasma cells and perineurial cells in the tonsil.
- A moderate to strong predominantly cytoplasmic staining reaction of the epithelial cells of the renal collecting tubules and at least weak to moderate staining reaction of most cells lining the loops of Henle.
- A moderate to strong, distinct, predominantly cytoplasmic staining of virtually all the neoplastic cells of the colon adenocarcinoma.
- A strong, distinct, predominantly cytoplasmic staining of virtually all the neoplastic cells of the lung adenocarcinoma.
- An at least moderate predominantly membranous but also cytoplasmic staining reaction of the majority of the neoplastic cells in the clear cell renal cell carcinoma.
- No staining of lymphocytes in the tonsil and of the epithelial cells in the proximal tubules in kidney.

In this assessment, all EMA clones produced distinct membranous staining reaction in smooth muscle cells in some vessels in the tonsil specimen. This was not expected and not observed in the previous assessments and the reaction was fully accepted provided that an otherwise expected staining pattern was obtained.

KEY POINTS FOR EMA IMMUNOASSAYS
- Clone **E29** were the most widely used antibody for demonstration of EMA applied by 86% of the laboratories.
- The mAb clone **GP1.4** did not provide any sufficient results and cannot be recommended for detection of EMA.
- 3-layer detection systems provided the highest pass rates among all clones assessed.
- In this assessment, tonsil as single control material was found less reliable as critical positive tissue control and should preferably be used in combination with kidney.

Participation

Number of laboratories registered for EMA, run 77	470
Number of laboratories returning slides	427 (91%)

Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results
427 laboratories participated in this assessment. One laboratory applied its staining protocol to an incorrect slide. This was not included in the analysis. Of the remaining 426 laboratories 325 (76%) achieved a

sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarise the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab e.g. mAb clone GP1.4 both as concentrate and RTU.
- Use of 2-step detection systems.
- Insufficient HIER e.g. too short retrieval time.

Controls
Normal tonsil and kidney in combination are recommended as positive and negative tissue controls. Virtually all intermediate and superficial squamous epithelial cells must show a strong cytoplasmic staining reaction, and most importantly, the plasma cells must display at least weak to moderate but distinct membranous staining reaction. Tonsil is thus primarily used to identify that the two expected staining patterns (cytoplasmic and membranous) have been obtained. The demonstration of plasma cells in previous assessment has been reliable as critical control with respect to analytical sensitivity for EMA but was in this assessment found less useful. No staining should be seen in lymphocytes. In kidney, a moderate to strong predominantly cytoplasmic staining of the epithelial cells of the renal collecting tubules must be present, whereas the cells lining the loops of Henle should show an at least weak to moderate staining reaction. The epithelial cells of the proximal tubules must be negative.

Conclusion
The widely used mAb clone **E29**, but also the newly introduced clones **GM25**, **QR118**, **MX132**, **C12A29** and **BY138** could all be used to produce optimal staining results for EMA. Irrespective of the clone applied, efficient HIER, use of a sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Among Ready-to-Use (RTU) systems from the major vendors, and applying vendor recommended protocol settings, the RTU systems **790-4463** (Ventana/Roche), **IR629** and **GA629** (Dako/Agilent) all based on the clone **E29** provided high pass rates especially with vendor recommendations and use of 3-layer detections systems. The mAb clone **GP1.4** both used as a concentrate and as RTU **PA0035** (Leica Biosystems) did not produce any sufficient results and laboratories using this product should consider changing to a more robust antibody.

Table 1a. **Overall results for EMA, run 77**

	n	Optimal	Good	Borderline	Poor	Sufficient results[1]	Optimal results
Concentrated antibodies	102	43	31	26	2	73%	42%
Ready-To-Use antibodies	324	161	91	72	-	78%	50%
Total	426	204	121	99	2		
Proportion		48%	28%	23%	1%	76%	

