Assessment Run B6 2008

Cytokeratin, high molecular weight (CK-HMW)

The slide to be stained for CK-HMW comprised:
1. Esophagus, 2. Breast lobular carcinoma in situ (LCIS)*, 3-4. Breast ductal carcinoma NOS, 5. Breast hyperplasia.
* In the accompany letter, the tumour erroneously was marked breast ductal carcinoma in situ (DCIS).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK-HMW staining as optimal included:

- A strong and distinct cytoplasmic staining of all the squamous epithelial cells of the esophagus throughout all the cell layers.
- A strong and distinct cytoplasmic staining of the basal/myoepithelial cells in the breast hyperplasia and the breast LCIS.
- A negative (or very weakly positive) staining in the neoplastic cells of LCIS and breast ductal carcinomas no. 3 & 4, while the basal/myoepithelial cells in remnants of normal ducts should show a distinct staining.

103 laboratories submitted stains. 6 laboratories used an inappropriate Ab (such as CK-LMW or CK-PAN). Of the remaining 97 laboratories 10 achieved optimal marks (11%), 13 good (13%), 63 borderline (65 %) and 11 poor marks (11 %).

The following Abs were used:

mAb clone	Reactivity	Producer and number
34BE12	CK 1, 5, 10, 14	Dako, n=63; Ventana, n=4; NeoMarkers/Thermo, n=1; Enzo, n=1; BioCare, n=1
D5/16 B4	CK 5, 6	Dako, n=13; Ventana, n=1
XM26	CK 5	Novocastra/Leica, n=6; Vector, n=1
LL002	CK 14	Novocastra/Leica, n=3, NeoMarkers/Thermo, n=1; Ventana, n=1
XM26 & LL002	CK 5, 14	Zytomed, n=1

Optimal staining for CK-HMW in this assessment was obtained with the mAb **D5/16 B4** (4 out of 14), **XM26** (3 out of 7), **LL002** (3 out of 5) and **XM26 & LL002** (1 out of 1).

All optimal protocols were based on heat induced epitope retrieval (HIER).

D5/16 B4: The HIER buffers used were Tris-EDTA/EGTA pH 9.0 (1/6)*; Cell Conditioning1 (BenchMark, Ventana) (1/5), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2) or Target Retrieval Buffer pH 9, (Dako) (1/1). The mAb was typically diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. With these settings 7 out of 10 (93 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

XM26: The HIER buffers used were Tris-EDTA/EGTA pH 9.0 (2/2) or Citrate pH 6.0 (1/3). The mAb was typically diluted in the range of 1:100 – 1:300 depending on the total sensitivity of the protocol employed. With these settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

LL002: The HIER buffers used were Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1), Cell Conditioning1 (BenchMark, Ventana) (1/1) or Target Retrieval Buffer pH 9, (Dako) (1/1). The mAb was typically diluted in the range of 1:30 – 1:300 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab. With these settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

XM26 & LL002: The HIER buffer used was Target Retrieval Solution pH 6.1 (Dako). The mAb was diluted 1:100.

The most frequent causes of insufficient staining were:
- Less successful primary Ab (clone 34BE12 – 65 out of 70 protocols gave an insufficient staining)
- Too low concentration of the primary Ab

- Insufficient HIER – usage of Citrate pH 6.0 and/or too short efficient HIER time

In this assessment the prevalent feature of an insufficient staining was a strong cytoplasmic staining of the neoplastic cells in the breast carcinomas no. 2 and 4 hampering the identification of the myoepithelial cells in the normal glands and LCIS. This reaction pattern was only seen with the mAb clone 34BE12 and observed in 60 out of 70 protocols using the clone. In only 5 out of 70 protocols the mAb clone 34BE12 gave a sufficient staining assessed as good. The remaining 5 protocols were assessed as insufficient due to a weak or a false negative staining. The clone 34BE12 reacts with CK 1, 5, 10, 14 but also with a yet unidentified CK-subtype. HIER seemed to amplify both the specific reaction and the cross reaction, whereas protelolytic pre-treatment either as a single pre-treatment or in combination with HIER seemed to reduce the cross reactivity.
56 out of 57 stains (98 %) based on HIER were assessed as insufficient, compared to 3 out of 10 (70 %) of the stains based on a combined proteolysis and HIER. 2 out of 3 of the stains based on proteolysis without HIER were assessed as insufficient. Due to this cross reaction the utility of the clone to demonstrate myoepithelial cells in breast pathology has previously been questioned, both in NordiQC (CK-HMW run 16 and CK5 run 12) and in the literature (Lacroix-Triki et al., Virchows Arch 2003; 442:548–554), whereas the clone is excellent in for the demonstration of basal cells in prostate – providing that HIER is used (CK-HMW run 16).

