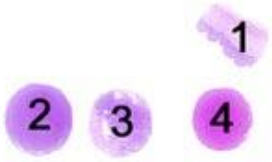
Assessment Run B5 2008

E-cadherin (ECAD)

The slide to be stained for E-cadherin (ECAD) comprised:
1. Colon, 2. Breast ductal carcinoma, 3. Breast lobular carcinoma, 4. Liver.
All tissues were fixed in 10% neutral buffered formalin for 24-48 hours.



Criteria for assessing a membranous ECAD staining as optimal included:

- A strong, distinct membranous staining of virtually all the columnar epithelial cells in the appendix.
- A strong, distinct membranous staining of the epithelial cells of the bile ducts and a moderate membranous staining of the hepatocytes in the liver.
- A moderate to strong, distinct membranous staining of virtually all the neoplastic cells of the breast ductal carcinoma.
- No staining or at maximum a focal membranous staining of the neoplastic cells of the breast lobular carcinoma.

94 laboratories submitted stains. At the assessment 38 achieved optimal marks (40 %), 33 good (35 %), 12 borderline (13 %) and 11 poor marks (12 %).

The following Abs were used:
mAb clone **NCH-38** (Dako, n=48; NeoMarkers/Thermo Scientific, n=4)
mAb clone **HECD-1** (Zymed, n=10; Immunologic, n=3; Abcam, n=2; BioCare, n=1)
mAb clone **ECH-6** (Ventana, n=11; Immunovision, n=1)
mAb clone **36B5** (Novocastra, n=6)
mAb clone **4A2C7** (Zymed, n=4)
mAb clone **36** (BD Transduction Lab, n=2)
mAb clone **SPM471** (NeoMarkers, n=1; Spring Bioscience, n=1)

Optimal staining for ECAD in this assessment was obtained with the mAb **NCH-38** (24 out of 52), the mAb **HECD-1** (7 out of 16), the mAb **ECH-6** (5 out of 12) and the mAb **36B5** (2 out of 6).

All optimal protocols, independent of the Ab, were based on heat induced epitope retrieval (HIER) as follows:

NCH-38:Tris-EDTA/EGTA pH 9.0 (14/25)*, Cell Conditioning1 (BenchMark, Ventana) (3/12), Target Retrieval Solution pH 9.0 (Dako) (3/3), Bond Epitope Retrieval Solution 2 (Bond, Leica Microsystems) (1/1) or Citrate pH 6.0 (3/7). The mAb was diluted in the range of 1:5 – 1:200 depending on the total sensitivity of the protocol employed.
Using these protocol settings 38 out of 47 (81 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

HECD-1: Tris-EDTA/EGTA pH 9.0 (4/8), Target Retrieval Solution pH 9.0 (Dako) (1/1), EDTA/EGTA pH 8.0 (1/1) or Citrate pH 6.0 (1/3). The mAb was diluted in the range of 1:50 – 1:2,500 depending on the total sensitivity of the protocol employed.
Using these protocol settings 11 out of 13 (85 %) laboratories produced a sufficient staining.

ECH-6: Cell Conditioning1 (BenchMark, Ventana) (5/11) and used as a Ready-To-Use Ab.
Using these protocol settings 9 out of 11 (82 %) laboratories produced a sufficient staining.

36B5: Tris-EDTA/EGTA pH 9.0 (1/2) or Bond Epitope Retrieval Solution 2 (Bond, Leica Microsystems) (1/1). The mAb was diluted 1:50.
Using these protocol settings both of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab
- Too high concentration of the primary Ab

- Less successful Ab
- Excessive counterstaining.

The prevalent feature of an insufficient staining was a too weak or a completely false negative staining of the breast ductal carcinoma and of the remnants of the benign ductal glands in the lobular carcinoma. This pattern was seen in 20/24 of the insufficient results. Excessive counterstaining (especially combined with excessive HIER) complicated the identification the neoplastic cells in the lobular carcinoma and thus the interpretation of the ECAD. In 4/24 an excessive background reaction and a diffuse cytoplasmic staining of the neoplastic cells of the lobular carcinoma was observed. In the correctly calibrated protocols the majority of the neoplastic cells of the lobular carcinoma were negative and only scattered neoplastic cells showed a disrupted membranous reaction, while the neoplastic cells of the ductal carcinoma showed a distinct continuous membrane reaction.

