Assessment Run 16 2006

Cytokeratin, high molecular weight (CK-HMW)

The slide to be stained for CK-HMW comprised:
1. Tonsil, 2. Esophagus, 3. Breast ductal carcinoma, 4. Prostate hyperplasia,
5. Prostate adenocarcinoma.
All specimens were fixed in 10 % NBF.

Criteria for assessing an optimal CK-HMW staining included:

- A strong and distinct cytoplasmic staining of the squamous epithelial cells of the tonsil and esophagus
- A strong and distinct cytoplasmic staining of the basal cells in the prostate hyperplasia.
- A negative (or very weak positive) staining in the prostate secretory epithelial cells and prostate adenocarcinoma

92 laboratories submitted stains. Of these 5 used a CK-HMW antibody considered inappropriate (see below). The remaining 87 laboratories were assessed as follows: 55 achieved optimal marks (63 %), 22 good (25 %), 7 borderline (8 %) and 3 poor marks (3 %).

The following appropriate Abs were used:

mAb	Reactivity	Producer and number
34βE12	CK 1, 5, 10, 14	Dako, n=61; Ventana, n=6; ENZO, n=4; Cell Marque, n=2; NeoMarkers, n=1
D5/16 B4	CK 5, 6	Dako n=5
DE-SQ	CK 13, 14, 15, 16	NeoMarkers n=1
LL002	CK 14	Novocastra n=2, Serotec n=1
XM26	CK 5	Novocastra n=3

The Ab's **AE3** (reacts with CK 1, 4, 5, 7, 8) **CAM 5.2** (reacts with 7, 8, 19) and the pAb **Z0622** (reacts a wide range of cytokeratins) were considered inappropriate due to reactions with CK-LMWs.

In this assessment an optimal staining could be achieved with the mAb clone **34BE12** (49 out of 74 were optimal), clone **D5/16 B4** (4 out of 5 were optimal) and clone **XM26** (2 out of 3 were optimal).

Using the mAb clone **34BE12** both heat induced epitope retrieval (HIER) as pre-treatment, HIER combined with proteolytic pre-treatment and proteolytic pre-treatment alone could be used.

44 out of 58 laboratories using HIER (76 %) obtained an optimal staining reaction. Several HIER buffers could be used, but the majority used either a Tris-EDTA/EGTA pH 9 buffer (29 out of 40 using this obtained optimal results) or the CC1 buffer (Ventana Benchmark; 6 out of 7 using this were marked as optimal). Also Citrate pH 6 and EDTA pH 8 could be used. The Ab. could either be used as a RTU Ab. or as a concentrate in which the Ab. typically was diluted in the range of 1:50 – 600 depending on the total sensitivity of the protocol employed.

4 out of 10 laboratories using a combination of HIER and proteolytic pre-treatment (40 %) obtained an optimal staining reaction. Both Protease I or III (Ventana) combined with HIER in CC1 or Proteinase K (Dako) combined with HIER in Target Retrieval Solution pH 9 (Dako) could be used. The Ab. was typically used in the range of 1:20 - 1:100 depending on the total sensitivity of the protocol employed.

1 out of 6 laboratories using proteolytic pre-treatment (17 %) obtained an optimal staining reaction. The protocol was based on Protease I (Ventana) and a dilution of 1:50 of the primary Ab.

Using the mAb clone **D5/16 B4** the protocols resulting in an optimal staining were based on HIER using a Tris-EDTA/EGTA buffer pH 9 (3 out of 3 laboratories using this obtained an optimal mark). In the optimal protocols the mAb typically was diluted 1:100.

Using the mAb clone **XM26** the protocols resulting in an optimal staining reaction were based on HIER using a Tris-EDTA/EGTA buffer pH 9 (2 out of 2 laboratories using this obtained an optimal mark). In the optimal protocols the mAb typically was diluted in the range of 1:50 – 150 depending on the total sensitivity of the protocol employed.

The most frequent causes of insufficient stains were:
- Less successful primary Ab's
- Too low concentration of the primary Ab

In this assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the normal basal cells in the prostate. This false negative reaction can in a diagnostic setting be critical as a negative reaction with no demonstration of basal cells can be indicative of prostate neoplasia. In general the signal for CK-HMW in basal cells of the prostate glands should be as strong as possible without any or only a focal reaction of the prostate epithelial cells. All the mAb clones **34BE12**, **D5/16 B4** and **XM26** are useful for the demonstration of CK-HMW in the basal cells of the prostate glands. Concerning the reactivity of CK-HMW in the breast specimen the clone **34BE12** typically gave a strong staining in the neoplastic cells. The clones **D5/16 B4** and **XM26** reacted with a very limited proportion of the neoplastic cells of the breast (similar reactivity patterns were observed in NordiQC run 12 for CK5). It is noteworthy that the mAb clone **LL002** (detecting CK 14) was a less successful marker for CK-HMW as only a limited proportion of the basal cells in the prostate was demonstrated compared to the staining obtained using the above mentioned clones. At the same time only the basal cells in the esophagus were demonstrated while both the neoplastic cells of the breast and the squamous cells in the tonsil were strongly positive indicating that the protocols were optimized and that the decreased reactivity (compared to Bs detecting CK5) may be due to biological differences in the CK5 & CK14 distribution. However, a lower affinity for the Ab to detect CK14 cannot be ruled out.

