## Assessment Run 12 2004

# Cytokeratin 5 (CK5)


The slide to be stained for Cytokeratin 5 (CK5) comprised:
1. Breast ductal carcinoma, 2. Tonsil, 3. Malignant epithelial mesothelioma, 4. Lung adenocarcinoma, 5. Urothelial carcinoma.

Criteria for assessing a CK5 staining as optimal included: A strong and distinct cytoplasmic staining of the squamous epithelial cells of the tonsil, myoepithelial cells lining breast glands and ducts (with normal secretory epithelium or ductal carcinoma in situ), malignant mesothelioma and urothelial carcinoma, whereas no reaction should be seen in the breast in situ and invasive ductal carcinoma or lung adenocarcinoma.

74 laboratories submitted stainings. One lab used an inappropriate Pan-CK ab. At the assessment of the 73 stains, 34 achieved optimal (47 %), 22 good (30 %), 14 borderline (19 %) and 3 (4 %) poor result.

The following mAbs was used:

| Clone | Reactivity | Producer, number of stains |
|---|---|---|
| **XM-26** | CK5 | Novocastra, n=16 |
| **IVT-CK5** | CK5 | Immunovision, n=1 |
| **D5/16 B4** | CK5/6 | DakoCytomation, n=34; Zymed, n=3; Chemicon, n=2; Cell Marque, n=1 |
| **34BE12** | CK 1,5,10,14, and unidentified CK | DakoCytomation, n=12; Ventana, n=3; Cell Marque n=1; Enzo, n=1 |

In this assessment optimal staining could be obtained with the mAbs clone XM26 (12/16 = 75% using this clone), mAb clone D5/16 B4 (22/40 = 55% using this clone) and 34BE12 (1/17 = 6% using this clone).

In the optimal protocols with clone XM-26, all used HIER with the following heating buffers: Tris-EDTA/EGTA pH 9 (7 out of 7 using this buffer had an optimal result), CC1 (Ventana; 2 out of 2 using this buffer), TRS High pH 9,9 (DakoCytomation; 1 out of 1 using this buffer) and Citrate pH 6 (2 out of 7 using this buffer). mAb XM26 was typically used in the range of 1:25 – 150.
In the optimal protocols with clone D5/16 B4, all of 22 laboratories used Tris-EDTA/EGTA pH 9 as the heating buffer. D5/16 B4 was typically used in the range of 1:25 – 200.

The optimal protocol for 34BE12 was based on proteolytic pretreatment using Protease 2 (Ventana) combined with a prediluted primary Ab. In otherwise optimal stains, when HIER was used, clone 34BE12 gave a staining of breast secretory cells and ductal carcinoma in situ (which were negative when the CK5 og CK5/6 Abs were used), hampering the use of 34BE12 for the identification of CK5 and myoepithelial cells. It should be emphazised that this cross reaction is not seen in the prostate (sections not included in this run).

The majority of the laboratories were capable of detecting CK5 in the tonsil squamous epithelium, the malignant mesothelioma and urothelial carcinoma, whereas the insufficient staining typically revealed a too weak or false negative staining in the breast myoepithelial cells.

The most frequent causes of insufficient staining were:
- Omission of epitope retrieval
- Too low concentration of the primary Ab.
- False positive staining due to endogenous biotin.

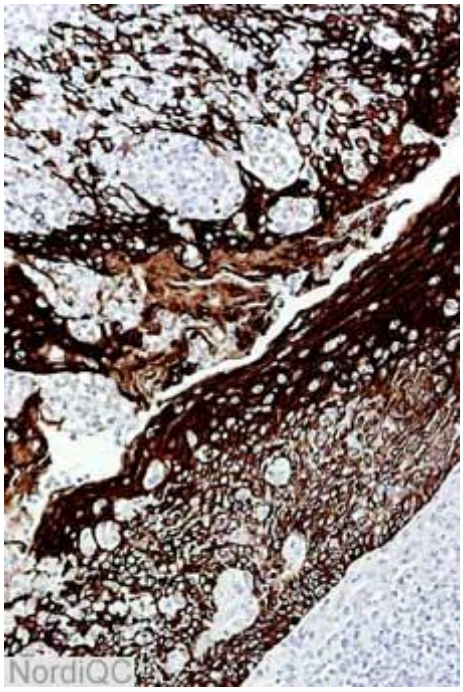


Fig. 1a
Optimal CK5/6 staining of the tonsil using clone D5/16 B4.
A moderate to strong cytoplasmic staining is seen all layers of the squamous epithelium.