1) Optimal and good

Table 1b. **Concentrated antibodies and assessment marks for EMA, run 77**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **E29**	70	Dako/Agilent	36	24	9	1	87%	49%
	10	Cell Marque	5	5	-	-		
	2	BioSB	-	2	-	-		
	1	Zytomed	-	-	1	-		
mAb clone **GP1.4**	13	Leica Biosystems	-	-	12	1	0%	0%
	1	BioGenex	-	-	1	-		
mAb clone **GM25**	1	Genebio Solution	1	-	-	-	-	-
rmAb clone **QR118**	1	Quartett	1	-	-	-	-	-
rmAb clone **ZR133**	1	Zeta Corporation	-	-	1	-	-	-
rmAb clone **BP6034**	1	Biolynx Biotechnology	-	-	1	-	-	-
Clone **11C11-B7**	1	Wondfo	-	-	1	-	-	-
Total	102		43	31	26	2		
Proportion			42%	30%	26%	2%	73%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for EMA, run 77**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **E29 790-4463 (VRPS)[3]**	45	Ventana/Roche	11	25	9	-	80%	24%
mAb clone **E29 790-4463 (LMPS)[4]**	122	Ventana/Roche	55	51	16	-	87%	45%
mAb clone **E29 GA629 (VRPS)[3]**	45	Dako/Agilent	43	2	-	-	100%	96%
mAb clone **E29 GA629 (LMPS)[4]**	27	Dako/Agilent	19	6	2	-	93%	70%
mAb clone **E29 IR629 (VRPS)[3]**	14	Dako/Agilent	14	-	-	-	100%	100%
mAb clone **E29 IR629 (LMPS)[4]**	15	Dako/Agilent	7	4	4	-	73%	47%
mAb clone **GP1.4 PA0035 (VRPS)[3]**	19	Leica Biosystems	-	-	19	-	0%	0%
mAb clone **GP1.4 PA0035 (LMPS)[4]**	14	Leica Biosystems	-	-	14	-	0%	0%
mAb clone **E29 8233-C010**	2	Sakura Finetek	2	-	-	-	-	-
mAb clone **E29 247M-90/97/98**	9	Cell Marque	3	3	3	-	67%	33%
mAb clone **E29 MAD-001100QDS**	2	Master Diagnostica/Vitro S.A.	2	-	-	-	-	-
mAb clone **E29 HAM107**	1	PathNSitu	-	-	1	-	-	-
mAb clone **E29 NOA-MSG059**	1	Zytomed Systems GmbH	-	-	1	-	-	-
mAb clone **E29 GM061302**	1	Gene Tech	1	-	-	-	-	-
mAb clone **MX132 MAB-1101**	1	Fuzhou Maixin	1	-	-	-	-	-
mAb clone **C12A29 CEM-0042**	1	Celnovte	1	-	-	-	-	-
mAb clone **BY138 BFM-0430**	1	Bioin Biotechnology	1	-	-	-	-	-
mAb clone **757F5D6 PA210**	1	Abcarta	-	-	1	-	-	-
mAb clone **757F5D6 BE5014**	1	Guangzhou Biotron	-	-	1	-	-	-
rmAb clone **QR118 P-E007-30**	1	Quartett	1	-	-	-	-	-
Clone **DY49499 491052**	1	Immunologic	-	-	1	-	-	-
Total	324		161	91	72	-		
Proportion			50%	28%	22%	0%	78%	

1) Proportion of sufficient results (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 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).

Detailed analysis of EMA, run 77

The following protocol parameters were central to obtaining optimal staining:

Concentrated antibodies

mAb clone **E29**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) High pH (11/16)*, TRS Low pH (1/2) (Dako/Agilent), Cell Conditioning 1 (CC1, Ventana/Roche) (8/39), BOND Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (13/15) or BOND Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (6/6) as retrieval buffer. The mAb was typically diluted between 1:50-1:800. Using these protocol settings, 78 of 88 (89%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for EMA on the four main IHC systems by optimal titration settings as listed above**

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	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **E29** 1:50-800	100% (5/5)	-	63% (5/8)	1/2	18% (6/33)	-	85% (11/13)	100% (6/6)

1) Autostainer Link 48.
2) BenchMark Ultra, Ultra plus
3) BOND III, Prime, Max

Ready-To-Use antibodies and corresponding systems

mAb clone **E29**, product no.**790-4463**, Ventana/Roche, BenchMark Ultra/Ultra Plus:
Optimal protocols using UltraView (760-500) with or without amplification (670-080) as detection system were typically based on HIER using CC1 (efficient heating time 64-76 min.) and 20-40 min. incubation of the primary Ab.
Optimal protocols using OptiView (760-700) as detection system were typically based on HIER using CC1 (efficient heating time 32-72 min.) and 12-40 min. incubation of the primary Ab.
Using these protocol settings, 107 of 121 (88%) laboratories produced a sufficient staining result.

mAb clone **E29,** product no. **GA629,** Dako, Omnis:
Optimal protocols were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30 min. at 97°C), 10-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 70 of 70 (100%) laboratories produced a sufficient result.

mAb clone **E29,** product no **IR629**, Dako/Agilent, Autostainer Link/Classic:
Optimal protocols were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 10-20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient result.
The product was used by 14 laboratories on a non-intended platform. This data is not included.

Table 3 summarises the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems with changes to basic protocol settings. Only protocols performed on the intended IHC stainer are included.

Table 3. **Proportion of sufficient and optimal results for EMA for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS BenchMark mAb **E29** **790-4463** **UltraView**	76% (28/37)	14% (5/37)	80% (49/62)	19% (12/62)
VMS BenchMark mAb **E29** **790-4463** **OptiView**	100% (8/8)	75% (6/8)	95% (57/60)	72% (43/60)
Dako Omnis mAb **E29** **GA629**	100% (45/45)	96% (43/45)	93% (25/27)	70% (19/27)
Dako AS mAb **E29** **IR629**	100% (14/14)	100% (14/14)	2/2	2/2
Leica BOND mAb **GP1.4** **PA0035**	0% (0/19)	0% (0/19)	0% (0/12)	0% (0/12)