In the literature clone 34BE12 has been described to be a valuable marker together with E-Cadherin to differentiate between lobular carcinoma (E-Cadherin negative and 34BE12 positive) and ductal carcinoma (E-Cadherin positive and 34BE12 negative). However this application was not the scope of the assessment, as the main task was to demonstrate myoepithelial cells and NordiQC did only evaluate the laboratories performance in that diagnostic application as the multi block was constructed with focus on the identification of CK-HMW in myoepithelial cells.

Esophagus is recommended control for CK-HMW: All squamous epithelial cells must show a strong staining without background reaction. In contrast to anti-CK5 or CK5/6 antibodies (clone XM26 or D5/16 B4), the anti-CK14 antibody (LL002) left focally the intermediate and superficial cells negative in the esophagus muoca, even in optimal protocols. This reaction pattern has also been shown in the previous assessments. It was also observed that anti-CK14 left the normal luminal breast epithelial cells unstained compared to anti-CK5 and anti-CK5/6 - see Fig. 6a and 6b.

With the mAb CK5/6 clone D5/16 B4 caution should be taken in interpretating the staining, as the antibody is an ascites format giving the Mouse Ascites Golgireaction (MAG) in tissue from patients with blood group A.

This was the third assessment of CK-HMW. No direct comparison can be made with the previous assessments as these were related to the general module and the assessment criteria and composition of the multitissue blocks were designed for these purposes. However, it shall be stressed that in run 16, 2006, it was concluded that the clone *"34BE12 may cross react with an unidentified CK particularly seen in breast (secretory cells and carcinomas). For this reason 34BE12 cannot be recommended in breast pathology".*

Conclusion
The mAb clones **XM26**, **D5/16 B4** and **LL002** are all well performing Abs for CK-HMW and should be the preferred choice for the demonstration of CK-HMW in breast myoepithelial cells. HIER is mandatory. The mAb clone 34BE12 can not be recommended for the demonstration of CK-HMW in breast myoepithelial cells due to an excessive cross reaction with other epithelial cells.

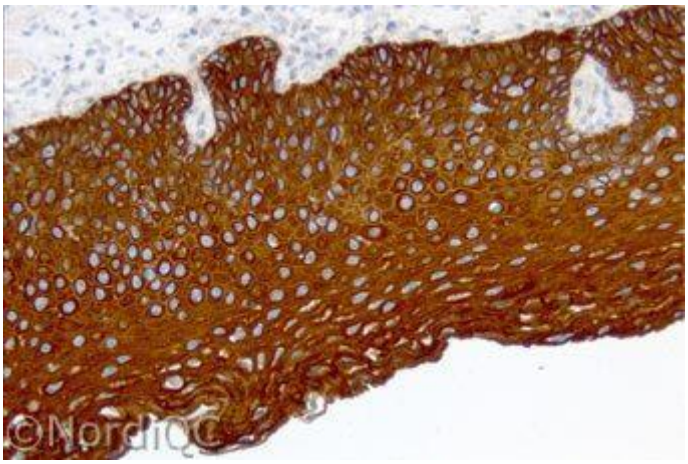

Fig. 1a
Optimal CK-HMW staining (mAb clone XM26 & LL002) of the esophagus. All the squamous epithelial cells show a strong cytoplasmic staining.

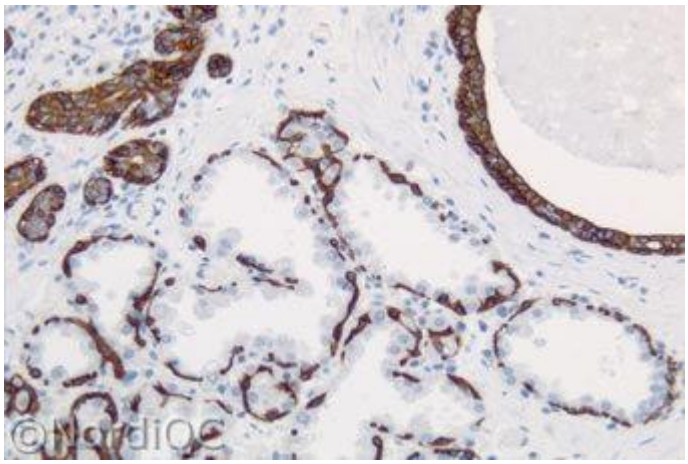

Fig. 1b
Optimal CK-HMW staining of breast hyperplasia using same protocol as in Fig. 1a. The myoepithelial cells show a strong and distinct cytoplasmic staining.