Conclusion
- The mAb clones **35BE12**, **D5/16 B4** and **XM26** seems to be the most sensitive and reproducible markers for CK-HMW. However, as described in the Run 12 assessment for CK5/CK-HMW, 35BE12 may cross react with an unidentified CK particularly seen in breast (secretory cells and carcinoma). For this reason 35BE12 cannot be recommended in breast pathology.
- Prostate is an appropriate control: The basal cells should stain as strongly as possible with minimal background reaction and only a focal reaction of the secretory cells.

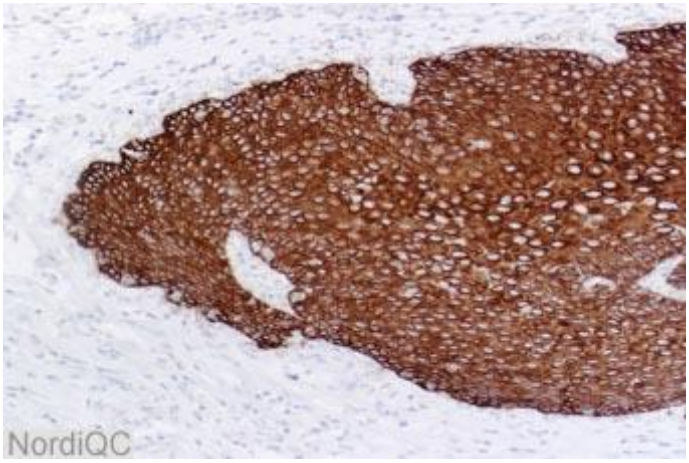

Fig. 1a
Optimal staining for CK-HMW (mAb clone 34BE12) of the esophagus. All the squamous epithelial cells show a strong cytoplasmic staining.

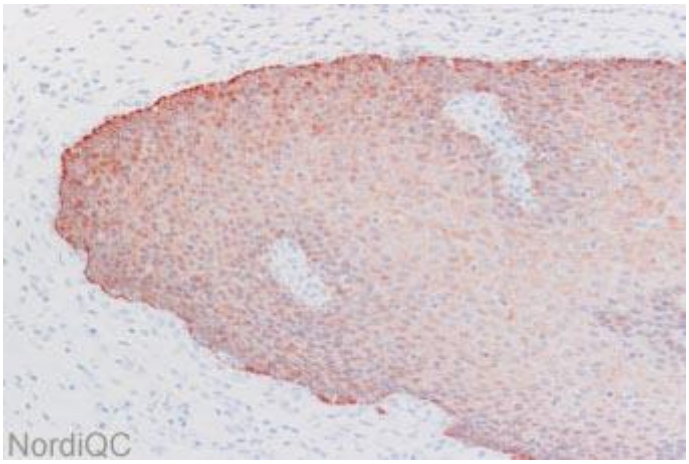

Fig. 1b
Staining for CK-HMW (mAb clone 34BE12) of the esophagus using an insufficient protocol (same field as in Fig. 1a.). The basal cells show a moderate staining reaction while the suprabasal cells are weakly stained.

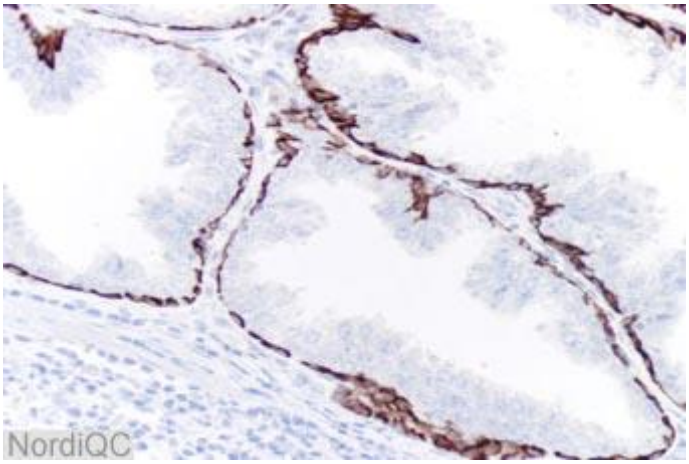

Fig. 2a
Optimal staining for CK-HMW (mAb clone 34BE12) of the prostate hyperplasia. The basal cells show a strong cytoplasmic reaction and a distinct continuous layer of basal cells is demonstrated (same protocol used in Fig. 1a).

