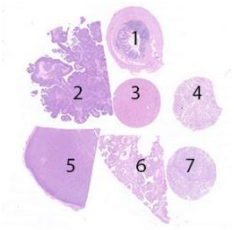
Assessment Run 42 2014

Glypican 3 (GLP3)

Material
The slide to be stained for GLP3 comprised:



1. Appendix, 2. Serous ovarian carcinoma, 3. Liver, 4. Placenta, 5. Hepatocellular carcinoma, 6-7. Clear cell ovarian carcinomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a GLP3 staining as optimal included:

- A moderate to strong predominantly cytoplasmic staining reaction of virtually all syncytiotrophoblasts and cytotrophoblasts in the placenta and at least a weak staining reaction in dispersed villous stromal and endothelial cells.
- A moderate to strong predominantly cytoplasmic but also membranous staining reaction of virtually all neoplastic cells in the hepatocellular carcinoma.
- An at least weak to moderate predominantly cytoplasmic staining reaction of the majority of the neoplastic cells in the two clear cell ovarian carcinomas.
- No staining reaction of normal liver cells and the vast majority of epithelial cells in the appendix.
- No staining reaction of the neoplastic cells in the serous ovarian carcinoma.

Participation

Number of laboratories registered for GLP3, run 42	89
Number of laboratories returning slides	80 (90%)

Results
80 laboratories participated in this assessment. Of these, 69 (86%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Unexplained (appropriate protocol settings but aberrant staining result)

Performance history
This was the first NordiQC assessment of GLP3. Table 2 shows the proportion of sufficient results

Table 2. **Proportion of sufficient results for GLP3 in the 1st NordiQC run performed**

	Run 42 2014
Participants, n=	80
Sufficient results	86%

Conclusion
The mAb clones **1G12**, **GC33** and **GC3-88** could all be used to obtain optimal staining results for GLP3. mAb clone **1G12** in concentrated format provided optimal results on all three main IHC systems. HIER preferable in an alkaline buffer and careful calibration of the primary antibody were the most important prerequisites for optimal staining results. The RTU systems of the mAb clones **GC33** and **GC3-88** (Ventana and BioGenex, respectively) provided the highest proportion of sufficient and optimal results. Placenta is recommended as positive tissue control for GLP3: Virtually all syncytiotrophoblasts and cytotrophoblasts must show a moderate to strong predominantly cytoplasmic but also membranous staining reaction. Dispersed villous stromal and endothelial cells must show an at least weak cytoplasmic staining recation.
Appendix can be used as negative tissue control. The vast majority of epithelial cells must be negative. Dispersed basal epithelial cells can show a weak cytoplasmic staining reaction.

Table 1. **Antibodies and assessment marks for GLP3, run 42**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1G12**	20 5 4 3 3 1 1 1 1 1	Cell Marque Santa Cruz BioMosaics Biocare Zytomed DCS Immunologic Monosan Zeta Zhongshan	15	18	5	2	83%	88%
Ready-To-Use antibodies								
mAb clone **1G12 760-4442**	5	Ventana/Cell Marque	2	3	0	0	100%	100%
mAb clone **1G12 261M-9x**	4	Cell Marque	1	1	2	0	-	-
mAb clone **1G12 PM396**	1	Biocare	1	0	0	0	-	-
mAb clone **1G12 B0134R**	2	BioMosaics	0	2	0	0	-	-
mAb clone **1G12 B1400**	1	BioMosaics	1	0	0	0	-	-
mAb clone **1G12 GI829R06**	1	DCS	1	0	0	0	-	-
mAb clone **1G12 MAD-000625QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **GC33 790-4564**	21	Ventana	11	9	1	0	95%	94%
mAb clone **GPC3-88 AM539-5M**	2	BioGenex	2	0	0	0	-	-
rmAb clone **SP86 MAD-000500QD**	2	Master Diagnostica	0	1	0	1	-	-
Total	80		35	34	8	3	-	
Proportion			44%	42%	10%	4%	86%	

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

Detailed analysis of GLP3, Run 42

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **1G12**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (3/5)*, TRS pH 9 (Dako) (3/6), TRS pH 6.1 (Dako) (1/2), Cell Conditioning 1 (CC1; Ventana) (3/10) Epitope Retrieval Solution 2 (BERS2; Leica) (1/7) or Tris-EDTA/EGTA pH 9 (4/7) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 29 of 33 (88%) laboratories produced a sufficient staining result (optimal or good).

