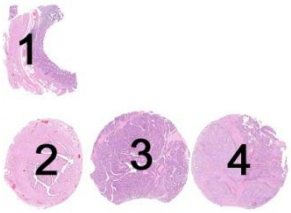
Assessment Run 29 2010

Cancer Antigen 125 (CA125)

The slide to be stained for CA125 comprised:
1. Appendix, 2. Salpinx, 3. Serous ovarian carcinoma grade II,
4. Serous ovarian carcinoma grade III
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CA125 staining as optimal included:

- A moderate to strong, predominantly membranous staining reaction in virtually all the epithelial cells of the salpinx.
- A moderate to strong distinct predominantly membranous staining reaction in the majority of the neoplastic cells in the two serous ovarian carcinomas.
- A weak to moderate staining of the follicular dendritic network in the germinal centres of the appendix.
- No staining reaction in the epithelial cells of the appendix.

112 laboratories participated in this assessment. 82 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CA125, run 29**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb[3)] clone **M11**	39 1 1	Dako Cisbio NeoMarkers	18	23	0	0	100 %	100 %
mAb clone **OV185:1**	26 2 1 1 1 1 1 1	Novocastra/Leica NeoMarkers BioGenex BioLogo Euro-Diagnostica Master Diagnostica Monosan Vector	2	21	10	1	68 %	83 %
mAb clone **OC125**	12 1 1 1	Dako Cisbio Immunologic Signet Lab	0	9	6	0	60 %	-
mAb clone **SPM111**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **OC125, 760-2610**	11	Ventana	0	8	3	0	73 %	-
mAb clone **OC125, PM101**	2	BioCare	0	2	0	0	-	-
mAb clone **OC125, 325M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **M11, IR701**	4	Dako	3	1	0	0	-	-
mAb clone **OV185:1, PA0539**	1	Novocastra/Leica	0	1	0	0	-	-
mAb clone **OV185:1, MS-1151-R7**	1	NeoMarkers	0	1	0	0	-	-
mAb clone **SPM111, ZM0019**	1	Unknown	0	1	0	0	-	-
Total	112		23	69	19	1	-	-
Proportion			20 %	62 %	17 %	1 %	82 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody.

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

Concentrated Abs
mAb clone **M11**: The protocols giving an optimal result were all based on HIER with either Tris-EDTA/EGTA pH 9 (2/7)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (5/7), Target Retrieval Solution pH 6.1 (S1699, Dako) (2/3), Cell Conditioning 1 (BenchMark, Ventana) (4/10), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/3) or Citrate pH 6 (3/8) as the retrieval buffer. The mAb was typically diluted in the range of 1:20–1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 39 (100 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **OV185:1**: The protocols giving an optimal result were both based on HIER with either Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/3) or EDTA/EGTA pH 8 (1/2) as the retrieval buffer. The mAb was diluted 1:5 - 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 6 (83 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **M11** (prod. no IR701, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings all of 3 laboratories produced an optimal staining.

The most frequent causes of insufficient staining were:

- Too low concentration of the primary Ab
- Omission of HIER
- Insufficient HIER - too short efficient HIER time and/or HIER in citrate pH 6.0
- Endogenous biotin

In this assessment and in concordance with the previous assessment (run 15, 2005) the prevalent feature of an insufficient staining was a generally too weak or completely false negative staining reaction of the cells expected to stain. This was observed in all the 20 stains, which in this run was assessed as insufficient. In 4 of the insufficient cases (20%) also a false positive staining due to endogenous biotin was observed. The weak or false negative staining was seen in both the epithelial cells of the salpinx and in the two serous ovarian carcinomas, whereas the false positive reaction of endogenous biotin was seen in the epithelial cells of the appendix.

The mAb clone M11, both as a concentrate and as a Ready-To-Use (RTU) format, gave a higher pass rate (100 %) and a higher proportion of optimal results than the other Abs (see Table 1), indicating that clone M11 is robust and applicable in all the IHC staining platforms and HIER settings used by the laboratories.

In comparison, the pass rate for the mAb clone OC125 with similar IHC staining platforms and HIER settings was markedly lower (69 %) and no optimal result obtained. However, mAb clone M11 from Dako and NeoMarkers is produced as an ascites format, which may cause a false positive MAG staining in tissues of blood type A (no data could be found on the Ab format from Cisbio).

The mAb clone OC125 from Dako, Cell Marque and Ventana is also produced as an ascites format (no data could be found on the Ab format from BioCare).
The mAb clone OV185:1 from e.g. Novocastra and NeoMarkers was the only Ab provided as a supernatant (thus without the potential MAG reaction) and giving an optimal staining result.