This was the first assessment of ECAD. As control, the liver tissue displayed the most informative reaction pattern as critical staining indicator for ECAD. In the optimal protocols virtually all the liver cells showed a distinct moderate membranous reaction, while the bile ducts showed a strong reaction. In the stains deemed too weak the liver cells were typically negative or only showed a weak patchy membranous reaction.

Conclusion
The mAb clones NCH-38, HECD-1, ECH-6 and 36B5 are all useful Abs for ECAD. HIER is mandatory to obtain an optimal result. Liver is a recommendable control: The liver cells are critical stain quality indicators, they must show at least a moderate membranous reaction with no or only minimal cytoplasmic staining.

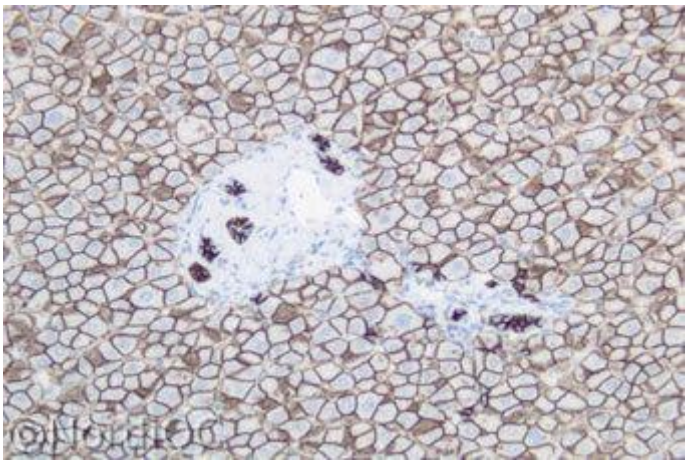


Fig. 1a
Optimal staining for ECAD of the liver using the mAb clone NCH-38 with HIER. Virtually all the hepatocytes show a moderate distinct membranous reaction, while the epithelial cells of the bile ducts show a strong staining.

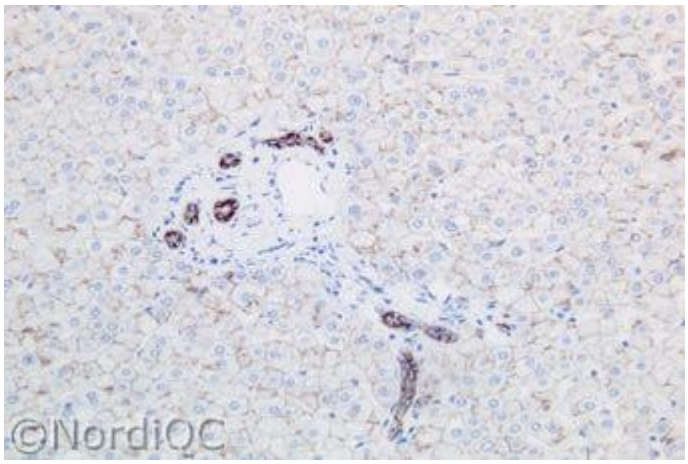


Fig. 1b
Staining for ECAD of the liver using an insufficient protocol based on the same mAb clone NCH-38 as in Fig. 1a, but in a too low concentration. The hepatocytes only show a weak disrupted membranous reaction – same field as in Fig. 1a.

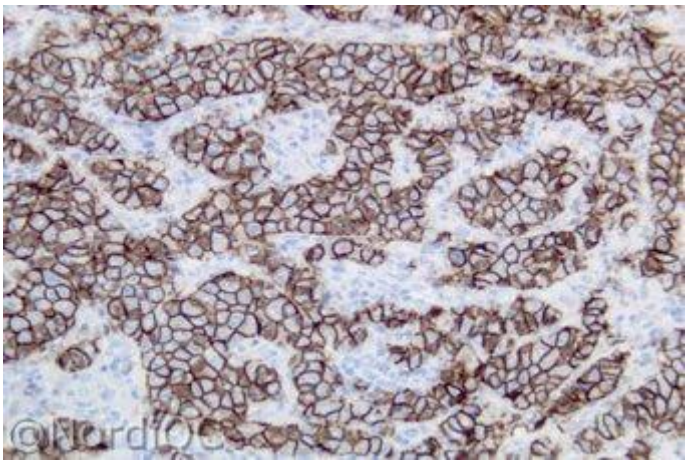


Fig. 2a
Optimal ECAD staining of the ductal breast carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a strong distinct membranous reaction with no background reaction.

