Assessment Run 36 2012

Gross cystic disease fluid protein-15 (GCDFP)

Material

The slide to be stained for GCDFP comprised:

1. Breast hyperplasia, 2. - 5. Breast ductal carcinomas.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a GCDFP staining as optimal were:

- A strong, distinct cytoplasmic staining reaction in scattered ductal epithelial cells and apocrine metaplastic cells of the hyperplastic breast.
- A moderate to strong, distinct cytoplasmic staining reaction in the majority of the neoplastic cells of the breast carcinomas no. 2, 4 and no. 5.
- At least a weak cytoplasmic and dot-like staining reaction in scattered neoplastic cells of the breast carcinoma no. 3.
- No more than moderate background staining in the vicinity of the positive cells. Background staining was accepted due to antigen diffusion.

Of 131 participating laboratories 86 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for GCD, run 36**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **23A3**	43 12 10 2 2 1 1 1	Leica/Novocastra Thermo/Neomarkers Dako Cell Marque Diagnostic Biosystems Labs Inc. Vector Lab. Abcam	25	33	8	6	81 %	88 %
mAb clone **D6**	6 2 1 1 1	Covance/Signet ID Labs Biocare Invitrogen Sanbio	4	5	1	1	82 %	86 %
mAb **SPM135**	1	Spring Bioscience	0	1	0	0	-	-
rmAb **EP1582Y**	2 1	Cell Marque Zytomed systems	1	2	0	0	-	-
rmAb **EP95**	1	Epitomics	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **23A3 IS/IR077**	20	Dako	10	10	0	0	100 %	100 %
mAb clone **23A3 PA0350**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **23A3 257M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **23A3 MS-1170**	1	Thermo/Neomarkers	0	0	1	0	-	-
mAb clone **23A3 MAD-001638QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone **EP1582Y 760-4386**	18	Ventana	10	7	1	0	94 %	94 %

rmAb clone **EP1582Y AN481-5M**	1	Biogenex	0	1	0	0	-	-
Total	131		50	62	12	7		
Proportion			38 %	48 %	9 %	5 %	86 %	

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

Following protocol parameters were central to obtain an optimal staining:

Concentrated Abs
mAb clone **23A3**: Protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9;Dako) (8/18)*, Bond Epitope Retrieval Solution 2 (BERS2; Leica) (6/9), Cell Conditioning 1 (CC1; Ventana) (8/25), Tris-EDTA/EGTA pH 9 (2/10) or Bond Epitope Retrieval Solution 1 (BERS1; Leica) (1/3)as the retrieval buffer. The mAb was typically diluted in the range of 1:10-1:75 depending on the total sensitivity of the protocol employed. Using these protocol settings 30 of 34 (88 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **D6**: Protocols giving an optimal result were all based on HIER using either Tris pH 9 (1/1), BERS2 (Bond, Leica) (1/1), CC1 (Ventana) (1/1) or Tris-EDTA/EGTA pH 9 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:4-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 of 7 (86 %) laboratories produced a sufficient staining.

rmAb **EP1582Y**: Protocol giving an optimal result was based on HIER with CC1 (Ventana) (1/1) as the retrieval buffer. The rmAb was diluted 1:1.000 and OptiView (Ventana, 760-700) was used as detection system.

Ready-To-Use Abs
mAb clone **23A3** (product.no. IS / IR077, Dako): Protocols giving an optimal result were based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (heating time 10-30 min at 95-97°C), 20 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 20 of 20 (100 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **EP1582Y** (prod. no. 760-4386, Ventana): Protocols giving an optimal result were all based on HIER using mild or standard CC1, 8-36 min incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 15 of 16 (94 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Use of low sensitive detection systems.

In concordance with the previous NordiQC assessment for GCDFP, the prominent feature of an insufficient staining was a too weak or false negative staining of structures expected to be demonstrated. The majority of laboratories were able to demonstrate GCDFP in apocrine metaplastic cells and the majority of neoplastic cells of the breast carcinomas no. 2, 4 and 5. Demonstration of GCDFP in normal ductal epithelial cells of the hyperplastic breast specimen and in particular neoplastic cells of breast carcinoma no. 3 was more challenging and required an optimally calibrated protocol.