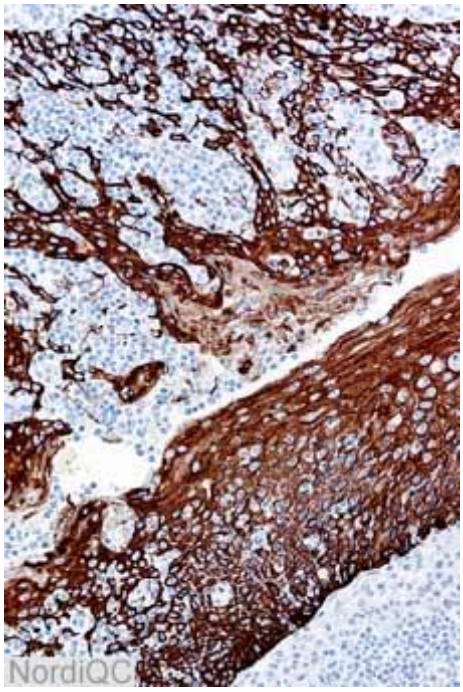


Fig. 1b
CK5/6 staining of the tonsil using clone D5/16 B4 and an insufficient protocol. The staining is comparable to that in Fig. 1a. However compare with Fig. 3b.

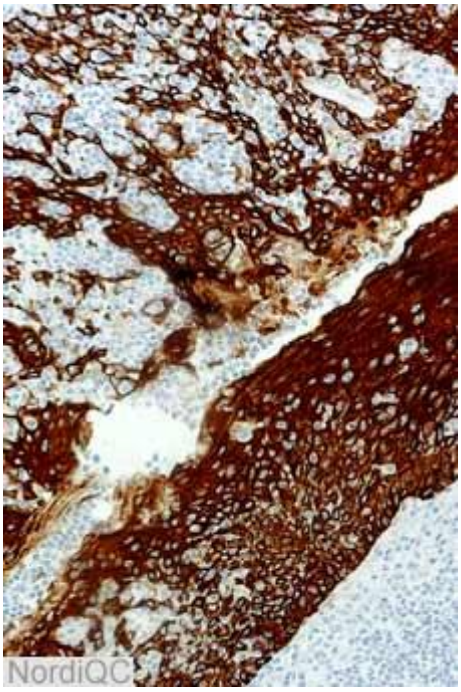


Fig. 1c
Clone 34BE12 staining of the tonsil similar to that in Fig. 1a and 1b. However, compare with Fig. 3c left.

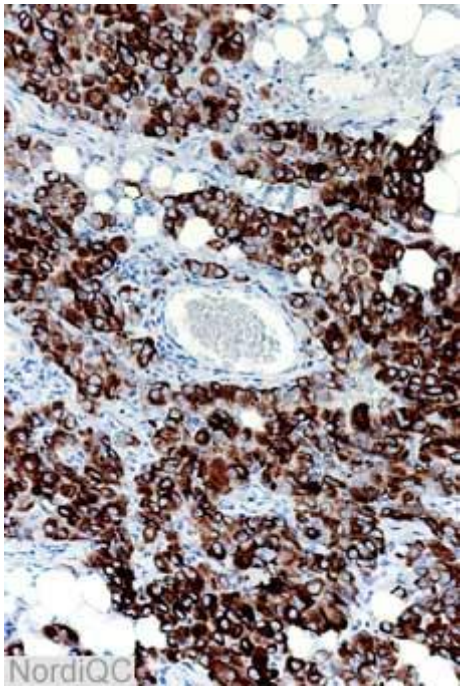


Fig. 2a
Optimal CK5/6 staining of the mesothelioma. A moderate to strong cytoplasmic staining is seen in almost all of the neoplastic cells. Same protocol as in Fig. 1a.

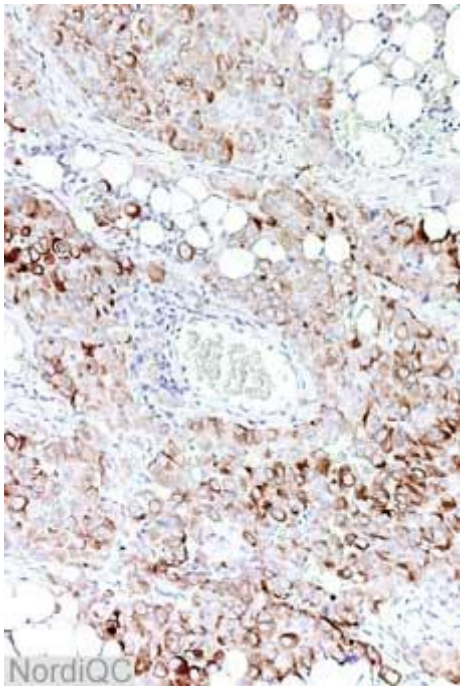


Fig. 2b
Insufficient CK5/6 staining of the mesothelioma. A staining of the neoplastic cells is too weak, compared to Fig. 2a. Furthermore compare with Fig. 3b. Same protocol as in Fig. 1b.