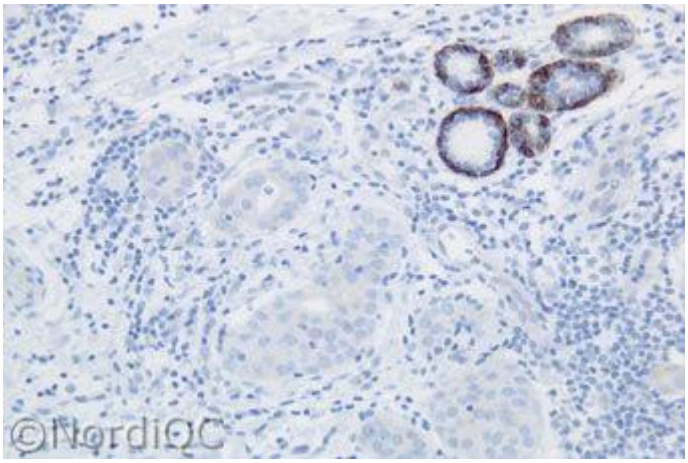

Fig. 2a
Optimal CK-HMW staining of the breast ductal carcinoma using same protocol as in Fig. 1a and b. The neoplastic cells are negative and only the myoepithelial cells of remnants of normal glands are stained.

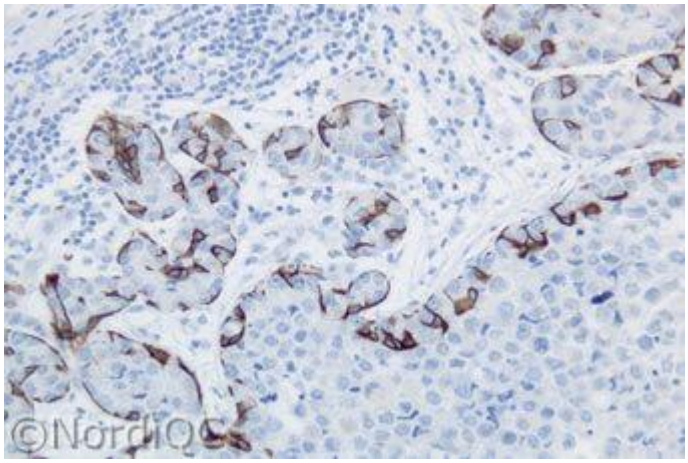

Fig. 2b
Optimal CK-HMW staining of the breast lobular CIS using same protocol as in Fig. 1 a and b and 2a.
The myoepithelial cells at the periphery of the nests of neoplastic cells show a distinct cytoplasmic staining, while the neoplastic cells are negative.

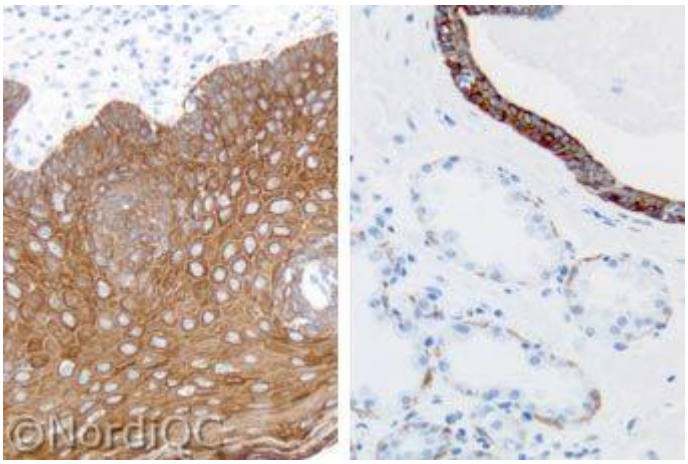

Fig. 3a
CK-HMW staining using an insufficient protocol based on the mAb clone D5/16 B4. The Ab is too diluted.
Left: Esophagus: The squamous cells are stained but only with a moderate intensity. Also compare with Fig. 3b.
Right: Breast hyperplasia: The myoepithelial cells only show a weak staining.

