## Assessment Run 32 2011

# Cytokeratin, high molecular weight (CK-HMW)

### Material

The slide to be stained for CK-HMW comprised:


1. Esophagus, 2. Liver, 3. Prostate hyperplasia / Prostate intraepithelial neoplasia (PIN), 4. Breast ductal carcinoma (+ few normal ducts), 5. Lung squamous cell carcinoma.

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 majority of the basal cells of the prostate hyperplastic glands and the PIN lesions.
- A moderate to strong cytoplasmic staining of the majority of the neoplastic cells of the lung squamous cell carcinoma.
- A negative staining of the liver and of the neoplastic cells of the breast ductal carcinomas, while the myopepithelial cells in normal ducts should show a distinct staining and the luminal cells a faint or heterogenous staining.

168 laboratories participated in this assessment. 5 laboratories used an inappropriate Ab (typically CK-Pan). Out of the remaining 163 labs 23 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CK-HMW, run 32**

| Concentrated Abs | Reactivity CK type | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **34BE12** | 1, 5, 10, 14, (19)* | 76<br>4<br>3<br>3<br>2<br>1 | Dako<br>Leica/Novocastra<br>ENZO<br>NeoMarkers<br>Biocare<br>Master Diagnostica | 1 | 6 | 75 | 7 | 8 % | 40 % |
| mAb clone **D5/16B4** | 5, 6 | 16<br>1 | Dako<br>Cell Marque | 6 | 7 | 3 | 1 | 76 % | 82 % |
| mAb clone **XM26** | 5 | 6 | Leica/Novocastra | 4 | 1 | 1 | 0 | 83 % | 83 % |
| mAb clone **LL002** | 14 | 2 | Leica/Novocastra | 0 | 0 | 2 | 0 | - | - |
| mAb clone **DE-SQ** | 13, 14, 15, 16 | 1 | NeoMarkers | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **EP1601Y** | 5 | 1 | Cell Marque | 0 | 1 | 0 | 0 | - | - |
| mAb clone cocktail **XM26/LL002** | 5, 14 | 3 | Diagnostic Biosystems | 1 | 2 | 0 | 0 | - | - |
| mAb clone cocktail **XM26/LL002** | 5, 14 | 1 | Homemade<br>XM26:Leica/Novocastra<br>LL002: Cell Marque | 0 | 1 | 0 | 0 | - | - |
| **Ready-To-Use Abs** | | | | | | | | | |
| mAb clone **34BE12 790-4373** | 1, 5, 10, 14, (19) | 16 | Ventana | 0 | 1 | 14 | 1 | 6 % | - |
| mAb clone **34BE12 IR051** | 1, 5, 10, 14, (19) | 14 | Dako | 0 | 0 | 14 | 0 | 0 % | - |
| mAb clone **34BE12 PM127** | 1, 5, 10, 14, (19) | 2 | Biocare | 0 | 0 | 2 | 0 | - | - |
| mAb clone **34BE12 N1553** | 1, 5, 10, 14, (19) | 2 | Dako | 0 | 0 | 2 | 0 | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **34BE12 760-2636** | 1, 5, 10, 14, (19) | 1 | Ventana/Cell Marque | 0 | 0 | 1 | 0 | - | - |
| mAb clone **D5/16B4 760-4253** | 5, 6 | 4 | Ventana/Cell Marque | 0 | 3 | 0 | 1 | - | - |
| mAb clone **D5/16B4 IR780** | 5, 6 | 2 | Dako | 1 | 1 | 0 | 0 | - | - |
| mAb clone **D5/16B4 790-4554** | 5, 6 | 1 | Ventana | 1 | 0 | 0 | 0 | - | - |
| rmAb/mAb clone cocktail **EP1601Y/LL002, 905H-08** | 5, 14 | 1 | Cell Marque | 1 | 0 | 0 | 0 | - | - |
| **Total** | 163 | | | 15 | 23 | 115 | 10 | - | |
| **Proportion** | | | | 9 % | 14 % | 71 % | 6 % | 23 % | |

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Apart from reacting with CK types 1, 10, 5 and 14, the Ab also reacts with an unknown CK type, possibly a denaturated CK19.

