

The slide to be stained for Pan-cytokeratin (pan-CK) comprised:

1. Appendix, 2. Liver, 3. Tonsil, 4. Breast lobular carcinoma, 5. Seminoma

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

- A strong and distinct cytoplasmic reaction in the appendix enterocytes (including crypt basis) and bile ducts, and a moderate predominantly membranous reaction of the large majority of hepatocytes.
- A strong and distinct cytoplasmic reaction of the squamous epithelial cells in the tonsil and at least a faint staining of fibroblastic reticulum cells in the lymphoid tissue of the tonsil.
- A strong and distinct cytoplasmic reaction of the breast lobular carcinoma.
- Focally, a strong and distinct cytoplasmic reaction of the seminoma.



85 laboratories submitted stains. At the assessment 21 achieved optimal marks (25 %), 28 good (33 %), 29 borderline (34 %) and 7 (8 %) poor marks.

The following Abs were used:

mAb clone cocktail **5D3 + LP34** (Novocastra, n=3)

mAb clone **Ab-2** (NeoMarkers, n=2)

mAb clone cocktail **AE1/AE3** (Boehringer, n=1; Chemicon, n=1; Dako, n=43; NeoMarkers, n=1; Zymed, n=3)

mAb clone cocktail **AE1/AE3 + 5D3** (Biocare, n=3)

mAb clone cocktail **AE1/AE3 + PCK26** (Ventana, n=7)

mAb clone **KL1** (Immunotech n=9, Serotec n=2)

mAb clone **MNF116** (Dako, n=8)

mAb clone **Lu-5** (NeoMarkers, n=2)

Furthermore, two laboratories used mAb clone **CAM5.2** (Becton Dickinson) which is considered inappropriate as a pan-CK marker as it only detects low molecular weight CKs.

Optimal staining for pan-CK in this assessment was obtained with the following mAb clones/clone cocktails: **Ab-2** (2 out of 2), **AE1/AE3** (13 out of 48) **AE1/AE3 + 5D3** (2 out of 3), **KL1** (2 out of 11) and **MNF116** (3 out of 8). However, for the other clones, we consider it likely that optimal results could have been obtained using other protocols (e.g., using HIER instead of proteolytic epitope retrieval). However, we have not yet tested this in our own laboratories.

All optimal protocols for the above mentioned clones were based on HIER except for the clone **MNF116**, for which the optimal protocols were based on proteolytic pre-treatment.

In the optimal protocols using the mAb clone **Ab-2** Tris-EDTA/EGTA pH 9 was used as HIER buffer (2 out of 2), with the mAb used in the range of 1:300 – 1:400 depending on the sensitivity of the protocol applied.

In the optimal protocols using the mAb clone **AE1/AE3** both citrate pH 6,0, CC1 (Ventana), and Tris-EDTA/EGTA pH 9 could be used as the HIER buffer (1 out of 5, 1 out of 2, and 10 out of 22 were optimal, respectively), with the mAb used in the range of 1:20-1:250 depending on the sensitivity of the protocol applied.

In the optimal protocols using the mAb clone **AE1/AE3 + 5D3** Tris-EDTA/EGTA pH 9 was used as the HIER buffer (2 out of 2) with the mAb used in the range of 1:400-1:800 depending on the sensitivity of the protocol applied.

In the optimal protocols using the mAb clone **KL1** Tris-EDTA/EGTA pH 9 was used as HIER buffer (2 out of 9) with the mAb used in the range of 1:50 – 1:75 depending on the sensitivity of the protocol applied.

In the optimal protocols using the mAb clone **MNF116** the protocol was based on proteolytic pre-treatment using Proteinase K and a dilution of the mAb in the range of 1:50 – 1:400 depending on the sensitivity of the protocol

applied.