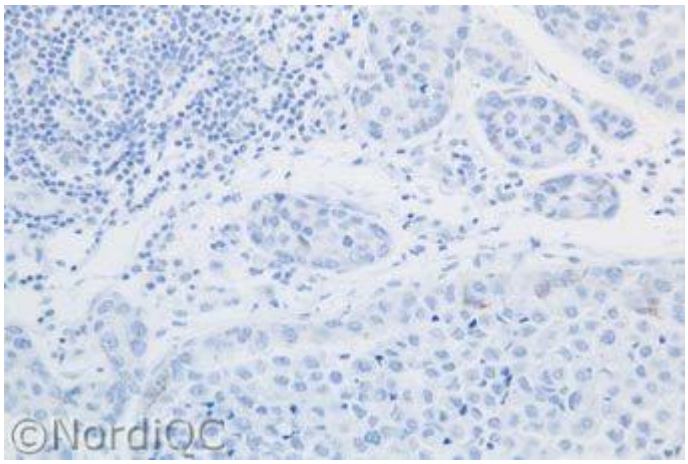

Fig. 3b
Insufficient CK-HMW staining of the breast lobular CIS using same protocol as in Fig. 3a.
The myoepithelial cells at the periphery of the nests of the neoplastic cells are virtually negative - same field as in Fig. 2b.

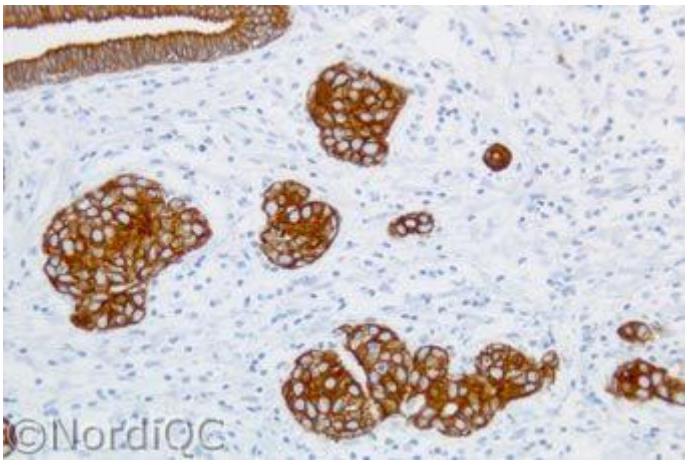

Fig. 4a
Insufficient CK-HMW staining using the mAb clone 34BE12 with HIER. The myoepithelial cells lining the breast duct (upper left) show a distinct staining, but also the normal luminal ductal epithelial cells and all the neoplastic cells show a distinct cytoplasmic staining. Compare with Fig. 4b – same protocol.

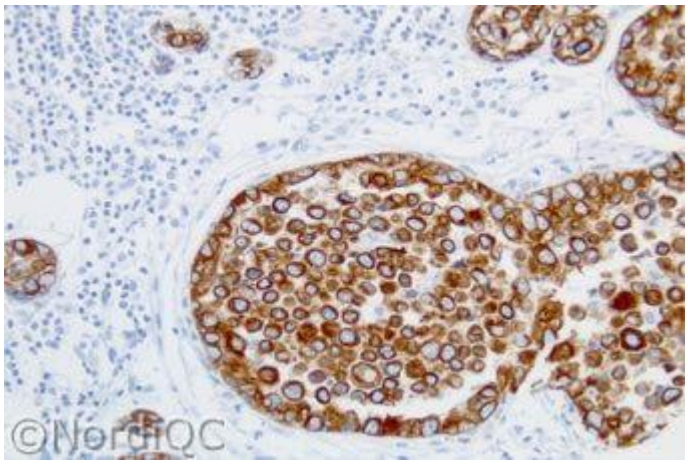

Fig. 4b
Insufficient CK-HMW staining of the breast lobular CIS using same protocol as in Fig. 4a.
The myoepithelial cells at the periphery of the nests of neoplastic cells cannot be identified due to a strong cytoplasmic staining of the majority of neoplastic cells.
Also compare with Fig. 2b – same tumour.

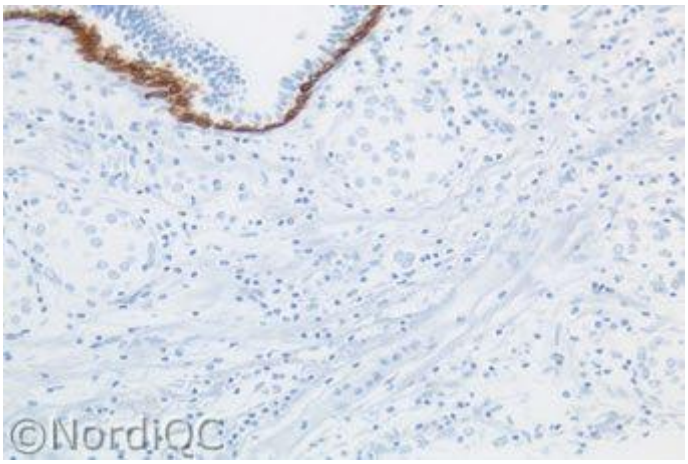

Fig. 5a
CK-HMW staining assessed as good. The mAb clone 34BE12 was used with proteolysis. The myoepithelial cells lining the breast duct (upper left) show a distinct staining, while the neoplastic cells are negative. However, note the impact of the proteolysis - the cytoplasm is markedly digested. Also compare with Fig. 5b – same protocol.

