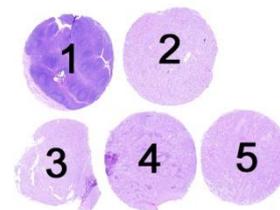


The slide to be stained for EMA comprised:

1. Tonsil, 2. Kidney, 3. Renal clear cell carcinoma, 4. Lung adenocarcinoma,

5. Meningioma

All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing an EMA staining as optimal included:

- A moderate to strong cytoplasmic staining of the majority of the intermediate and superficial squamous epithelial cells and at least a weak predominantly membranous staining of the plasma cells in the tonsil.
- A moderate to strong predominantly cytoplasmic staining of the epithelial cells of the renal collecting tubules, and no staining in the epithelial cells of the proximal tubules.
- A strong, distinct, predominantly cytoplasmic staining of virtually all the neoplastic cells of the lung adenocarcinoma.
- An at least weak to moderate predominantly membranous and dot-like cytoplasmic staining of the majority of the neoplastic cells in the meningioma and the renal cell carcinoma.

154 laboratories participated in this assessment. 94 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for EMA, run 28**

| Concentrated Abs               | N   | Vendor              | Optimal | Good | Borderl. | Poor | Suff. <sup>1</sup> | Suff. OPS <sup>2</sup> |
|--------------------------------|-----|---------------------|---------|------|----------|------|--------------------|------------------------|
| mAb clone <b>E29</b>           | 108 | Dako                | 76      | 34   | 5        | 1    | 95 %               | 99 %                   |
|                                | 5   | NeoMarkers          |         |      |          |      |                    |                        |
|                                | 1   | BioGenex            |         |      |          |      |                    |                        |
|                                | 1   | Cell Marque         |         |      |          |      |                    |                        |
|                                | 1   | Master Diagnostica  |         |      |          |      |                    |                        |
| mAb clone <b>GP1.4</b>         | 2   | Novocastra          | 2       | 2    | 0        | 0    | -                  | -                      |
|                                | 2   | NeoMarkers          |         |      |          |      |                    |                        |
| mAb clone <b>Mc5</b>           | 2   | Biocare             | 1       | 1    | 1        | 0    | -                  | -                      |
|                                | 1   | BioGenex            |         |      |          |      |                    |                        |
| <b>Ready-To-Use Abs</b>        |     |                     |         |      |          |      |                    |                        |
| mAb clone <b>E29, IR629</b>    | 12  | Dako                | 12      | 0    | 0        | 0    | 100 %              | 100 %                  |
| mAb clone <b>E29, 760-4259</b> | 15  | Ventana/Cell Marque | 4       | 9    | 2        | 0    | 87 %               | 100 %                  |
| mAb clone <b>E29, N1504</b>    | 1   | Dako                | 0       | 1    | 0        | 0    | -                  | -                      |
| mAb clone <b>GP1.4, PA0035</b> | 2   | Leica               | 1       | 1    | 0        | 0    | -                  | -                      |
| mAb clone <b>Mc-5, PM143</b>   | 1   | Biocare             | 0       | 1    | 0        | 0    | -                  | -                      |
| <b>Total</b>                   | 154 |                     | 96      | 49   | 8        | 1    | -                  | -                      |
| <b>Proportion</b>              |     |                     | 62 %    | 32 % | 5 %      | 1 %  | 82 %               | -                      |

1) Proportion of sufficient stains (optimal or good)

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

### Concentrated Abs

mAb clone **E29**: The protocols giving optimal results were mainly based on heat induced epitope retrieval (HIER) using one of the following retrieval buffers: Tris-EDTA/EGTA pH 9 (15/22)\*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (25/29), Target Retrieval Solution pH 6.1 (Envision FLEX TRS low pH/S1699, Dako) (3/3), Cell Conditioning 1 (BenchMark, Ventana) (14/22), Bond Epitope Retrieval Solution 2 (Bond, Leica)

(5/7), Bond Epitope Retrieval Solution 1 (Bond, Leica) (3/4), Antigen DECLOAKER pH 6 (BioCare) (1/1), Trilogy pH 7,5 (Cell Marque) (1/1), EDTA/EGTA pH8 (1/2) or Citrate pH 6 (7/13). The mAb was typically diluted in the range of 1:50– 1:2,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 101 out of 102 (99 %) laboratories produced a sufficient staining (optimal or good).

One laboratory with an optimal staining result used no pre-treatment, the dilution was 1:300.

\* (number of optimal results/number of laboratories using this buffer)

mAb clone **GP1.4**: The protocols giving an optimal result were both based HIER using either EDTA/EGTA pH 8 (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/1) as the retrieval buffer. The mAb was diluted 1:50.

mAb clone **Mc5**: The protocol giving an optimal result was based HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1) as the retrieval buffer. The mAb was diluted 1:50.