* (number of optimal results/number of laboratories using this HIER buffer)

Table 3. **Proportion of optimal results for GLP3 for the mAb clone 1G12 used as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **1G12**	6/11** (55%)	1/2	2/8 (25%)	-	1/6 (17%)	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **1G12**, product no. **760-4442**, Ventana/Cell Marque, BenchMark XT/Ultra: Protocols with optimal result were based on HIER using Cell Conditioning 1 (efficient heating time 32-64 min.), 16-32 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using these protocol settings 4 of 4 (100%) laboratories produced a sufficient staining result.

mAb clone **1G12**, product no. **PM396**, Biocare, intelliPATH: One protocol with optimal result was based on HIER using Diva pH 6.2 (Biocare) in a pressure cooker, 30 min. incubation of the primary Ab and MACH4 (M4U534) as detection system.

mAb clone **GC33**, product no. **790-4564**, Ventana, BenchMark XT/Ultra: Protocols with optimal result were all based on HIER using Cell Conditioning 1 (efficient heating time 24-72 min.), 16-48 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) +/- amplification kit as detection systems. Using these protocol settings 16 of 17 (94%) laboratories produced a sufficient staining result.

mAb clone **CPC3-88**, product no. **AM539-5M**, BioGenex, Xmatrx Elite: One protocol with optimal result was based on HIER using EZ-AR3 Citrate based pH7 (BioGenex), 30 min. incubation of the primary Ab and Super Sensitive (QD400-60KE) as detection system.

Comments
In this first NordiQC assessment of GLP3, the prevalent features of an insufficient staining result were either characterized by a generally too weak staining reaction of the cells expected to be demonstrated or a poor signal-to-noise ratio.
Too weak staining results were seen in 55% of the insufficient results (6 of 11). Virtually all laboratories were able to demonstrate GLP3 in high-level expressing cells as the neoplastic cells of the hepatocellular carcinoma and the syncytiothrophoblastic cells of the placenta, whereas cells with reduced or low-level GLP3 expression as villous stromal cells and neoplastic cells of the clear cell carcinomas, in particular, tissue core no 7, could only be demonstrated using an optimal and carefully calibrated protocol. A too low concentration of the primary Ab was the main reason causing a too low sensitivity for the demonstration of GLP3.
Poor signal-to-noise ratio and/or false positive staining reaction were seen in the remaining 45% of the insufficient results. These patterns were typically characterized by a diffuse moderate background staining and/or diffuse cytoplasmic staining reaction in e.g. the normal liver cells and epithelial cells of the appendix. Too high concentration of the primary Ab or application of a (Ready-To-Use) antibody not validated for the IHC system typically caused a poor signal-to-noise ratio.
In a few cases weak to moderate aberrant nuclear staining reaction was seen in virtually all cells. This aberrant pattern was only observed for the mAb clone 1G12. Paradoxically, the aberrant nuclear staining pattern was seen in some protocols almost identical to optimal protocols. Due to the limited number of participants, it was not possible to investigate if the aberrant staining pattern for the mAb clone 1G12 was related to lot-to-lot variations.

The optimal staining pattern was as listed in the criteria above. In addition to this, a weak to moderate cytoplasmic staining reaction in dispersed basal epithelial cells, ganglion cells and axons of peripheral nerves of the appendix was seen. This was fully accepted provided that the staining pattern in other structures was as described above. GLP3 has been described to be expressed in nerves.
The mAb clone 1G12 was the most frequently used Ab for demonstration of GLP3. Used as a concentrate, this clone could be used to produce optimal staining results on all three main IHC systems used (see table 3). No special requirements concerning HIER buffer and detection system were needed to provide optimal staining results provided that careful calibration of the primary Ab titer was performed.
Corresponding RTU systems for the mAb clone 1G12 from Ventana and Biocare also provided optimal results. For the Ventana RTU system, optimal results were generated by laboratory modified protocol settings using a more sensitive detection system than recommended by the producer.

The relative newly introduced Ventana RTU system, based on the mAb clone GC33, provided a high proportion of sufficient and optimal results. Optimal results could be obtained both by the official recommendations and by modifications adjusting HIER time, incubation time of the primary Ab and/or detection system.

Controls
Placenta is recommended as positive tissue control for GLP3. Virtually all syncytiotrophoblasts and cytotrophoblasts must show a moderate to strong predominantly cytoplasmic but also membranous staining reaction. Dispersed villous stromal cells must show an at least weak cytoplasmic staining reaction. Appendix can be used as negative tissue control. The vast majority of epithelial cells must be negative. Dispersed basal epithelial cells can show a weak cytoplasmic staining reaction.