The most frequent causes of insufficient stainings were (often in combination):

- Inappropriate choice of primary Ab
- Too low concentration of the primary antibody
- Inappropriate epitope retrieval (proteolysis for the mAb clone **AE1/AE3**)

The prevalent feature of an insufficient staining was a too weak or negative reaction of cells/structures supposed to be demonstrated. The majority of the laboratories (95 %) were capable to demonstrate CK in the enterocytes of the appendix and the bile ducts of the liver as well as the lobular breast carcinoma. However, demonstration of CK in the seminoma was more difficult and only demonstrated in approximately 60 % of the laboratories. All laboratories demonstrating CK in the seminoma were capable of detecting the relatively low expression of CK (CK type 8 and 18) in hepatocytes, indicating that liver may be a reliable control for the demonstration of low molecular weight CKs, provided that the protocol is calibrated to stain the liver cells. In this assessment all laboratories using the most widely used mAb clone **AE1/AE3** (n=59) either alone or in cocktail with **PCK26** basing the protocol on proteolytic pre-treatment (n=12) had an insufficient staining due to a negative staining reaction of the hepatocytes.

Simultaneously with the demonstration of low molecular weight CK a pan-CK Ab should also identify the high molecular weight CK in squamous epithelial cells. In this assessment only few laboratories had problems with the detection of CK in the squamous epithelial cells of the tonsil. The reason for insufficient demonstration of high molecular weight was typically due to an inappropriate Ab (as the mAb clone **CAM5.2** which is a marker for low molecular weight CK subtypes only) or a too low concentration of the Ab, especially when using the clone **KL1**, which reacts with a more limited number of the high molecular weight CK subtypes.

Pan-CK was also assessed in run 8. In that run 72 laboratories participated out of which 47 % (34 laboratories) was marked insufficient. Each laboratory was given a specific recommendation to improve their protocol. 29 laboratories, which obtained an insufficient result in run 8, submitted a new pan-CK stain in run 15. 18 of the laboratories followed the recommendation and 13 improved the result from insufficient to either good or optimal (72 %). 11 laboratories did not follow the recommendation and 1 of these (9 %) obtained a sufficient staining in run 15.

However, the overall proportion of insufficient staining was in this run only reduced to 42 % from 47 % in run 8, and as the proportion of insufficient staining still is relative high, pan-CK will be repeated in 2006 or 2007.

## Conclusion

The mAb clones **Ab-2**, **AE1/AE3**, **AE1/AE3 + 5D3**, and **KL1** was found to be the most successful markers for pan-CK using HIER. The mAb clone **MNF116** was found to be useful for pan-CK using proteolytic pre-treatment. Liver and tonsil in combination are appropriate control tissues. The large majority of hepatocytes should be stained.

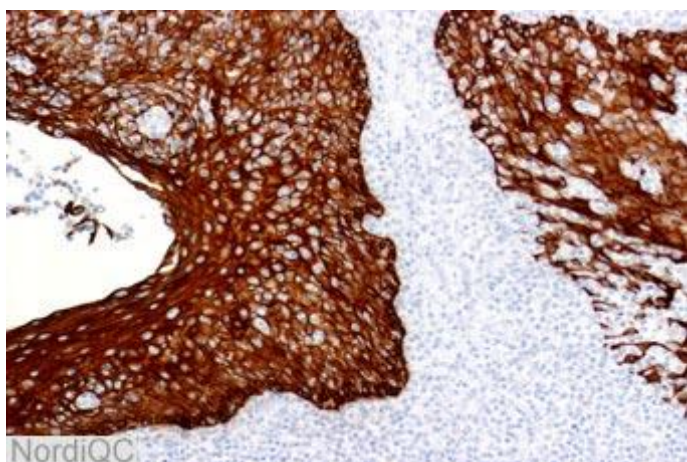


Fig. 1a  
Optimal staining for pan-CK in the tonsil. All the squamous epithelial cells throughout the entire epithelial layer are stained.