A sufficient result (optimal or good) could be obtained with all the clones used. In this assessment 126 of 131 participants used HIER. 49 of 50 optimal protocols were based on the use of an alkaline HIER buffer. Applied as a concentrate with an in-house calibrated system, the most widely used Abs were the mAb clones 23A3 and D6 showing a relative high pass rate of 81% and 82%, respectively.

The proportion of sufficient results was influenced by the choice of the detection system used. If the mAb clone 23A3 was used as a concentrate and applied with a 2-step polymer-/multimer-based detection system (EnVision FLEX (Dako) or UltraView (Ventana)), 74% (28/38) of laboratories obtained a sufficient staining result, and 22 % were optimal. If same protocol settings were applied with a more sensitive 3-step polymer-/multimer-based detection system (EnVision FLEX+ (Dako), Bond Refine (Leica) or OptiView (Ventana)), 94 % (29/31) of the laboratories obtained a sufficient staining result and 55% obtained an optimal mark. Compared to the overall pass rate of 86%, the pass rate was significant lower for the participants using a biotin based detection systems, as 6 out of 10 laboratories obtained an sufficient staining result (60%) out of which none was assessed as optimal.

11 participants used the mAb clone D6 as a concentrate of which 3 protocols were based on proteolytic digestion and 2 protocols used no pre-treatment at all. Of the protocols based on non-HIER, 4 out of 5 laboratories produced a sufficient staining result (80%) but none (0%) were assessed as optimal, as an excessive background staining typically was seen compromising the interpretation. Of the remaining 6 protocols, all based on HIER, 4 out of 6 laboratories produced a sufficient staining result (67%) and all (100%) were assessed as optimal. The slightly lower pass rate, compared to participants using non-HIER, was caused by use of less sensitive protocol settings as too low concentration of the primary Ab and/or insufficient HIER.

The most successful and robust assay for GCDFP in this assessment was obtained by the Dako RTU format based on mAb clone 23A3 (IS / IR077) giving a pass rate of 100% (20/20) of which 50% were assessed as optimal. The Ventana/Cell Marque RTU format based on rmAb clone EP1582Y (760-4386) also provided a high pass rate of 94% (17/18) out of which 53% (10/18) were assessed as optimal.

Controls
Normal skin is the preferred positive control for GCDFP. The epithelial cells of the eccrine sweat glands must show an as strong as possible positive cytoplasmic staining reaction, while all other cells should be negative. As in this assessment, normal breast tissue can also be used as control in which scattered epithelial cells of the ductal glands must show an as strong as possible staining reaction.
For both skin and breast tissue a weak to moderate background staining in the vicinity of a strong staining reaction must be accepted due to antigen diffusion – an aberrant nuclear staining reaction in these areas may also be observed and has to be accepted. It has to be emphasized that the number and intensity of the ductal epithelial cells may vary throughout these two tissues.

Effect of external quality assessment
This was the second assessment of GCDFP in NordiQC. A significant increase in the proportion of sufficient results was observed compared to the previous run 25 (Table 2).

Table 2. **Proportion of sufficient results for GCDFP**

	Run 25 2009	**Run 36 2012**
Participants, n=	43	130
Sufficient results	61 %	87 %

The pass rate of 87 % in this assessment was high and was obtained despite many new laboratories participating for the first time. The explanation could be due to several factors including extended use of properly calibrated commercially available RTU formats or less challenging tissue material circulated.

Conclusion
The mAb clones **23A3**, **D6** and the rmAb clone **EP1582Y** are all recommendable Abs for GCDFP. Efficient HIER in an alkaline buffer in combination with a sensitive and specific IHC system is mandatory for optimal performance. Biotin based detection systems can not be recommended due to a reduced sensitivity and the risk of a false positive staining reaction caused by endogenous biotin. In this assessment the Ready-To-Use systems based on the mAb clone **23A3** from Dako and rmAb clone **EP1582Y** from Ventana or Cell Marque gave the highest proportion of sufficient results.
Normal skin is an appropriate control: The epithelial cells of the eccrine sweat glands must show the strongest possible cytoplasmic reaction, while all other cells should be negative. A weak background staining can be expected.

