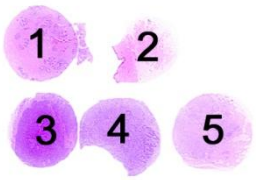
Assessment Run 25 2009

Gross cystic disease fluid protein-15 (GCDFP)

The slide to be stained for Gross cystic disease fluid protein-15 (GCDFP) comprised:
1. Breast, 2. Skin, 3-5. Breast ductal carcinoma.
All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing a GCDFP staining as optimal included:

- A strong, distinct cytoplasmic reaction in scattered ductal epithelial cells and apocrine metaplastic cells of the breast.
- A moderate to strong, distinct cytoplasmic reaction of the majority of the epithelial cells of the eccrine sweat glands in the skin.
- A strong, distinct cytoplasmic reaction of virtually all the neoplastic cells of the breast carcinoma no. 3 and the majority of the neoplastic cells of the breast carcinoma no. 4.
- At least a weak to moderate cytoplasmic and a dot-like reaction in scattered neoplastic cells of the breast carcinoma no. 5.
- No more than moderate background reaction in the vicinity of the positive cells was accepted (antigen diffusion).

43 laboratories participated in the assessment. 61 % achieved a sufficient mark. The results are summarized in Table 1:

Table 1. **Abs and scores for GCDFP, run 25**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **23A3**	19 6 2 1 1 1	Novocastra NeoMarkers Cell Marque Abcam Dako Vector	11	8	7	4	63 %	85 %
mAb **D6**	4 3 2 1 1	Signet Zymed ID Labs Biotech. BioCare BioGenex	4	1	5	1	46 %	100 %
Ready-To-Use Abs								
mAb clone **23A3**	1	Novocastra	0	1	0	0	-	-
rmAb clone **EP1582Y**	1	Ventana, 760-4386	0	1	0	0	-	-
Total	43		15	11	12	5	-	-
Proportion			35 %	26 %	28 %	12 %	61 %	88 %

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **23A3**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (5/9)*, Cell Conditioning 1 (BenchMark, Ventana) (2/8), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (2/6) or Citrate pH 6 (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 17 out of 20 (85 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **D6**: The protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 (2/2) or

Cell Conditioning 1 (BenchMark, Ventana) (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab.
- Omission of HIER (5/5 laboratories using the clone D6 without HIER obtained an insufficient mark)

In this assessment the prevalent feature of an insufficient staining was a general too weak or false negative staining, occasionally accompanied by an excessive background reaction. The false negative reaction was in particular observed in the breast carcinoma no. 5, while a proper staining of the two other breast carcinomas were demonstrated by the majority of the participants. With mAb clone D6 omission of HIER also gave an intense background reaction in the normal breast specimen compromising the interpretation. For both mAb clones D6 and 23A3, the background reaction was significantly reduced with HIER, and was only seen in the vicinity of the GCDFP positive glands.
Skin was found to be an appropriate control: The epithelial cells of the eccrine sweat glands should show an as strong as positive cytoplasmic reaction, while all other cells should be negative.
Normal breast tissue was less useful as control, as the number and intensity of the ductal epithelial cells varied throughout the tissue.

Conclusion
The mAb clones **23A3** and **D6** are both useful markers for GCDFP. HIER - preferable in an alkaline buffer - seems mandatory to obtain an optimal staining. Skin is recommended as positive control: The eccrine sweat glands shall show an as strong as positive cytoplasmic reaction, while all other cells shall be negative.