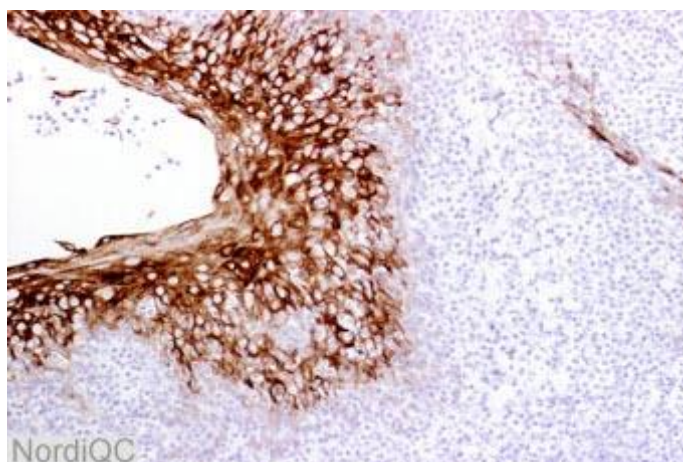
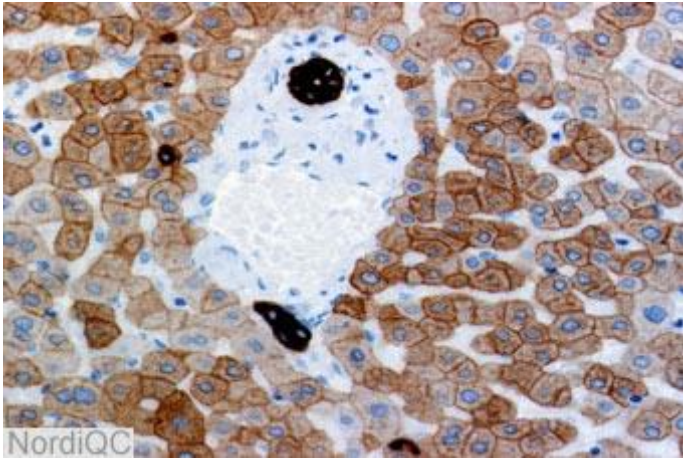
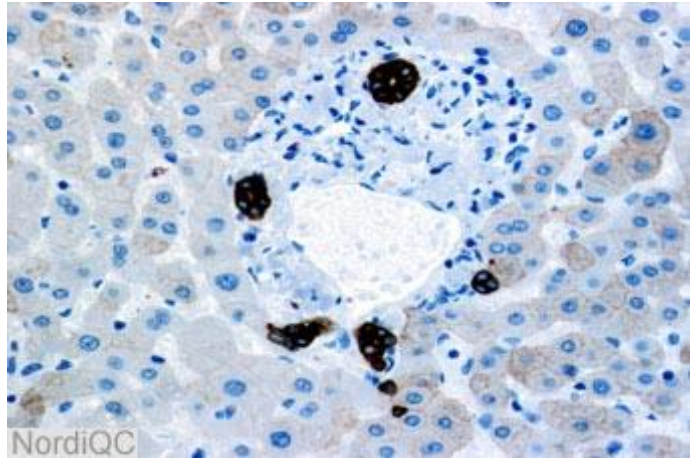


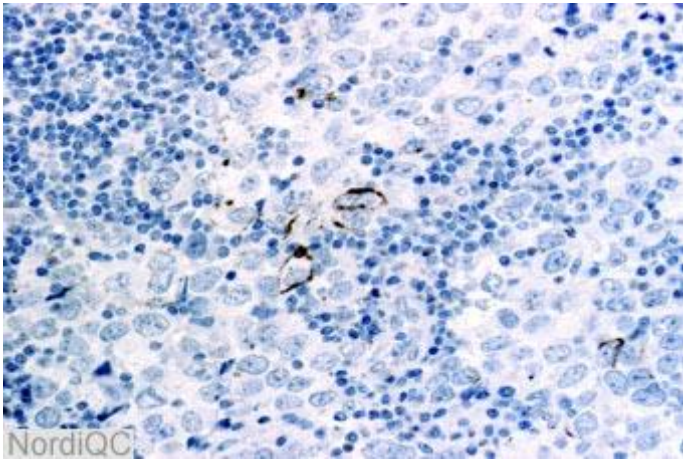
Fig. 1b  
Staining for pan-CK in the tonsil using an insufficient protocol - same field as in Fig. 1a. Only the superficial squamous epithelial cells are stained.