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

Comments
In this third NordiQC assessment for EMA, the prevalent features of insufficient staining results were characterised either by a generally too weak/false negative staining reaction of the cells expected to be demonstrated or a poor signal-to-noise ratio/excessive background. Too weak or false negative staining results were observed in 92% of the insufficient results (93/101). Virtually all laboratories were able to detect EMA in high-level antigen expressing cells such as the intermediate and superficial squamous epithelium of the tonsil, collecting tubules of the kidney and in this case also the neoplastic cells of the clear cell renal cell carcinoma and lung adenocarcinoma. Demonstrating moderate-to-strong cytoplasmic staining in the colon adenocarcinoma and the loops of Henle were more challenging and could only be obtained with optimally calibrated protocols. Protocols with reduced and too low level of analytical sensitivity also demonstrated fewer plasma cells and perineurial cells than expected. The remaining 8% of the insufficient results were caused by poor-signal-to noise ratio or excessive background.
In general, the mAb clone GP1.4 struggled to maintain a cytoplasmic reaction in all tissues but especially pronounced in the lung adenocarcinoma where a strong luminal reaction was observed with only a faint to weak cytoplasmic staining reaction. This was observed both within laboratory developed (LD) and Ready To-Use (RTU) assays (See Figs 6a+b).
In this assessment using the plasma cells in the tonsil did not provide the same insight as a low expressor as in the previous assessment run 28 (2010) and therefore the main reason for downgrading was related to the staining patterns of EMA in kidney and the lung adenocarcinoma.
In this assessment smooth muscle cells in some - especially large - vessels of the tonsil was observed. In literature and publications, EMA is historically described as negative in normal smooth muscle cells. However, all clones both applied as concentrate and RTU formats gave this unexpected staining reaction. It was also revealed by the validation process of the NordiQC IHC assay for EMA, that EMA in addition to the expected reaction in epithelial cells, plasma cells and perineurial cells did demonstrate a weak but distinct predominantly membranous staining reaction of few normal smooth muscle cells e.g. in esophagus and prostate (see Fig. 7b). It is difficult to identify the specific reason for this aberrant and previously unreported positive staining reaction in smooth muscle cells, but it might be related to the increased analytical sensitivity of current IHC assays compared with the corresponding EMA IHC assays used in the older publications describing EMA staining patterns.

The mAb clone **E29** was the most widely used antibody for demonstration of EMA and applied by 86% (368/426) of the laboratories (see Tables 1b-c). Used as concentrated format within laboratory developed (LD) assays the mAb clone **E29** gave the highest proportion of optimal results - 87% (72/83).
As shown in Table 2, optimal results were obtained on all four main IHC platforms from Dako/Agilent, Leica Biosystems and Ventana/Roche. However, the overall pass rate was lower on the Ventana platforms. Among the 39 laboratories using the concentrated format of **E29** on a Ventana platform, 20 applied a 2-layer detection system, achieving a pass rate of 70%, with 10% of protocols assessed as optimal. In comparison, 19 laboratories used a 3-layer detection system, resulting in a pass rate of 84%, of which 32% were assessed as optimal. All insufficient results were marked as too weak and observed even with a highly sensitive 3-layer detection system and a relatively high concentration of the primary Ab in the range of 1:100-200.
The Dako Omnis also provided a somewhat lower pass rate (63%) comparing to the Dako Autostainer platform (100%). This was primarily related to omission of the linker on Omnis, as the pass rate increased to 86% (6/7) when the FLEX+ protocol was applied.

The mAb clone **GP1.4** was used as a concentrate by 14 laboratories. The product was applied on both Autostainer Link 48 (Dako/Agilent), Ventana BenchMark Ultra Plus (Ventana/Roche) and BOND III and Prime (Leica Biosystems). Even though the primary antibody was used in dilution range of 1:50-300, both high and low pH HIER and with both 2- and 3-layer detection systems, no sufficient protocols were observed. For all protocols the demonstration of EMA expression was as previously mentioned too weak and, in most cases, only displayed as a membranous staining reaction instead of a cytoplasmic reaction as expected. In the last assessment run 28, a total of 6 participants used this clone, all receiving sufficient results. However, as the tissues included and purpose were changed in this run 77 compared to run 28, this might have affected the performance of the antibody. In run 28 the purpose of IHC for EMA was related to both identification of carcinomas and neurological lesions, while run 77 only focused on EMA to identify epithelial origin in cancers of unknown primary. Given the fact that no sufficient protocols were achieved even with a wide range of protocol settings the mAb clone **GP1.4** cannot be recommended for detection of EMA.