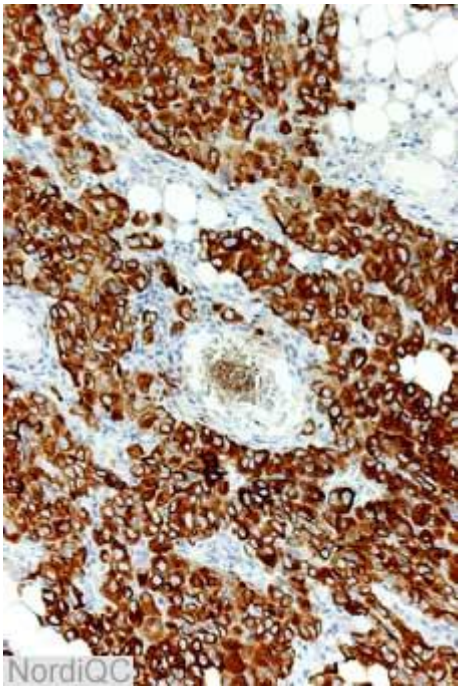


Fig. 2c
Clone 34BE12 staining of the mesothelioma similar to that in Fig 2a. However, compare with Fig. 3c.

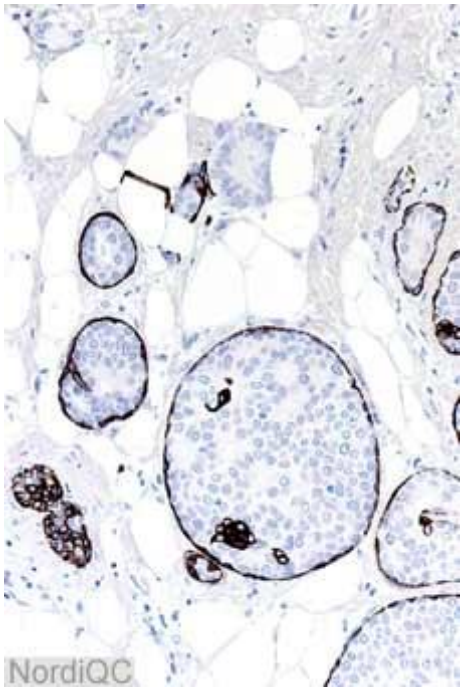


Fig. 3a
Optimal CK5/6 staining of the breast ductal carcinoma.
A strong cytoplasmic staining is seen in the myoepithelial cells, with no staining of the carcinoma in situ and the invasive carcinoma (upper part of the photo).
Same protocol as in Fig. 1a.

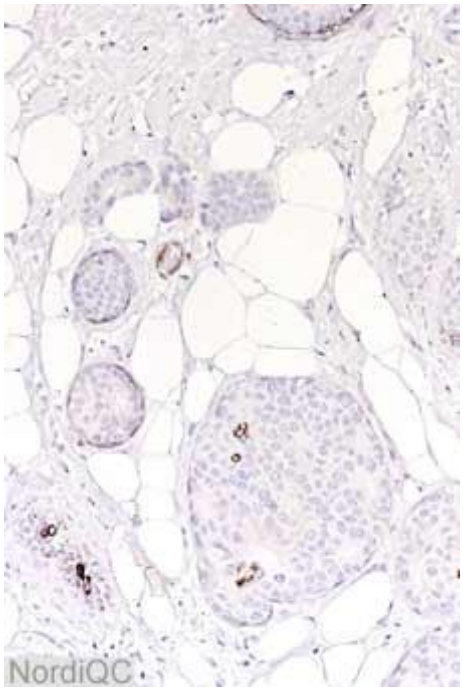


Fig. 3b
Insufficient CK5/6 staining of the breast ductal carcinoma (compare with Fig. 3a, same field).
The myoepithelial cells are virtually unstained. Same protocol as in Fig. 1b and 2b.

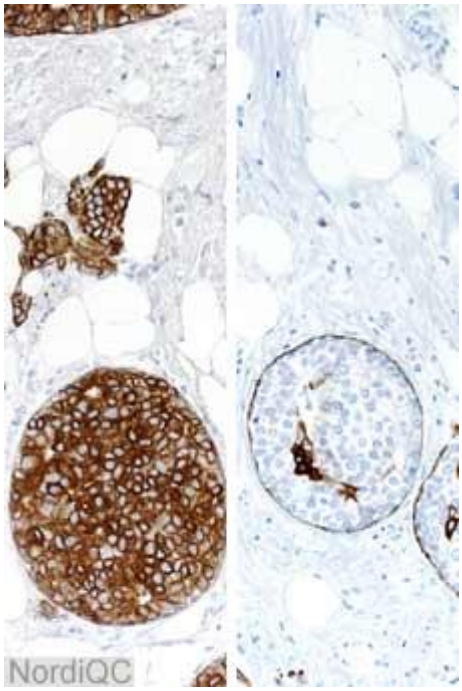


Fig. 3c
Left: Clone 34BE12 staining of the breast ductal carcinoma using HIER. A strong cytoplasmic staining is seen in the CIS.
Right: Clone 34BE12 staining of the breast ductal carcinoma using proteolytic pretreatment. Staining is seen in the myoepithelial cells, whereas no staining is seen in the CIS.

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