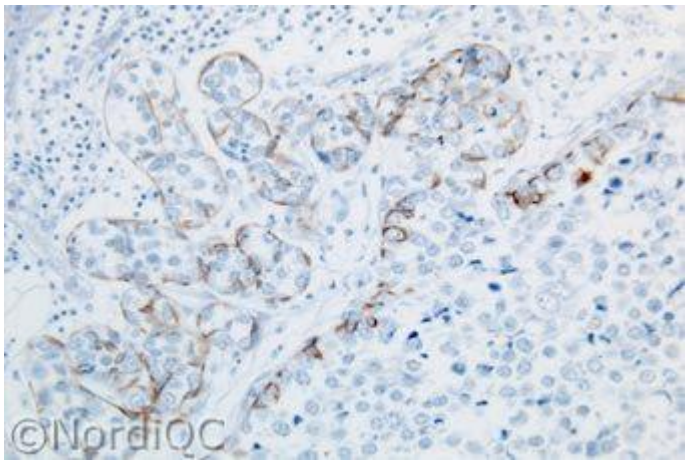

Fig. 5b
CK-HMW staining of the breast LCIS assessed as good using same protocol as in Fig. 5a. The myoepithelial cells at the periphery of the nests of neoplastic cells show a weak to moderate staining, with no staining of the neoplastic cells, facilitating the interpretation. Also compare with Fig. 4b.

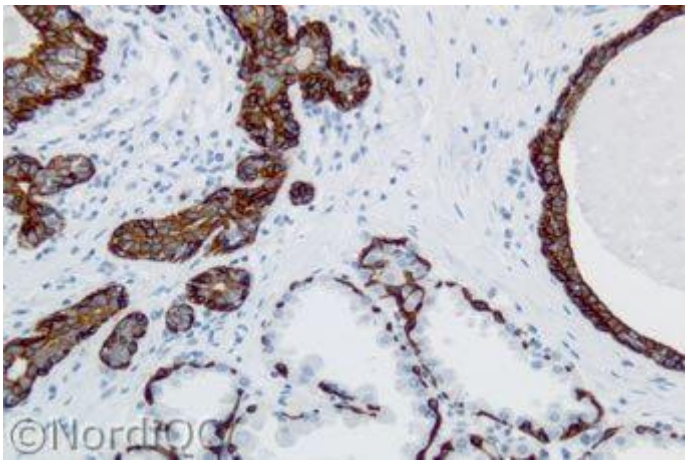

Fig. 6a
Optimal CK-HMW staining of the breast hyperplasia using the mAbs clones XM26 & LL002 (CK5/14). The myoepithelial cells show a strong and distinct cytoplasmic staining. Note also the luminal epithelial cells in the cyst and the normal glands are stained, while the epithelial cells lining the apocrine metaplastic glands are negative. This pattern was seen with mAbs to CK5, CK5/6 and CK5/14.
Compare with Fig. 6b – mAb to CK14

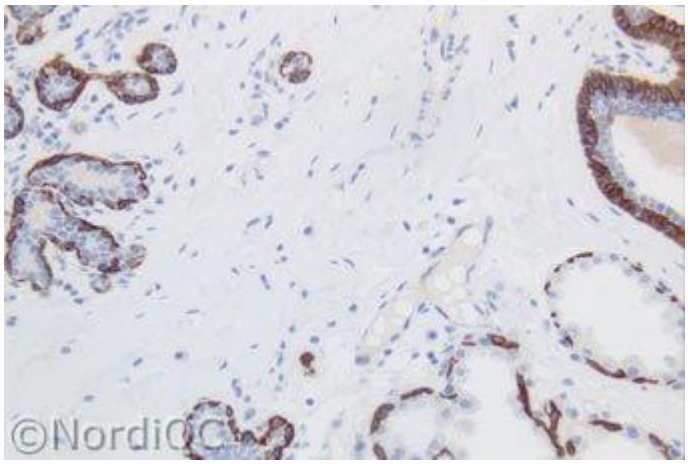

Fig. 6b
Optimal CK-HMW staining of the breast hyperplasia using mAb clone LL002 (CK14). The myoepithelial cells show a strong and distinct cytoplasmic staining while no staining is seen in the luminal epithelial cells.

SN/HN/MV/LE 8-12-2008