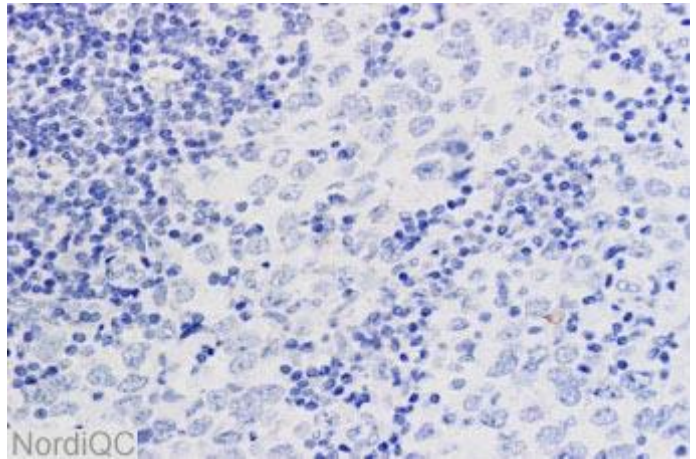
**Fig. 2a**  
Optimal staining for pan-CK in the liver, using clone AE1/AE3 after HIER. The hepatocytes show a moderate to strong distinct predominantly membranous reaction and the bile duct epithelial cells are intensively stained.



**Fig. 2b**  
Staining for pan-CK in the liver using an insufficient protocol (probably due to a too low concentration of the primary Ab.). Only the bile duct epithelial cells are distinctively demonstrated while the hepatocytes are unstained or only weakly positive.



**Fig. 3a**  
Optimal staining for pan-CK in the seminoma. A few neoplastic cells show a strong distinct reaction (same protocol used in Fig. 1a and 2a).



**Fig. 3b**  
Insufficient staining for pan-CK in the seminoma – same field as in Fig. 3a. The neoplastic cells are unstained. (same protocol used in Fig. 2b).

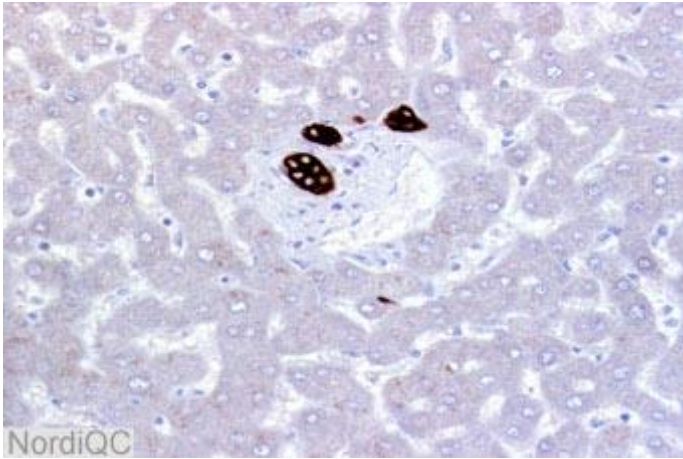


Fig. 4a  
Staining for pan-CK in the liver using an insufficient protocol, namely AE1/AE3 with proteolytic pre-treatment. Only the bile duct epithelial cells are distinctively demonstrated while the hepatocytes are unstained or only weakly positive.

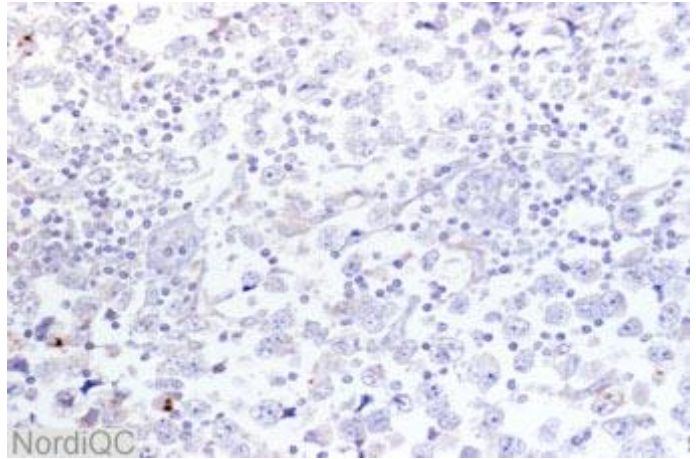


Fig. 4b  
Staining for pan-CK in the seminoma using the same protocol as Fig. 4a. The morphology of the neoplastic cells is severely impaired due to excessive proteolytic pre-treatment.

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