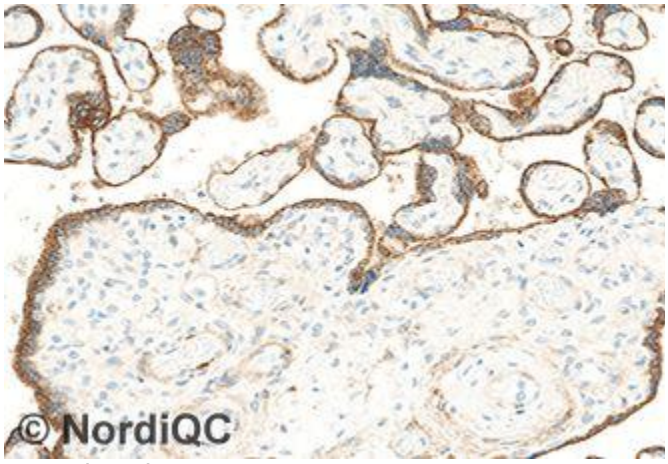


Fig. 1a (X200)
Optimal staining result for GLP3 of the placenta applying the mAb clone CG33, Ready-To-Use (Ventana) using HIER in an alkaline buffer (CC1 pH 8.5 Ventana) and a 3-step polymer based detection system (OptiView, Ventana). Virtually all syncytiotrophoblasts and cytotrophoblasts show a moderate to strong cytoplasmic staining reaction, while a weak but distinct staining reaction in dispersed villous stromal and endothelial cells is seen. No background staining is observed – also compare with Figs. 2a - 4a, same protocol.

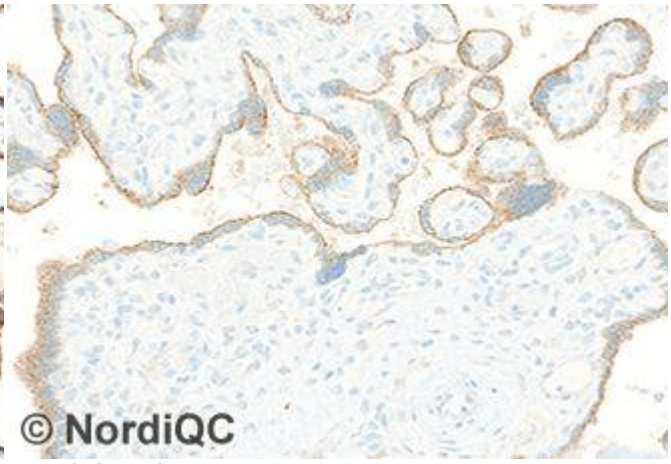


Fig. 1b (X200)
Staining result for GLP3 of the placenta using an insufficient protocol based on the mAb clone 1G12. The protocol provided a too low sensitivity most likely due to a too low titre of the primary Ab - same field as in Fig. 1a. The majority of syncytiotrophoblasts and cytotrophoblasts are demonstrated, but the intensity is significantly reduced and the villous stromal and endothelial cells are negative – also compare with Figs. 2b - 3b, same protocol.

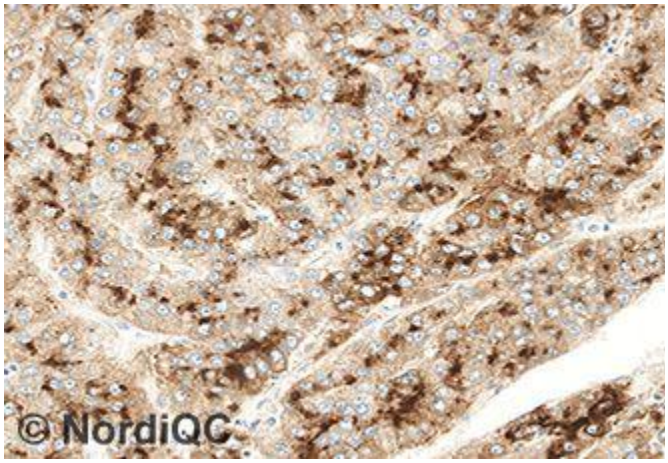


Fig. 2a (X200)
Optimal staining result for GLP3 of the hepatocellular carcinoma using same protocol as in Fig. 1a. The vast majority of the neoplastic cells show a moderate to strong predominantly cytoplasmic staining reaction.