### Ready-To-Use Abs

mAb clone **E29** (prod. no IR629, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 12 out of 12 (100 %) laboratories produced an optimal staining.

mAb clone **E29** (prod. no. 760-4259, Ventana/Cell Marque), The protocols giving an optimal result were based on HIER using standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 18-40 min in the primary Ab and Ultra View (760-500) as the detection system. 1 laboratory used amplification kit. Using these protocol settings 9 out of 9 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **GP1.4** (prod. no. PA0035, Leica), The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 15 min. in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings 2 out of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:

- Omission of HIER
- Too low concentration of the primary antibody.

In this assessment and in concordance with the observations in the previous assessment of EMA, run 10 2004, the prevalent feature of the insufficient results was a too weak or false negative staining in the cells expected to be demonstrated. This was especially observed in the meningioma and the renal cell carcinoma, whereas the staining of the lung adenocarcinoma was sufficient in virtually all the stains submitted. A too low sensitivity was most commonly seen when HIER was omitted: 5 out of 13 protocols omitting HIER gave an insufficient result and only one resulted in an optimal staining.

Normal tonsil was found to be a reliable positive control for EMA, provided that the majority of the intermediate and superficial squamous epithelial cells showed a strong cytoplasmic staining and most importantly that the plasma cells showed a distinct membranous staining. Virtually all labs obtaining this reaction pattern in the tonsil and plasma cells were assessed as sufficient.

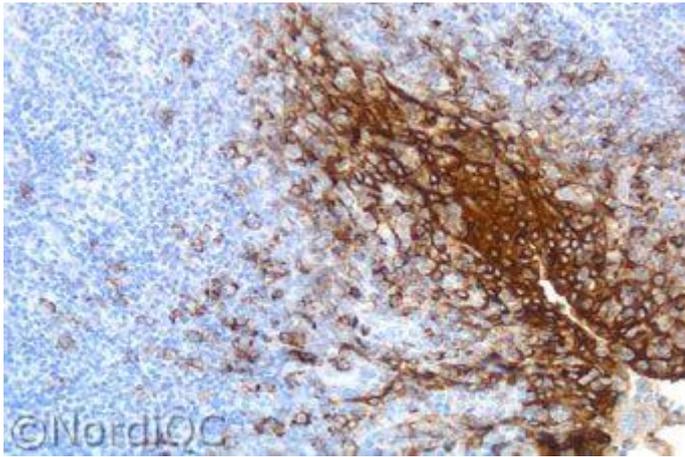
This was the second assessment of EMA in NordiQC, and the proportion of sufficient results increased from 59 % in run 10, 2004 to 94 % in the current run. The higher pass rate is probably due to many factors including changes in the protocols used by the participants: In run 10, 2004, 21 % of the protocols were based on omission of HIER and in the current run, only 8 % was without HIER. The applied titre of the primary Abs has also been increased from an average of 1:850 in run 10 to 1:550 in this run, which combined with the use of more sensitive detection systems definitely has had a positive impact on the performance.

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

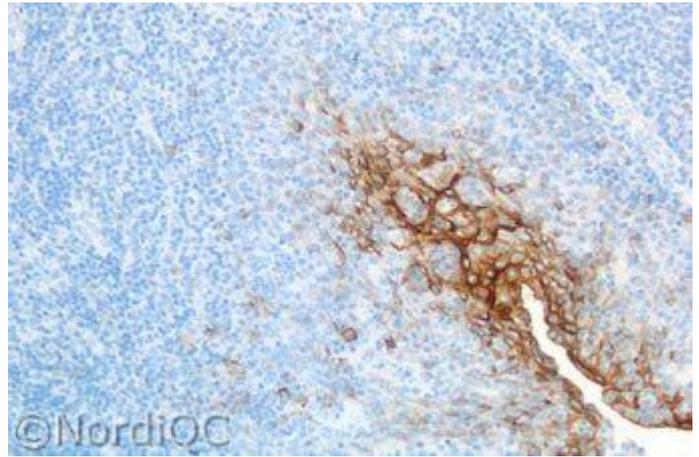
|                    | Run 10 2004 | Run 28 2010 |
|--------------------|-------------|-------------|
| Participants, n=   | 78          | 154         |
| Sufficient results | 59 %        | 94 %        |

### Conclusion

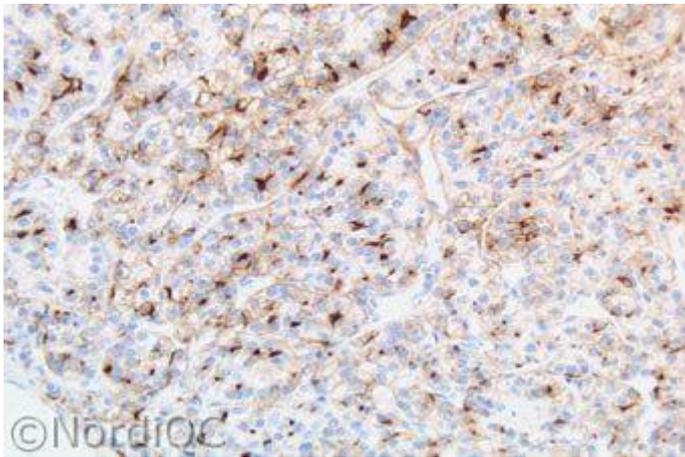
The mAb clones E29, GP1.4 and Mc5 are all recommendable antibodies for EMA. HIER should be used to obtain an optimal staining. Tonsil is recommended as positive control: the intermediate and superficial squamous epithelial cells must show a strong cytoplasmic staining and the plasma cells at least a weak but distinct membranous staining.