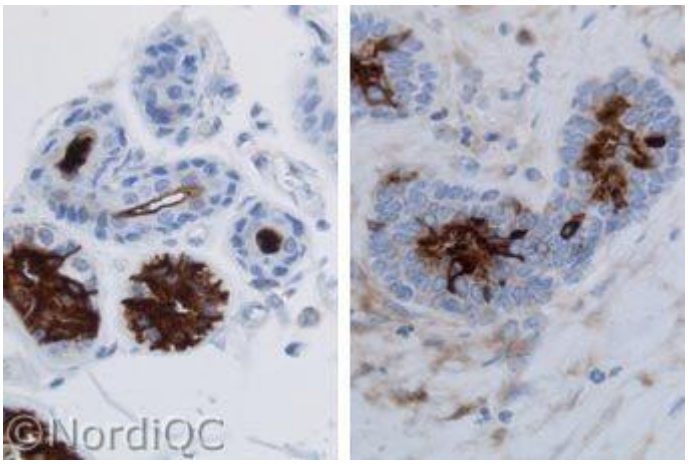


Fig. 1a
Optimal staining for GCDFP using the mAb clone 23A6 optimally calibrated and with HIER.
Left: Skin: The majority of the epithelial cells of the eccrine sweat glands show a strong and distinct cytoplasmic reaction.
Right: Breast: Scattered ductal epithelial cells show a distinct cytoplasmic reaction and also a strong reaction is seen in the luminal extracellular mucus. A weak background reaction is seen.
Also compare with Figs. 2a & 3a – same protocol.

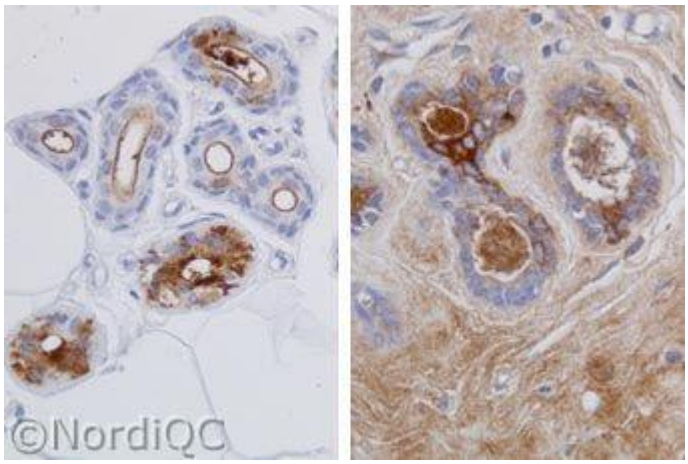


Fig. 1b
Insufficient staining for GCDFP using the mAb clone D6 with omission of HIER.
Left: Skin: The majority of the epithelial cells of the eccrine sweat glands show a moderate cytoplasmic reaction.
Right: Breast: Scattered epithelial cells show a moderate cytoplasmic reaction, but also a moderate background reaction is seen.
Also compare with Figs. 2b & 3b – same protocol.

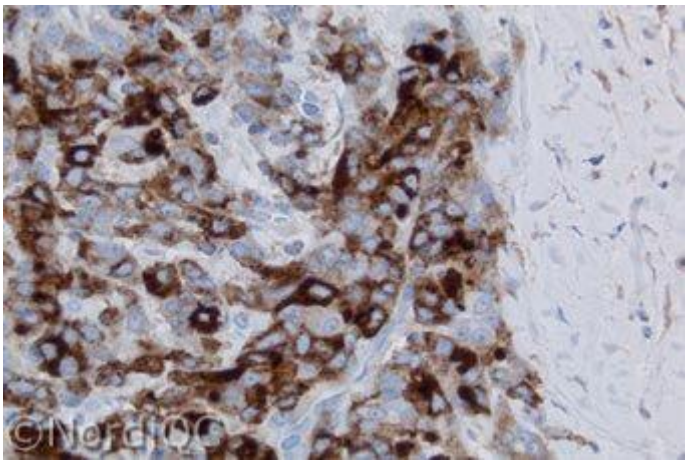


Fig. 2a
Optimal staining for GCDFP of the breast ductal carcinoma no. 3 using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong and distinct cytoplasmic reaction and only a minimal background reaction is seen.