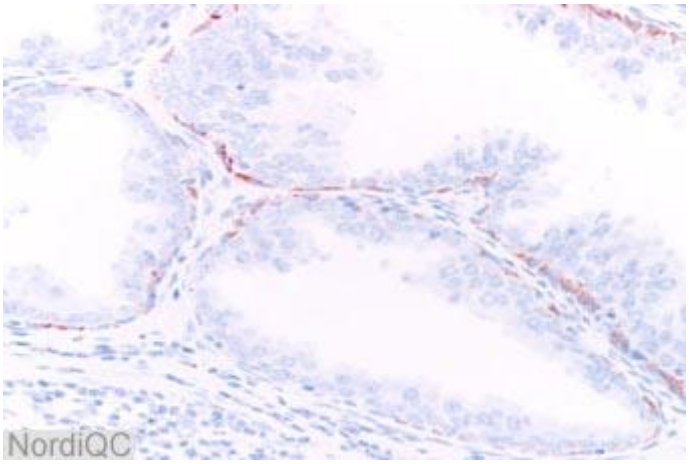

Fig. 2b
Insufficient staining for CK-HMW (mAb clone 34BE12) of the prostate hyperplasia. (same field as in Fig 2a). The basal cells are weakly stained or negative (same protocol used in Fig. 1b).

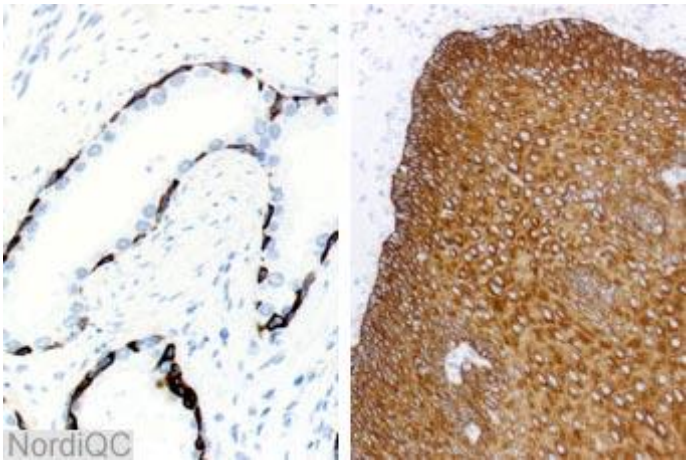

Fig. 3a
Left. Optimal staining for CK-HMW of the prostate hyperplasia using an Ab against CK5 (clone XM26). All the basal cells are stained. Compare with Fig. 3b.
Right. Optimal staining for CK-HMW of the esophagus using an Ab against CK5 (clone XM26). All the squamous epithelial cells show a strong staining. Compare with Fig. 3b.

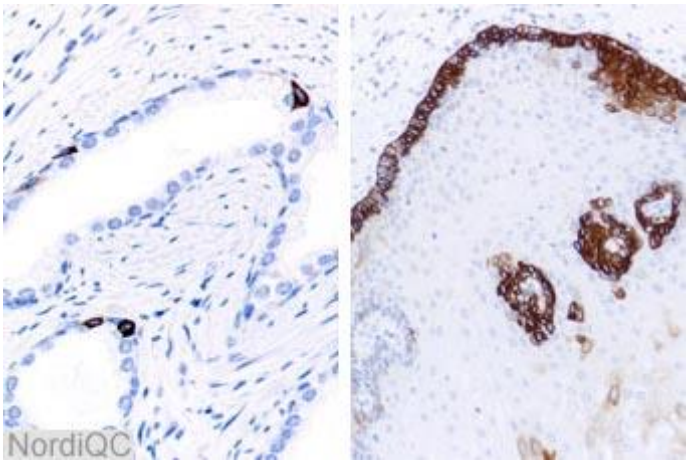

Fig. 3b
Left. Insufficient staining for CK-HMW of the prostate hyperplasia using an Ab against CK14 (clone LL002). Few basal cells are strongly stained, the others are negative. Compare with Fig. 3a.
Right. Insufficient staining for CK-HMW of the esophagus using an Ab against CK 14 (clone LL002). Only the basal cells show a strong staining. Compare with Fig 3a.

SN/HN/MV/LE 29-3-2006