In this assessment 76% (324/426) of the laboratories used an RTU format for detection of EMA. This is a significant increase compared to the former run 28 in which 20% (31/154) of the participants applied an RTU format.
The RTU mAb clone **E29** (ref. no.**790-4463**), developed for the BenchMark platform (Ventana/Roche), was the most commonly used RTU format, applied by 52% of laboratories (167/324). When vendor-

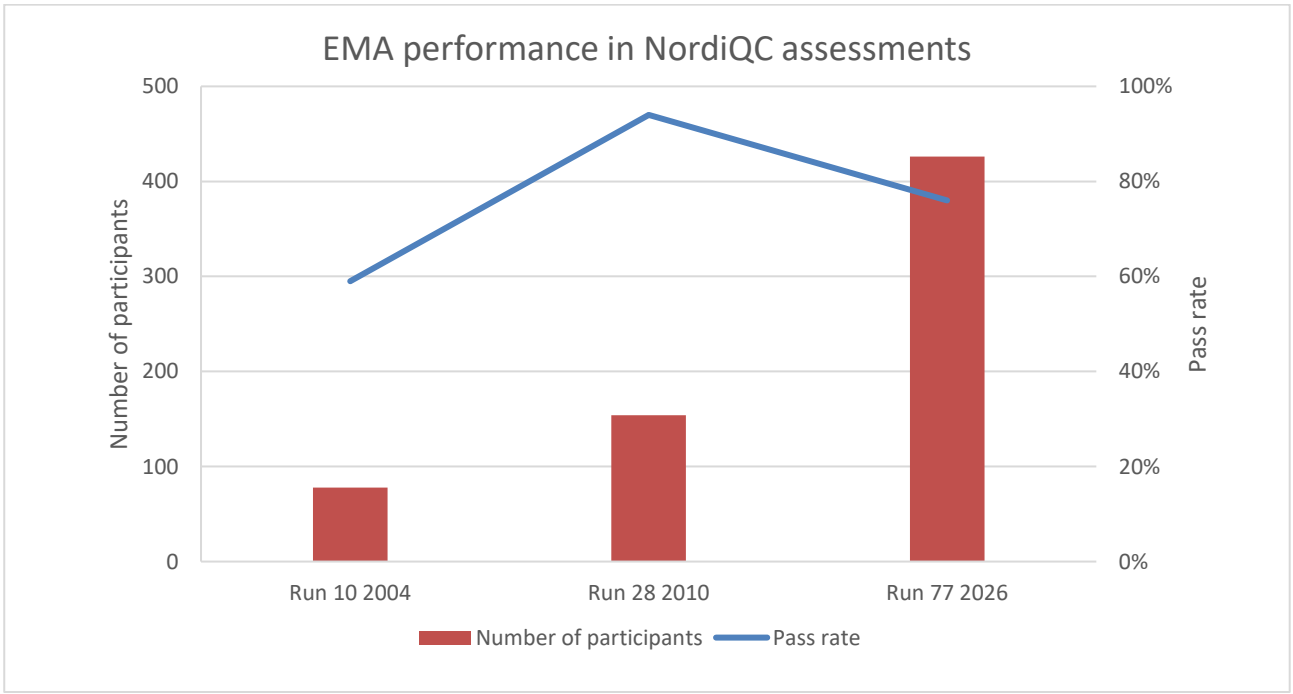
recommended protocol settings were followed on the BenchMark system, the overall pass rate was 80% (36/45), although only 24% of the results (11/45) were assessed as optimal (see Tabel 1c). The low level of optimal results was mainly an effect of the use of 2-layer detection systems as displayed in Tabel 3 and confirmed by earlier mentioned use of the corresponding concentrated format of clone E29. Using OptiView as detection system with vendor recommended protocol settings the pass rate increased to 100%, 75% being optimal, however only 8 laboratories applied these settings. 60 laboratories used OptiView within LD assays mostly increasing HIER to 48-64 min with a pass rate of 95%, 72% being optimal. In comparison, 62 participants used UltraView within LD modifications with a pass rate of 80%, but only 19% being optimal which demonstrates the importance of using the clone **E29** on the Ventana with 3-layer detection systems.

The two Dako/Agilent RTU systems for EMA, **IR629** (Autostainer product) and **GA629** (Omnis product), were also based on mAb clone E29 and provided the highest number of optimal results using vendor recommended protocol settings. On the Autostainer platform, all protocols were assessed as optimal when TRS pH 9 (3-in-1) was applied for 20 minutes, followed by 20 minutes of primary antibody incubation and EnVision FLEX as detection system. For the Dako Omnis, HIER was applied for 30 min, and primary Ab incubation was 25 min with the addition of Mouse Linker for 10 min. These settings provided a pass rate of 100% with 96% optimal results (See table 3). By contrast, omission of the linker reduced the proportion of optimal results to 33% (4/12) (See Figs 1-5a+b).
In addition, the product IR629 with intended use for Autostainer plus, was used by 14 laboratories on non-intended platforms. 6 used the product on Ventana BenchMark and even with the use of OptiView this product gave an inferior performance and cannot be recommended to be transferred to Ventana IHC platforms, as the Ventana Benchmark systems in this context reduced the analytical sensitivity most likely due to the fact that the primary Ab is diluted with residual buffer on the instrument compromising the initially calibrated assay. Ventana users should consider changing to the EMA RTU format 790-4463 which is based on the same clone but optimized for the Ventana BenchMark platforms.