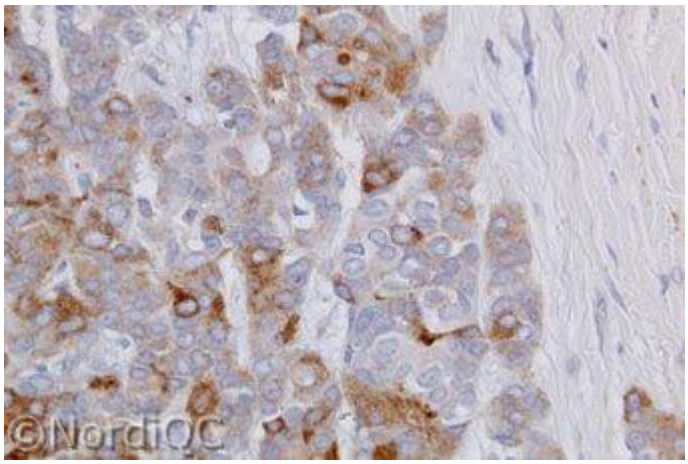


Fig. 2b
Insufficient staining GCDFP of the breast ductal carcinoma no. 3 using same protocol as in Fig. 1b. The neoplastic cells are demonstrated, but both the intensity and proportion is reduced - same field as in Fig. 2a. Also compare with Fig. 3b – same protocol.

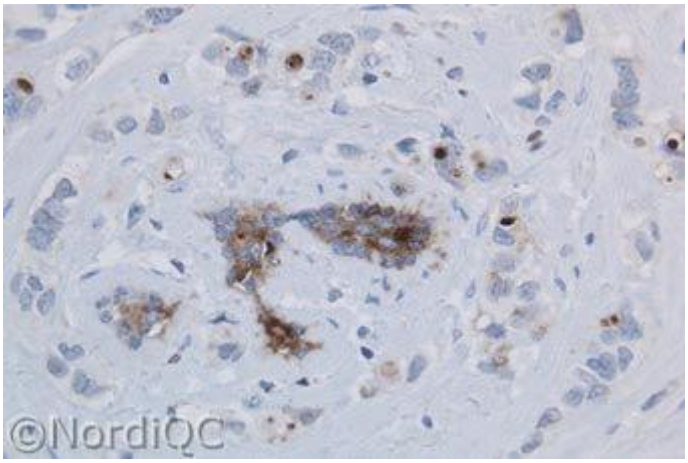


Fig. 3a
Optimal staining for GCDFP of the breast carcinoma no. 5 using same protocol as in Fig. 1a & 2a. The majority of the neoplastic cells show at least a weak cytoplasmic reaction and focally a dot-like staining. Also note the moderate to strong reaction of the normal ductal epithelial cells in the middle of the photo.

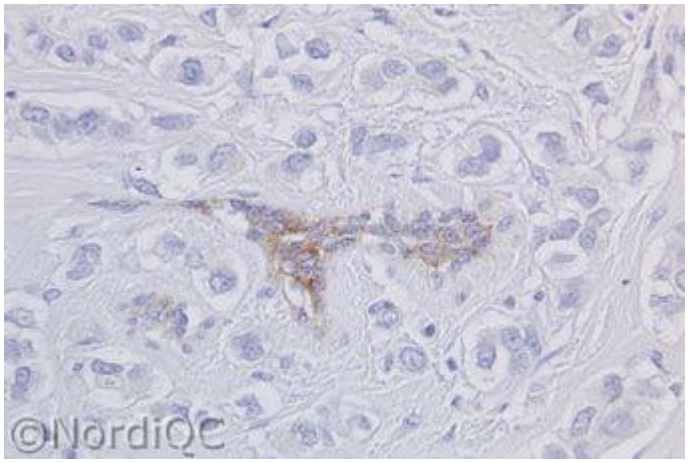


Fig. 3b
Insufficient staining for GCDFP of the breast carcinoma no. 5 using same protocol as in Fig. 1b & 2b. No reaction is seen in the neoplastic cells and only the normal ductal epithelial cells are demonstrated.

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