In accordance with the previous assessment of CA125, salpinx was found to be a reliable positive control: All laboratories obtaining a moderate to strong distinct membranous staining of virtually all the epithelial cells were assessed as sufficient. In the optimal stains also a weak to moderate staining reaction was seen in the follicular dendritic network of the germinal centres in the appendix.

This was the 2nd assessment of CA125 in NordiQC. The proportion of sufficient results increased from 67 % in run 15, 2005, to 82 % in the current run (table 2). The higher pass rate may in part be due to the increased use of the mAb clone M11 (6/48 = 13% in run 15, 41/112 = 37% in the current run).

Table 2. **Proportion of sufficient results for CA125 in the two NordiQC runs performed**

	Run 15 2005	Run 29 2010
Participants, n=	48	112
Sufficient results	67 %	82 %

Conclusion

The mAb clones M11 and OV185:1 are both recommendable Abs for CA125. However clone M11 is typically sold as ascites format, hence caution must be taken with tissue from patients with blood group A. HIER is mandatory for optimal performance. Normal salpinx is a recommendable positive control: Virtually all the epithelial cells must show a moderate to strong, distinct membranous staining.

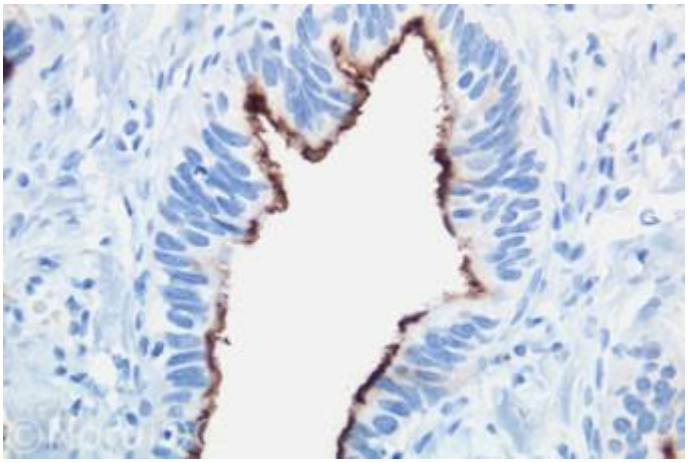


Fig. 1a
Optimal CD125 staining of the fallopian tube using the mAb clone M11 optimally calibrated and with HIER. Virtually all the epithelial cells show a moderate and distinct membranous staining and no background staining is seen.

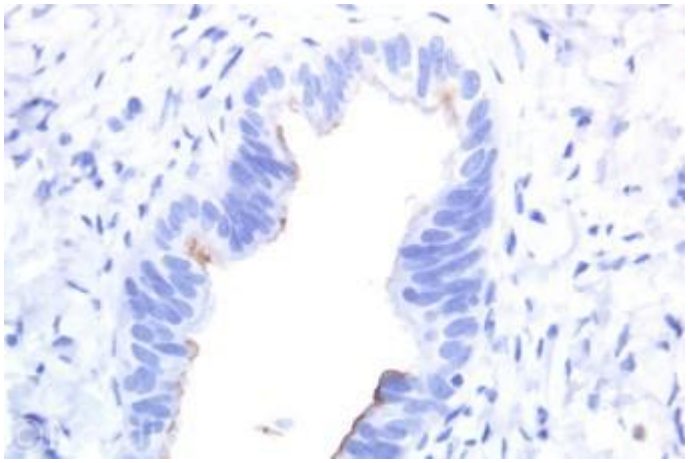


Fig. 1b
Insufficient CA125 staining of the fallopian tube using the mAb clone OV185:1 too diluted - same field as in Fig. 1a. Only scattered epithelial cells show a weak and equivocal membranous staining – also compare with Figs. 2b – 3b, same protocol.