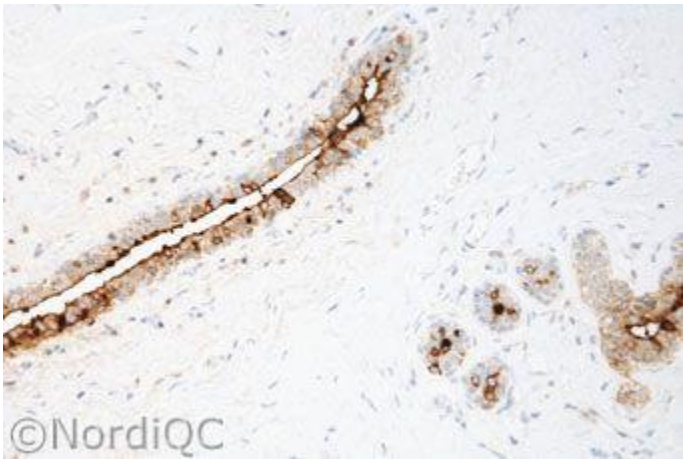

Fig. 1a
Optimal staining for GCDFP-15 of the breast hyperplasia using the mAb clone 23A3 optimally calibrated as a concentrate, HIER in an alkaline buffer and a polymer based detection system. The majority of the ductal epithelia cells show a distinct moderate to strong cytoplasmic staining reaction. Also compare with Fig. 2a – same protocol.

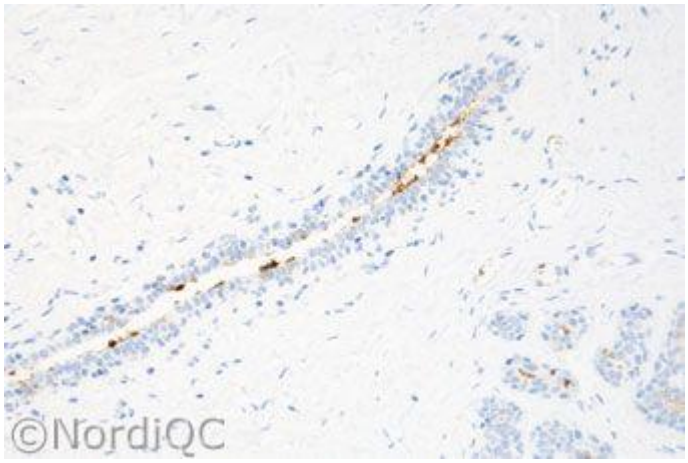

Fig. 1b
Insufficient staining for GCDFP-15 of the breast hyperplasia applying the mAb clone 23A3 as a concentrate using exactly the same protocol settings as used in Fig 1a, except for a 20 fold dilution of the primary antibody. The proportion and the intensity of the cells demonstrated are significantly reduced compared to the result in Fig. 1a. Also compare with Fig. 2b – same protocol.

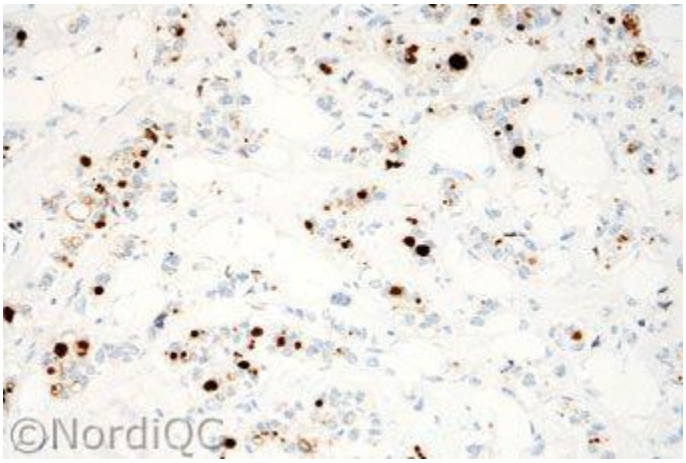

Fig. 2a
Optimal staining for GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1a.
The majority of the neoplastic cells show a moderate to strong dot-like cytoplasmic staining reaction.