The following central protocol parameters were used to obtain an optimal staining:

**Concentrated Abs**
mAb clone **34BE12**: The protocol giving an optimal result was based on a pre-treatment using a combination of heat induced epitope retrieval (HIER) and proteolysis. HIER was performed in a microwave oven using Citrate pH 6 followed by proteolysis in Protease 2 (Ventana). The primary mAb was diluted 1:100. 5 labs used a combined pre-treatment and 2 out of 5 (40 %) produced a sufficient staining (optimal or good).

mAb clone cocktail **D5/16B4**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/2)*, Bond Epitope Retrieval Solution 2 (BERS2) (Bond, Leica) (1/1) or Cell Conditioning 1 (CC1) (BenchMark, Ventana)(4/9) as the retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 11 (82 %) laboratories produced a sufficient staining.
*(number of optimal results/number of laboratories using this buffer)

mAb clone cocktail **XM26/LL002**: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (Dako) (1/1), as the retrieval buffer. The mAb was diluted 1:100. Using this protocol setting 1 laboratory produced an optimal staining.

mAb clone **XM26**: The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (1/1), TRS pH 9 (Dako) (1/1), BERS2 (Bond, Leica) (1/2) or CC1 (BenchMark, Ventana) (1/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 6 (83 %) laboratories produced a sufficient staining.

**Ready-To-Use Abs**
mAb clone cocktail **D5/16B4** (prod. no. IR780, Dako): The protocol giving an optimal result was based on HIER in PT-Link using TRS pH 9 (3-in-1) and an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining.

mAb clone cocktail **D5/16B4** (prod. no. 790-4554, Ventana): The protocol giving an optimal result was based on HIER using extended CC1, an incubation time of 44 min in the primary Ab and UltraView (760-500) with amplification as the detection system.

mAb clone cocktail **EP1601Y/LL002** (prod. no. 905H-08, Cell Marque): The protocol giving an optimal result was based on HIER using standard CC 1, an incubation time of 32 min in the primary Ab and UltraView (760-500) with amplification as the detection system.

The most frequent causes of insufficient stains were:

- Less successful primary Ab (for the mAb clone 34BE12 - 116/124 protocols gave an insufficient staining)
- Too low concentration of the primary Ab
- Insufficient HIER - too short efficient HIER time and/or use of Citrate pH 6 as HIER buffer

In this assessment the prevalent feature of an insufficient staining was an aberrant false positive cytoplasmic staining of the neoplastic cells of the breast ductal carcinoma and of the epithelial cells of the bile ducts in the liver. This pattern was seen in 91 out of the 125 insufficient results and was seen when the mAb clone 34BE12 was used with HIER (without proteolytic pretreatment). According to the company datasheets mAb clone 34BE12 reacts with the CK-HMW types 1, 5, 10 & 14, but when the Ab is applied with HIER it also cross-reacts with an yet unidentified CK-LMW subtype, possibly CK19 giving a false positive staining in epithelial cells not expressing CK-HMW. In this assessment and in concordance to the previous NordiQC run B6 assessment, this gave an aberrant staining of the ductal breast carcinoma of luminal type. The cross-reaction was not seen if proteolysis was used as pre-treatment for the mAb clone 34BE12. However if proteolysis was used as pre-treatment a too weak sensitivity typically was seen, as 8 out of 9 protocols based on proteolysis was assessed as insufficient due to a too weak or false negative staining. In total 116 out of 124 protocols based on the mAb clone 34BE12 gave an insufficient staining and only one protocol based on a combined pre-treatment of HIER followed by proteolysis gave an optimal staining. In this context it also has to be emphasized that the aberrant cross-reaction was not seen in the prostate epithelial cells. Thus, the mAb clone 34BE12 can be used for demonstration of CK-HMW in prostate specimens, but due to the above mentioned cross-reaction in breast epithelial cells/breast carcinoma it should not be used for breast tissue.