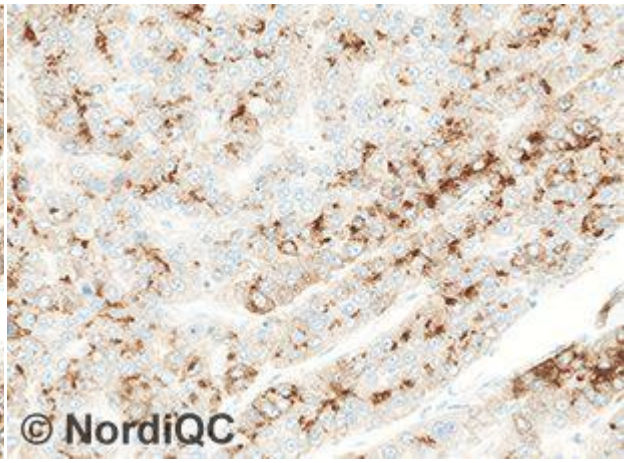


Fig. 2b (X200)
Staining result GLP3 of the hepatocellular carcinoma using same protocol as in Fig. 1b - same field as in Fig. 2a. The neoplastic cells are demonstrated but a reduced intensity is seen - also compare with Fig. 3b, same protocol.

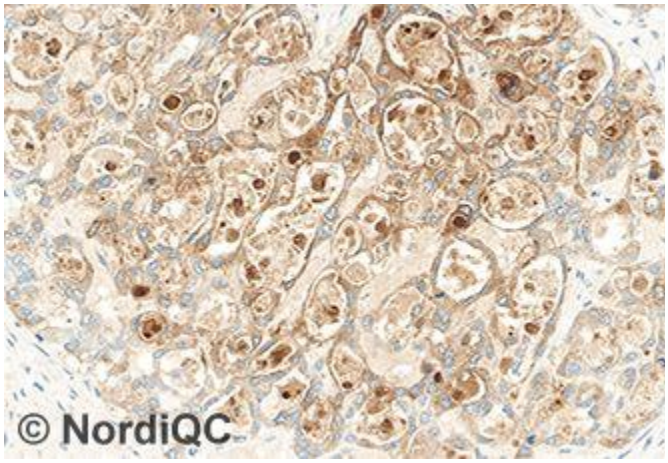


Fig. 3a (X200)
Optimal staining result for GLP3 of the ovarian clear cell carcinoma, tissue core no. 7 using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a weak to moderate cytoplasmic staining reaction.

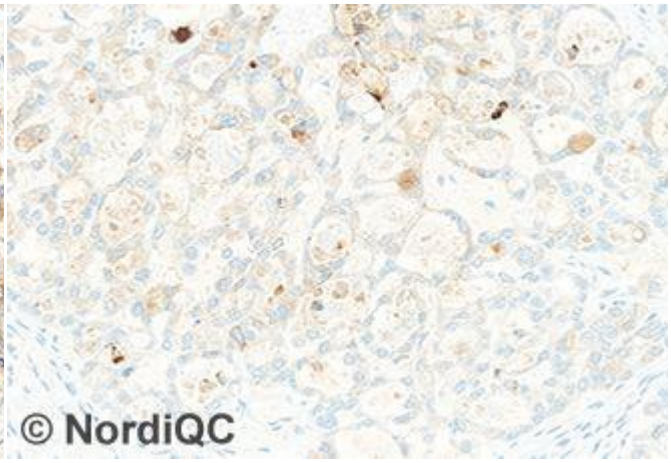


Fig. 3b (X200)
Insufficient staining result for GLP3 of the ovarian clear cell carcinoma, tissue core no. 7 using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. The proportion and staining intensity of the neoplastic cells is significantly reduced compared to the level expected and obtained in Fig. 3a.

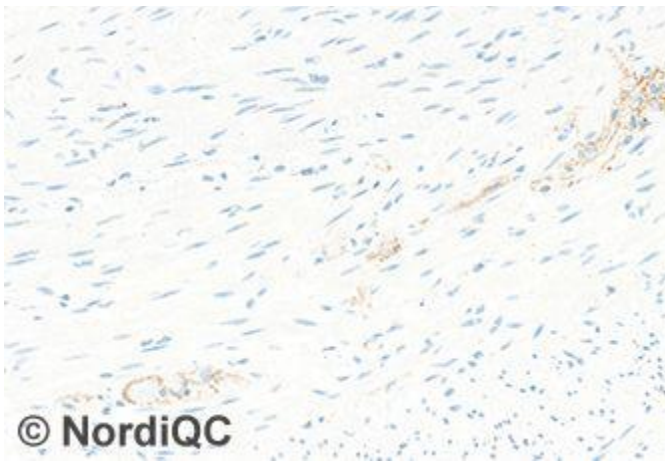


Fig.4a (X200)
Optimal staining result for GLP3 of the appendix using same protocol as in Figs. 1a - 3a. Ganglion cells and axons of the peripheral nerves show a weak to moderate staining reaction. No background staining is observed. Also see the result in Fig 4B.