On the Leica BOND platforms, the mAb clone **GP1.4** (**PA0035**) failed to produce any sufficient staining results. Consistent with the findings outlined above for the corresponding concentrated format, this clone lacked the analytical sensitivity required for reliable EMA detection and is therefore not recommended. Laboratories using BOND systems may benefit from switching to a concentrated format, preferably a mouse monoclonal antibody such as **E29**. Notably, Table 2 shows that clone **E29** performed well on the BOND platform with both acidic and alkaline retrieval conditions.

Performance history
This was the third NordiQC assessment of EMA. The pass rate decreased from 94% to 76% in this assessment compared with the previous assessment in run 28 as shown in Graph 1.

Graph 1**. Proportion of sufficient results for EMA in the three NordiQC runs performed.**



Summary

In this third assessment of EMA the pass rate decreased from 94% in run 28 to 76%. Several parameters contributed to the declined pass rate; 1. A high increase in the number of new participants of 77% (154 to 426), 2. Use of poorly performing Abs such as clone GP1.4. Previously only 4% of all participants (6/154) used this clone compared to 11% (47/426) in this assessment 3. Use of less sensitive 2-layer detection systems especially for the Dako Omnis and Ventana platforms and 4. Change of purpose where in the previous assessment the focus was both on neurological lesions and carcinomas, while the primary focus in this assessment was limited to identifying carcinomas of epithelial origin. The tissue material included in this run might have been more challenging than in the previous run.

At the moment, well performing antibodies and recommendations are available for most platforms. However, all protocol settings must be carefully calibrated according to the expected reaction patterns in tissues with the diagnostically relevant range of expression. In this context it should be emphasized that validation needs to include a sufficient number of appropriate neoplasms e.g., colon or lung adenocarcinomas to verify the performance (see below).

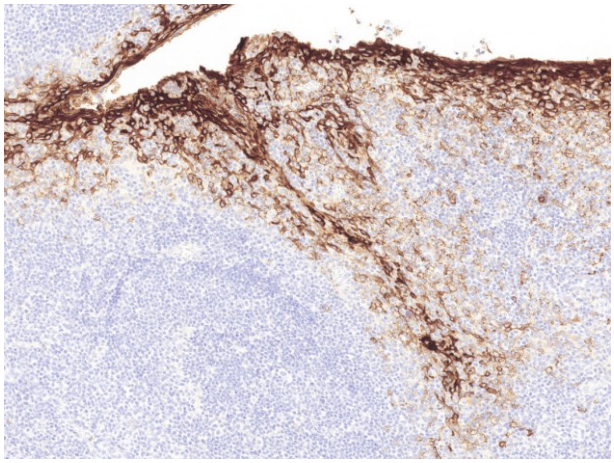


Fig. 1a

Optimal EMA staining reaction of the tonsil using the RTU system GA629 (Dako/Agilent), based on the mAb clone E29 on a Dako Omnis, applying vendor recommended protocol settings with the 3-layer polymer detection system, EnVision FLEX+. Same protocol settings used in Figs. 2a-5a. The squamous epithelial cells show a moderate to strong cytoplasmic staining, while the plasma cells show a moderate and distinct membranous staining. No background staining is seen.

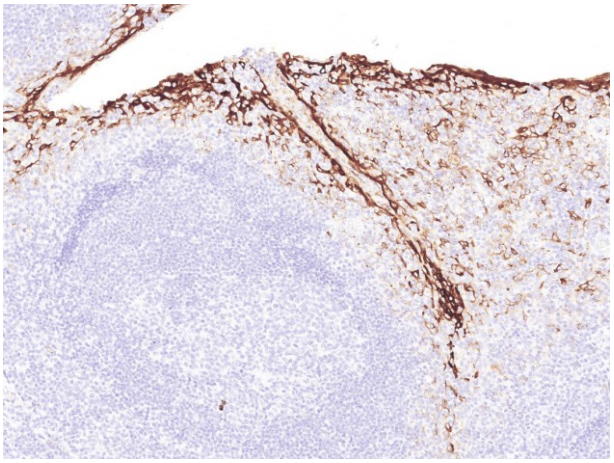


Fig. 1b

Insufficient EMA staining reaction of the tonsil using the RTU system GA629 (Dako/Agilent), based on the mAb clone E29 on a Dako Omnis, but only applying protocol settings with the 2-layer polymer detection system, EnVision FLEX. Same protocol settings used in Figs. 2b-5b.

Only a reduced number of the squamous epithelial cells show a cytoplasmic staining reaction, while many of the plasma cells are negative – also compare with Fig. 1a same field.