The most robust and specific Abs for CK-HMW were the mAb clones XM26 for CK5, the mAb clone D5/16B4 for CK 5/6 and the rmAb clone EP160Y for CK5 either applied as a single marker or in combination with e.g. the mAb clone LL002 for CK14. In total 30 out of 38 protocols based on one of these clones were assessed as sufficient giving a pass rate of 79 %. All of these clones could give an optimal staining with HIER in an alkaline buffer. The 8 insufficient results based on one of the above mentioned clones were all characterized by a too weak or false negative staining of the structures expected to be demonstrated, typically caused by insufficient HIER and/or a too low concentration of the primary Ab.

Esophagus is a recommendable positive control for CK-HMW in which the squamous epithelial cells must show an as strong as possible staining without background reaction. All cell layers are intensively stained with anti-CK5 Abs clone XM26 and anti-CK5/6 mAb clone D5/16 B4 when used with optimal protocol settings. In contrast, an optimal protocol for anti-CK14 mainly demonstrates the basal cells whereas only scattered intermediate and superficial cells show a positive staining reaction.
When the mAb clone D5/16 B4 is used, caution should be taken when interpreting the staining, as the antibody typically is provided as an ascites format that gives the Mouse Ascites Golgireaction (MAG) in tissue of blood group A.

This was the 4th assessment of CK-HMW in NordiQC. As shown in table 3, the proportion of sufficient results has declined dramatically. This is mainly due to inclusion of breast cancer in the test material, which strongly challenges the performance of clone 34BE12.

Table 2. **Proportion of sufficient results for CK-HMW in the four NordiQC runs performed**

| | Run 12 2004 | Run 16 2006 | Run B6 2008 | Run 32 2011 |
|---|---|---|---|---|
| Participants, n= | 73 | 87 | 97 | 163 |
| Sufficient results | 77 % | 88 % | 24 % | 23 % |

**Conclusion**
The mAb clones XM26 (CK5), D5/16 B4 (CK5/6), LL002 (CK14) and the rmAb clone EP160Y (CK5) are all well performing markers for CK-HMW and should be preferred for the demonstration of CK-HMW. HIER in an alkaline buffer is mandatory for an optimal performance. The mAb clone 34BE12 can not be recommended as a marker for CK-HMW due to an excessive cross reaction with CK-LMW in e.g. breast ductal epithelial cells giving a false positive staining reaction.

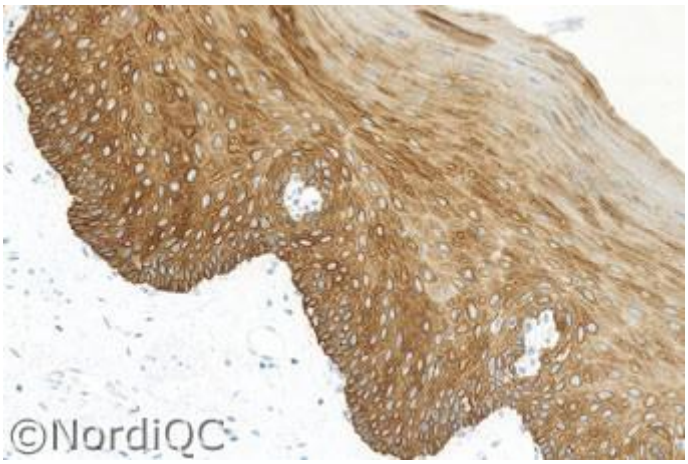


Fig. 1a
Optimal staining for CK-HMW of the tonsil using the mAb clone D5/16B4 against CK5/6 optimally calibrated and with HIER in an alkaline buffer.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen.

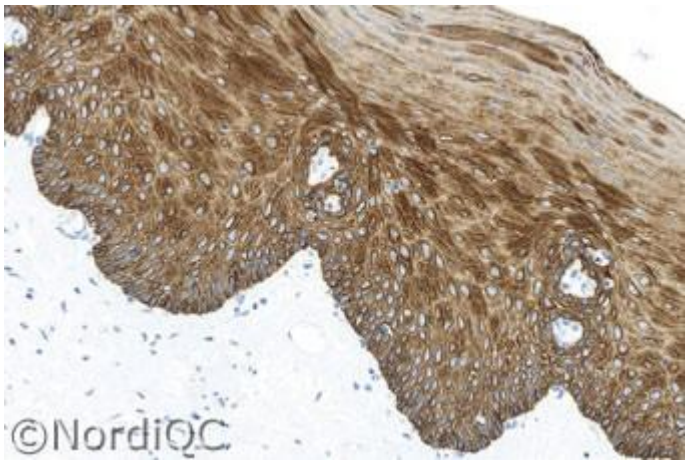


Fig. 1b
Staining for CK-HMW of the tonsil using an insufficient protocol based on the mAb clone 34BE12 against CK-HMW with HIER in an alkaline buffer, same field as in Fig. 1a.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen. However, compare with Fig. 3b, same protocol.