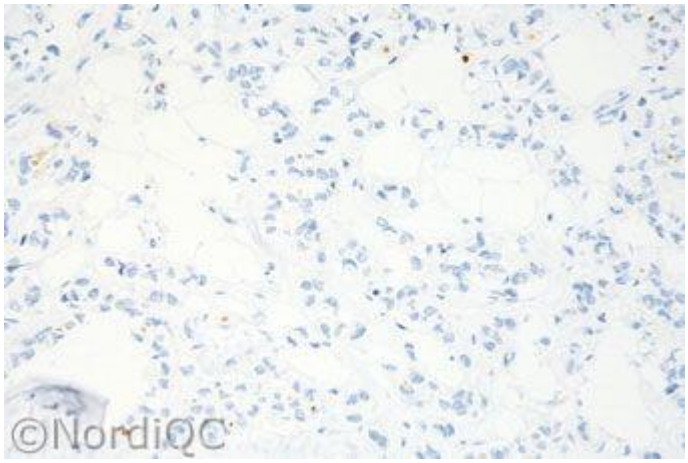

Fig. 2b
Insufficient staining GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1b. - same field as in Fig. 2a.
Only scattered neoplastic cells show a faint dot-like reaction.

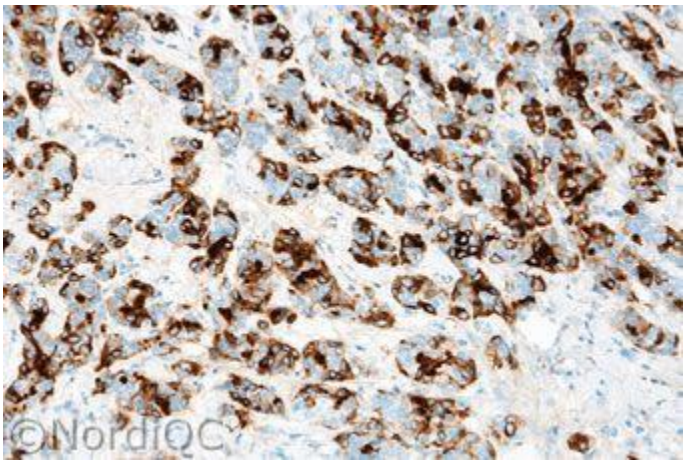

Fig. 3a
Optimal staining for GCDFP-15 of the breast carcinoma no. 4 using the mAb clone 23A3 optimally calibrated as a concentrate, HIER in CC1 and a 3-step multimer based detection system. Virtually all the neoplastic cells show a strong cytoplasmic staining reaction.

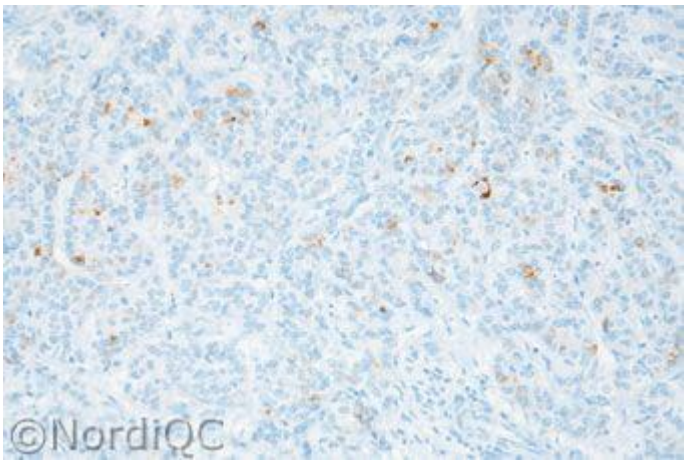

Fig. 3b
Insufficient staining for GCDFP-15 of the breast carcinoma no. 4 using the mAb clone 23A3 as a concentrate with a too low sensitivity (too low concentration of the primary Ab and a 2-step multimer based conjugate) – same field as in Fig. 3a. Only scattered neoplastic cells show a weak cytoplasmic staining reaction.

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