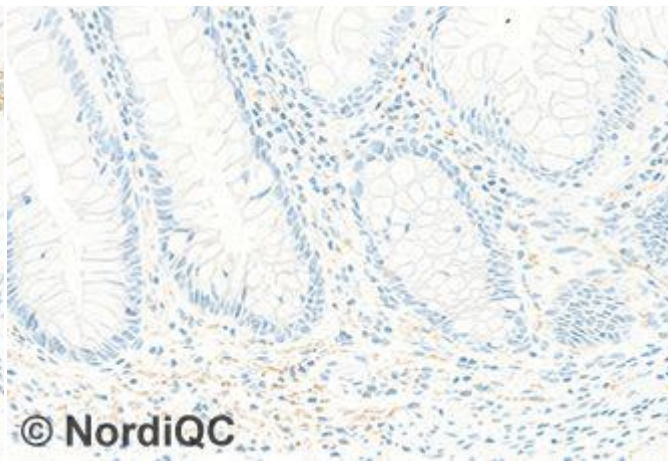


Fig. 4b (X200)
Optimal staining result for GLP3 of the appendix using same protocol as in Figs. 1a - 4a.
The peripheral nerves show a weak to moderate staining reaction, while no staining reaction of the epithelial cells are seen.

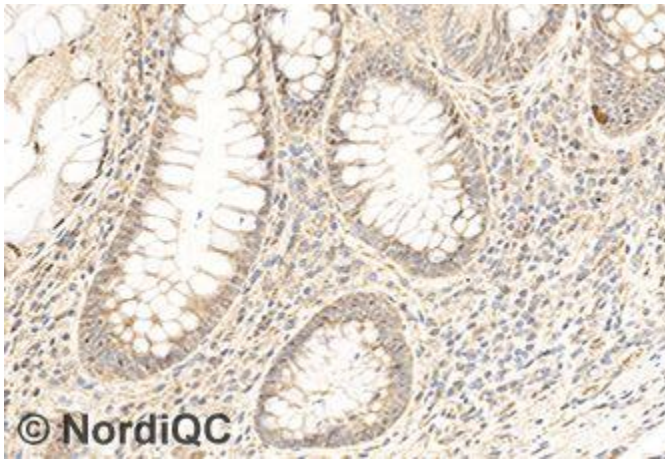


Fig.5a (X200)
Insufficient staining reaction for GLP3 of the appendix using the mAb clone 1G12 inadequately calibrated. An excessive background staining and aberrant cytoplasmic staining reaction of the epithelial cells is seen. This staining pattern most likely was due to a too high concentration of the primary Ab.

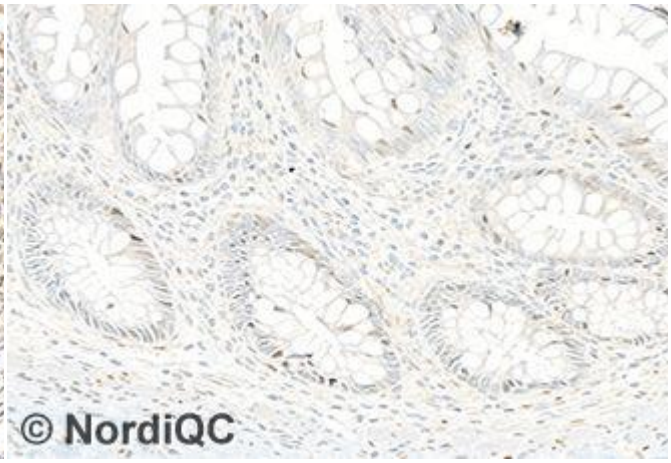


Fig.5b (X200)
Insufficient staining reaction for GLP3 of the appendix using th mAb clone 1G12.
An aberrant nuclear staining reaction of epithelial cells and stromal cells is seen. This staining pattern was only seen for the mAb clone 1G12. No single cause could be identified to provide this staining pattern but the combination of a high concentration of the primary Ab, efficient HIER and a highly sensitive detection system were typically used as protocol settings. However identical settings also provided optimal results.

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