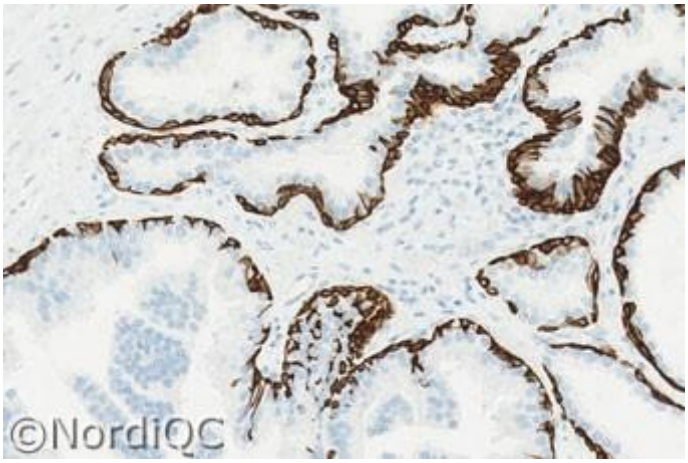


Fig. 2a
Optimal staining for CK-HMW of the prostate hyperplasia/PIN lesion using same protocol as in Fig. 1a.
Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen.

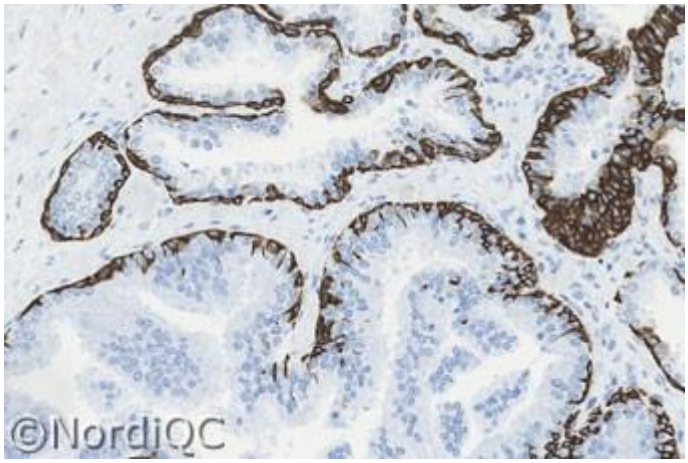


Fig. 2b
Staining for CK-HMW of the prostate hyperplasia/PIN lesion using same insufficient protocol as in Fig. 1b, same field as in Fig. 2a. Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen, same field as in Fig. 2a. However, compare with Fig. 3b., same protocol.

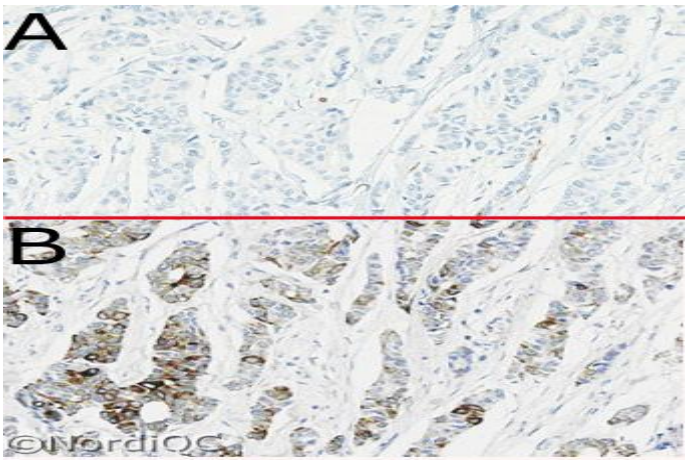


Fig. 3a
Optimal staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1a. & 2a.
The neoplastic cells expressing CK-LMW are negative, while the remnants of entrapped myoepithelial cells expressing the CK-HMW subtypes CK5 & CK14 show a moderate cytoplasmic staining.