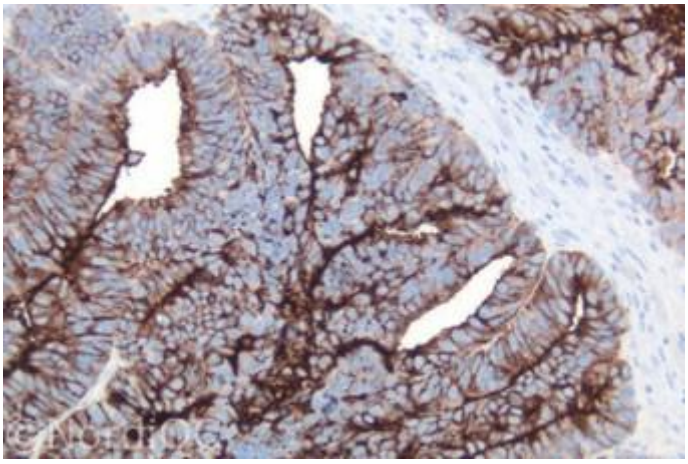


Fig. 2a
Optimal CA125 staining of the serous ovarian carcinoma grade II using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong and distinct staining and no background staining is seen.

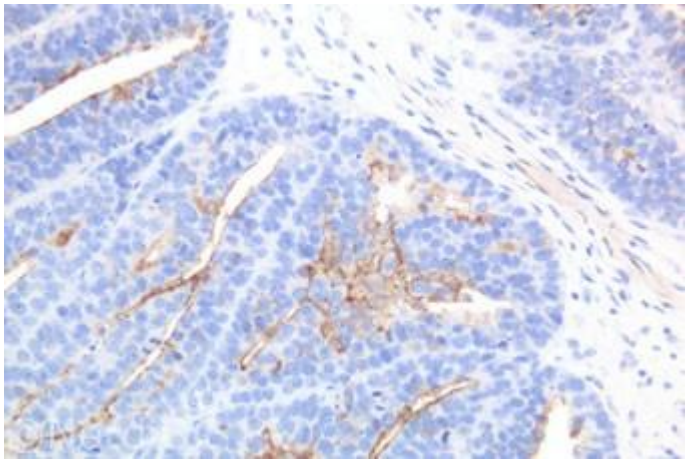


Fig. 2b
Insufficient CA125 staining of the serous ovarian carcinoma grade II using same protocol as in Fig. 1b - same field as in Fig 2a. Both the proportion and intensity of the positive cells is significantly reduced as compared to the result shown in Fig. 2a. Also compare with Fig. 3b, same protocol.

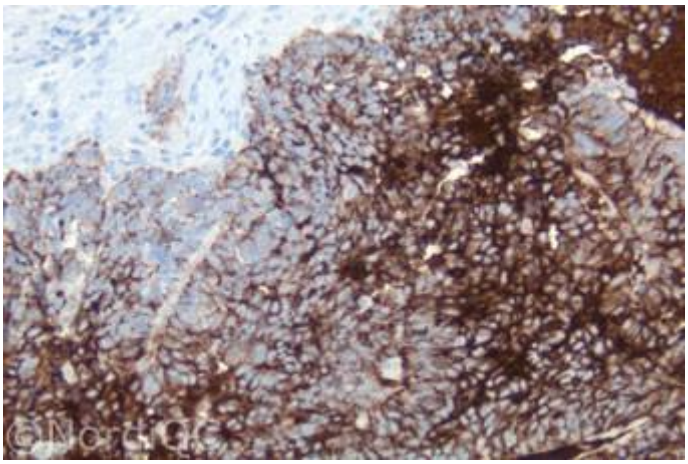


Fig. 3a
Optimal CA125 staining of the serous ovarian carcinoma grade III using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong and distinct staining and no background staining is seen.

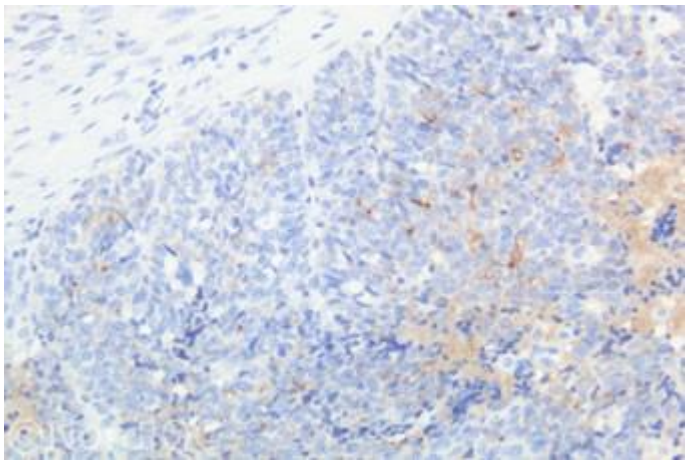


Fig. 3b
Insufficient CA125 staining of the serous ovarian carcinoma grade III using same protocol as in Fig. 1b - same field as in Fig 3a. Only scattered neoplastic cells show a weak dot-like staining.

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