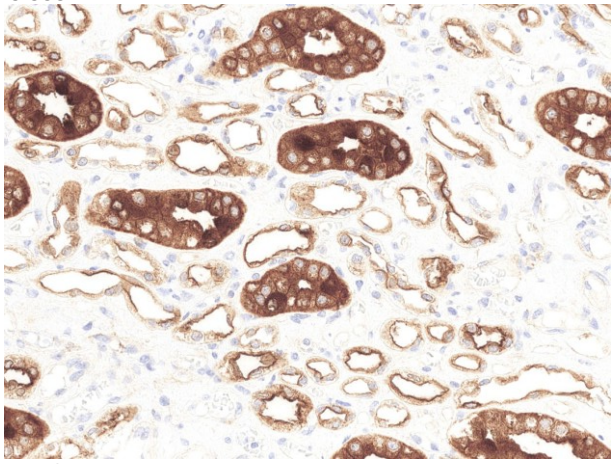


Fig. 2a

Optimal EMA staining reaction of the kidney using the same protocol as in 1a. A moderate to strong predominantly cytoplasmic staining reaction of the epithelial cells of the renal collecting tubules and a weak to moderate reaction in the loop of Henle is seen.

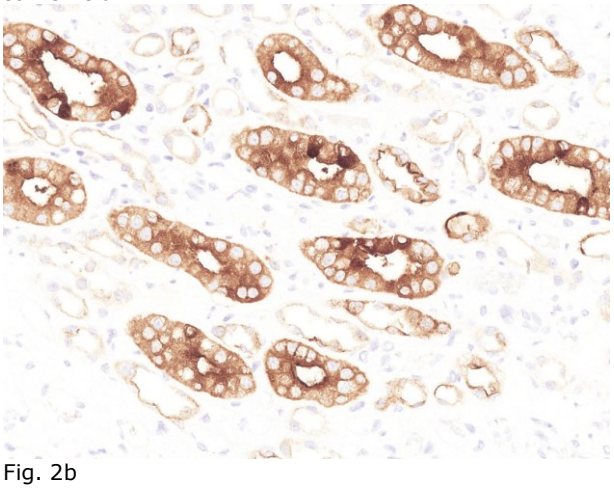


Fig. 2b

Insufficient EMA staining reaction of the kidney using the same protocol as in 1b. A moderate to strong predominantly cytoplasmic staining reaction of the epithelial cells of the renal collecting tubules was observed while the loop of Henle only displayed a faint to weak reaction – also compare with Fig. 2a same field.

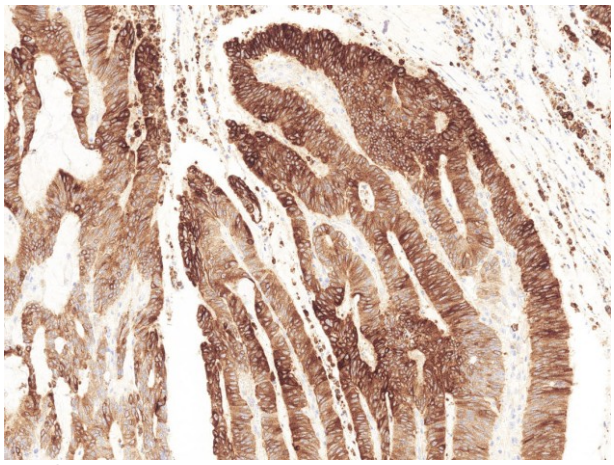


Fig. 3a
Optimal EMA staining reaction of the colon adenocarcinoma using the same protocol as in figs. 1a and 2a. A moderate to strong, distinct, predominantly cytoplasmic staining reaction of virtually all the neoplastic cells of the colon adenocarcinoma was observed.

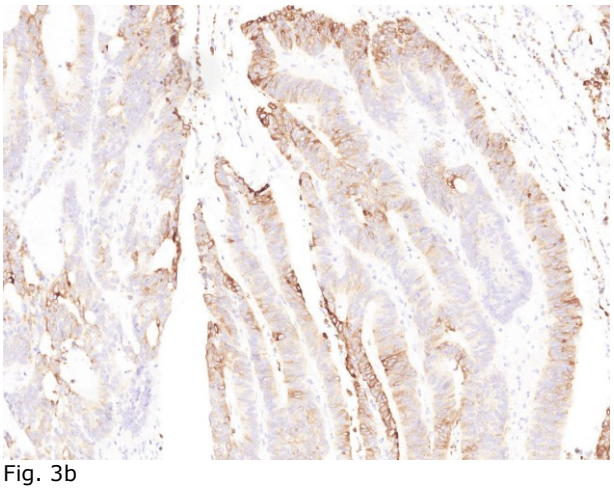


Fig. 3b
Insufficient EMA staining reaction of the colon adenocarcinoma using the same protocol as in figs. 1b and 2b. A largeproportion of the neoplastic cells were negative while the remaining positive cells only displayed a weak to moderate cytoplasmic reaction. Also compare with Fig. 3a - same field.