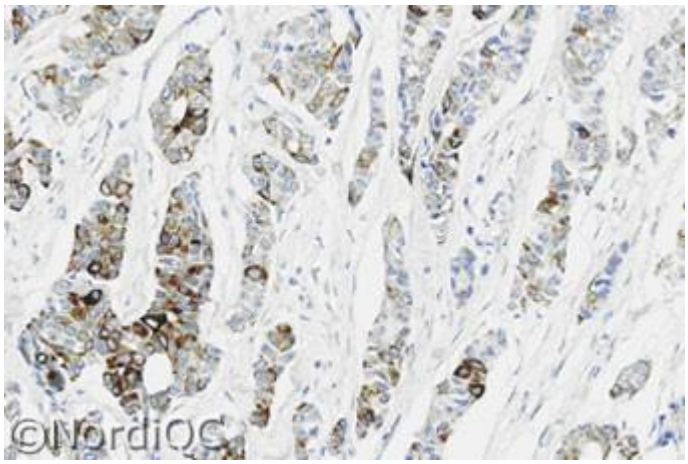


Fig. 3b
Insufficient staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1b. & 2b, same field as in Fig. 3a. A moderate to strong aberrant cytoplasmic staining is seen in the majority of the neoplastic cells. This false positive cross reaction with an unidentified subtype of CK-LMW was typically seen, when the mAb clone 34BE12 was used with HIER.

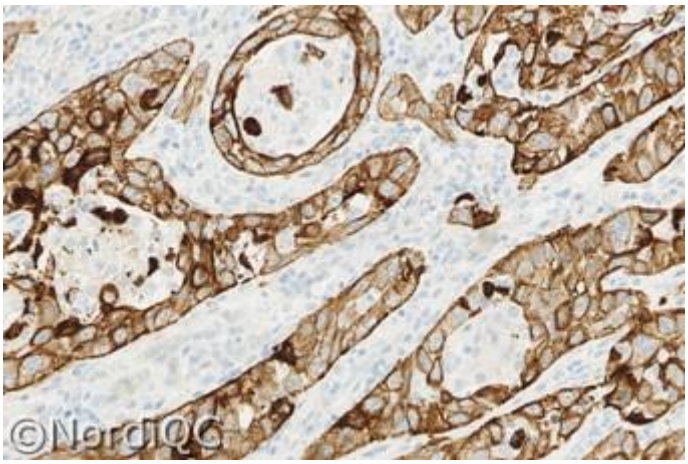


Fig. 4a
Optimal staining for CK-HMW of the lung squamous cell carcinoma using same protocol as in Figs. 1a. - 3a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining.

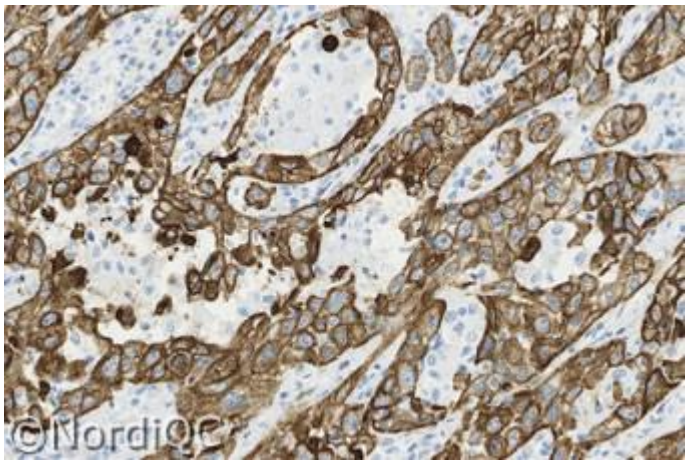


Fig. 4b
Staining for CK-HMW of the lung squamous cell carcinoma using same insufficient protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining. However, as the epithelial cells of the breast ductal carcinoma in Fig. 3b showed same staining characteristics, the staining for CK-HMW is not reliable.

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