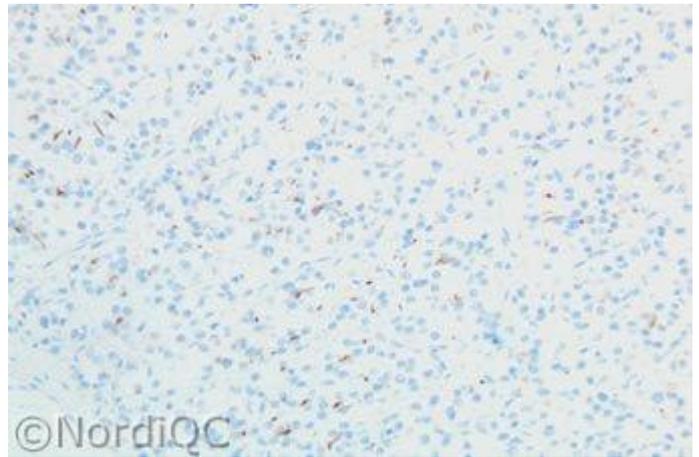
**Fig. 1a**  
Optimal staining for EMA of the tonsil using the mAb clone E29 optimally calibrated and with HIER. The squamous epithelial cells show a strong cytoplasmic staining, while the plasma cells show a moderate and distinct membranous staining. No background staining is seen.



**Fig. 1b**  
Insufficient staining for EMA of the tonsil using the mAb clone E29 too diluted - same field as in Fig. 1a. Only a reduced number of the squamous epithelial cells show a cytoplasmic staining, while virtually none of the plasma cells are stained - also compare with Figs. 2b & 3b same protocol.



**Fig. 2a**  
Optimal staining for EMA of the renal clear cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a membranous and also an intracytoplasmic dot-like staining.



**Fig. 2b**  
Insufficient staining for for EMA of the renal clear cell carcinoma using same protocol as in Fig. 1b - same field as in Fig 2a. The neoplastic cells only show a weak and equivocal staining.

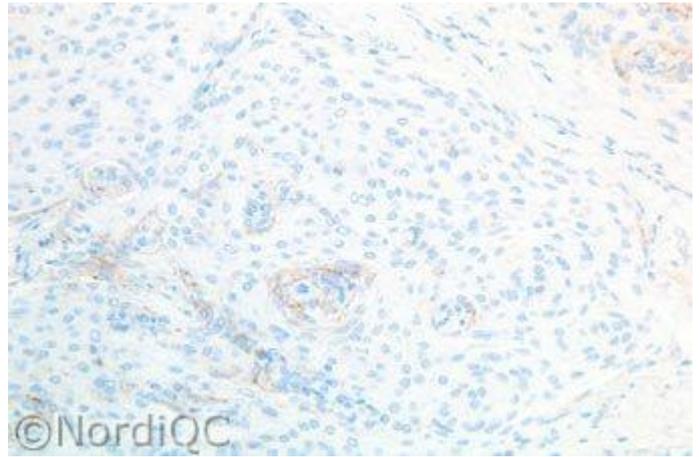
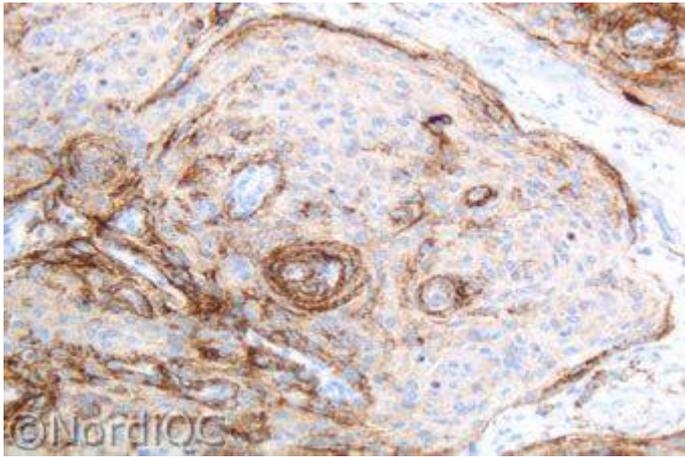


Fig. 3a  
Optimal staining for EMA of the meningeoma using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a moderate membranous staining. No background reaction is seen.

Fig. 3b  
Insufficient staining for EMA of the meningeoma sarcoma using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. The neoplastic cells only show a weak and equivocal staining.

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