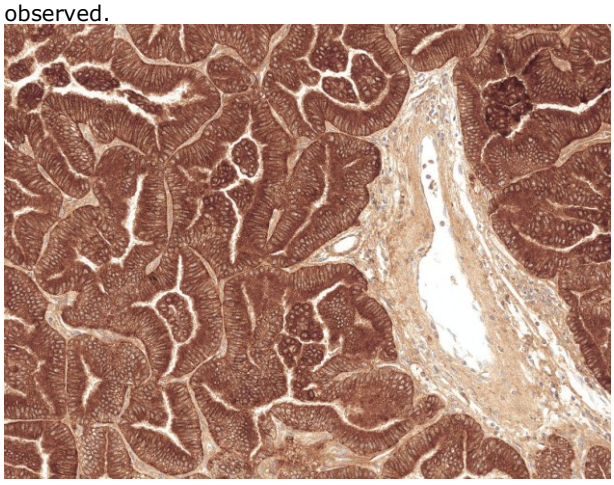


Fig. 4a
Optimal EMA staining reaction of the lung adenocarcinoma using the same protocol as in figs. 1a-3a. A strong, distinct, predominantly cytoplasmic staining reaction of all the neoplastic was observed. In this tissue core bleeding to the surrounding stroma was accepted and observed in all optimal protocols.

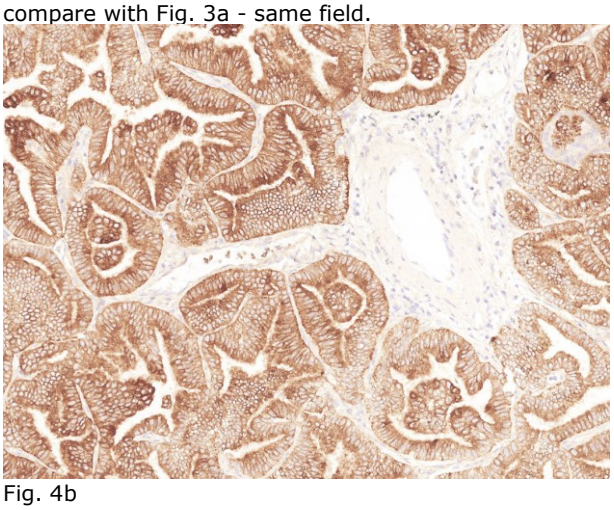


Fig. 4b
Insufficient EMA staining reaction in the lung adenocarcinoma using the same protocol as in Figs. 1b–3b. Although a moderate to strong cytoplasmic reaction was observed, the overall obtained analytical sensitivity compared with the optimal protocol was reduced. Also compare with Fig. 4a – same field.

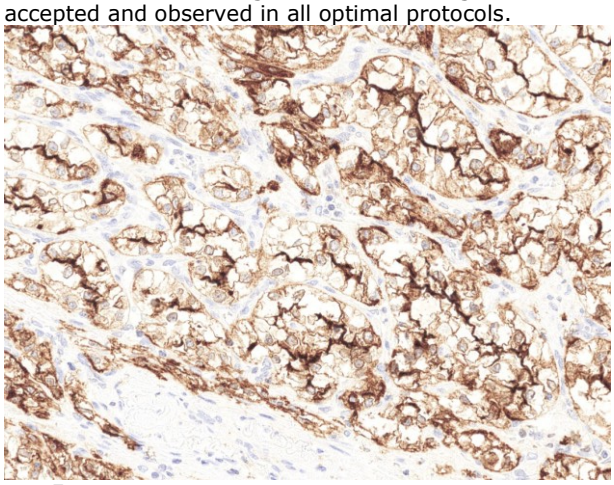


Fig. 5a
Optimal EMA staining reaction of the clear cell renal cell carcinoma using the same protocol settings as in figs. 1a-4a. The majority of the neoplastic cells show a

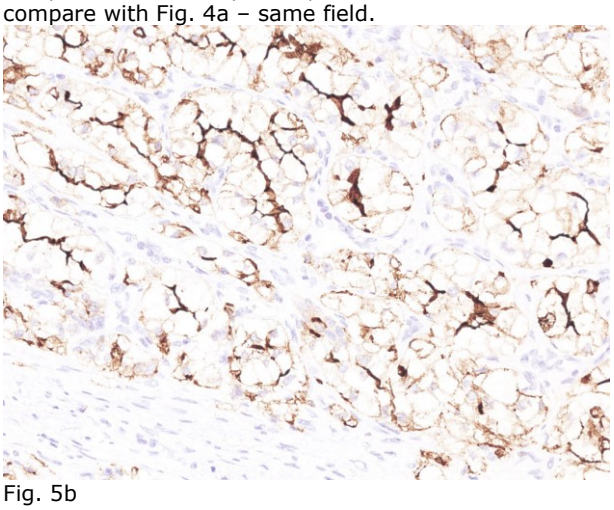


Fig. 5b
Insufficient EMA staining reaction in clear cell renal cell carcinoma using the same protocol as in Figs. 1b–4b. The majority of the neoplastic cells only display a

moderate to strong membranous and also intracytoplasmic staining reaction.