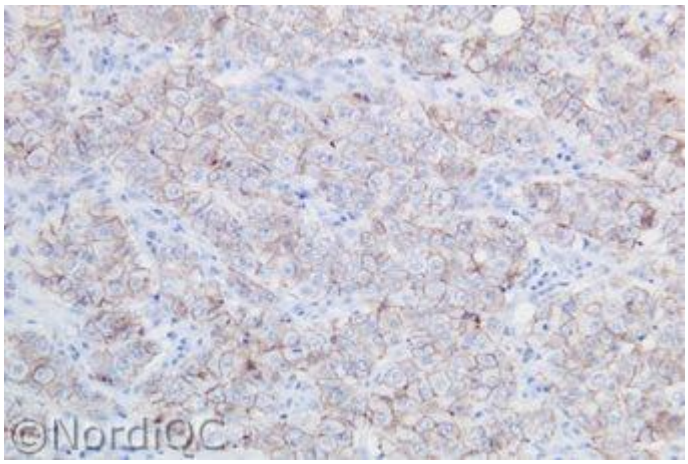


Fig. 2b
Staining for ECAD of the ductal breast carcinoma using same insufficient protocol as in Fig. 1b. The neoplastic cells only show a weak diffuse membranous reaction – also compare with Fig. 3b – same protocol.

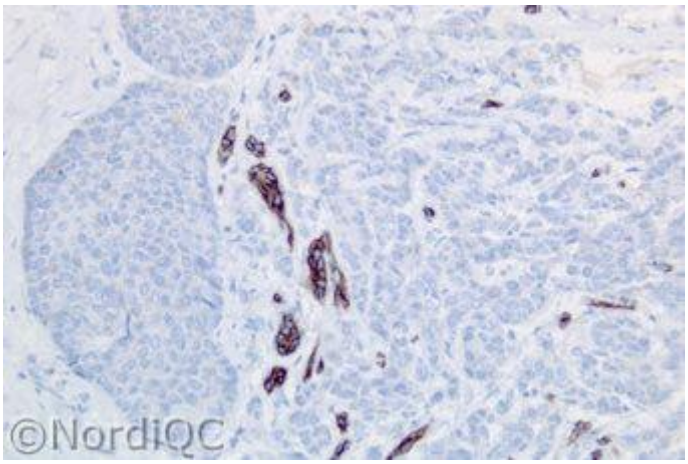


Fig. 3a
Optimal ECAD staining of the lobular breast carcinoma using same protocol as in Figs. 1a and 2a. Both the invasive neoplastic cells and the cells of the lobular carcinoma in situ component are negative and only the ductal epithelial cells of the entrapped benign glands show a strong distinct membranous reaction.

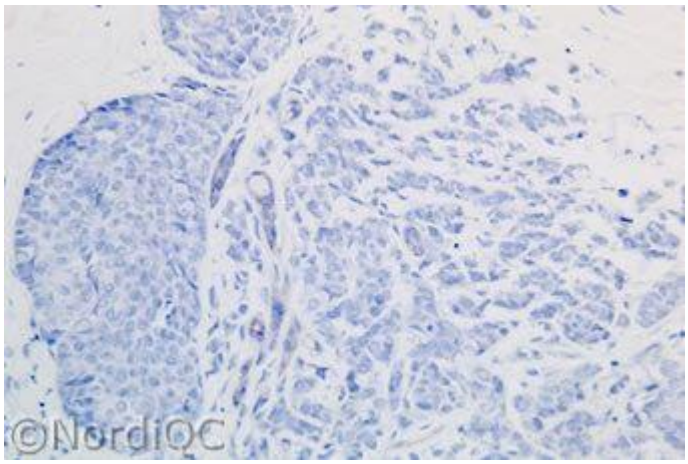


Fig. 3b
Insufficient ECAD staining of the lobular breast carcinoma using same protocol as in Figs. 1b and 2b. All cells are negative and the false negative reaction in the ductal epithelial cells of the entrapped benign glands complicates the interpretation of a loss of ECAD in the lobular carcinoma.

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