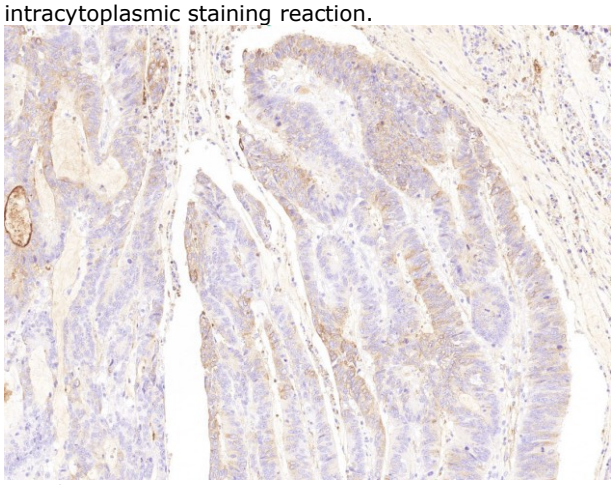


Fig. 6a
Insufficient EMA staining reaction of the colon adenocarcinoma using the RTU system PA0035 (Leica Biosystems), based on the mAb clone GP1.4 on a BOND III, applying vendor recommended protocol settings with the 3-layer polymer detection system, BOND Refine. Same protocol settings used in Fig. 6b.
A big proportion of the neoplastic cells were negative while the remaining positive cells only displayed a weak to moderate cytoplasmic reaction. Also compare with Fig. 3a - same field.

membranous staining reaction. Compare with Fig. 5a – same field.

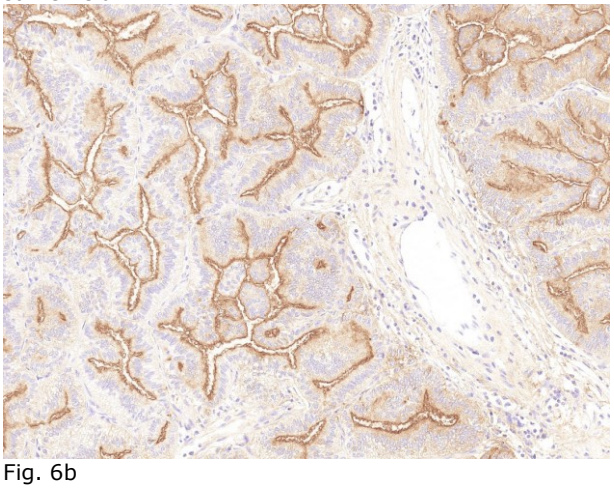


Fig. 6b
Insufficient EMA staining reaction in the lung adenocarcinoma using the same protocol as in Fig. 6a. A faint to moderate cytoplasmic reaction was observed; however, in most neoplastic cells the staining was mainly confined to a strong luminal membranous reaction, indicating insufficient cytoplasmic demonstration of EMA. Also compare with Fig. 4a – same field.

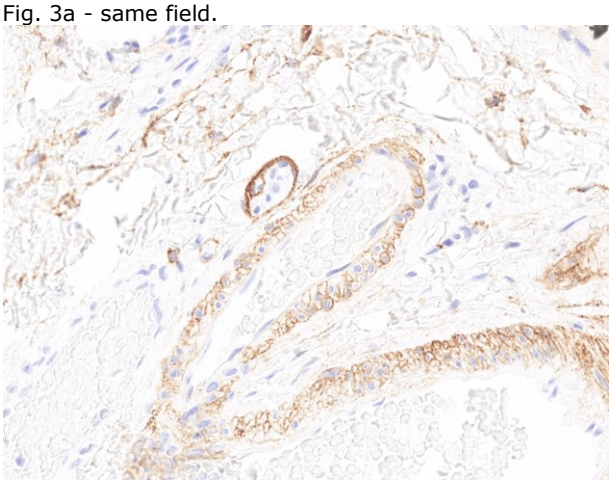


Fig. 7a
Staining reaction of EMA in tonsil displaying the positive reaction in perineurial cells lining nerve bundles, but also in smooth muscle cells of large vessels. The reaction was seen for all clones in this assessment.

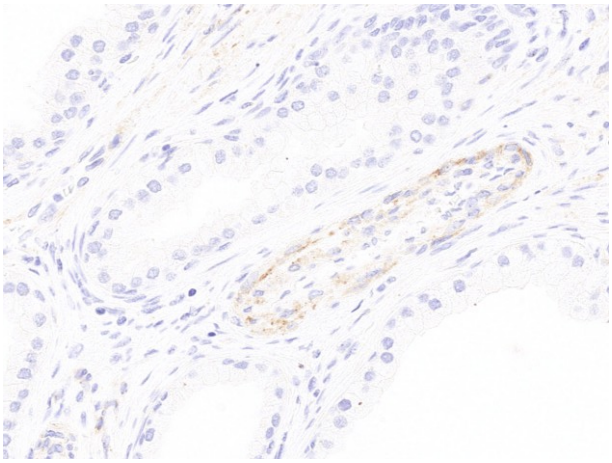


Fig. 7b
Staining reaction of EMA displaying a positive reaction in smooth muscle cells within vessel walls observed in normal prostate. This observation was achieved from the validation process of the NordiQC EMA IHC assay for this assessment and revealed that EMA can be expressed in few normal smooth muscle cells.

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