The slide to be stained for CA125 comprised:
1. Fallopian tube, 2. Appendix, 3. Colon adenocarcinoma, 4. Mesothelioma, 5. Ovarian serous carcinoma gr. 2, 6. Ovarian serous carcinoma gr. 3. All specimens were fixed in 10 % NBF for 24-48 h.

Criteria for assessing a CA125 staining as optimal included:
- A moderate to strong, predominantly membranous staining in the epithelium of the fallopian tube.
- A moderate to strong distinct predominantly membranous staining in the majority of the neoplastic cells in the two ovarian serous carcinomas.
- At least a focal membranous staining in the mesothelioma.
- No staining of the appendix or the colon adenocarcinoma.

48 laboratories submitted stains. At the assessment 18 achieved optimal marks (38 %), 14 good (29 %), 13 borderline (27 %) and 3 (6 %) poor marks.

The following Abs were used:
- mAb clone OC125 (Dako, n=21; Ventana, n=4; Laborel, n=1)
- mAb clone Ov185:1 (Novoceastra, n=14)
- mAb clone M11 (Dako, n=6; HistoCIS, n=1)
- mAb clone TA347 (Zymed, n=1)

Optimal staining for CA125 in this assessment was obtained with following mAbs: clone Ov185:1 (8 out of 14), clone M11 (6 out of 7) and clone OC125 (4 out of 26).

All optimal protocols irrespective of the clone used were based on Heat Induced Epitope Retrieval (HIER).

With clone Ov185:1 the following HIER buffers were used in the optimal protocols: Tris-EDTA/EGTA pH 9 (5 out of 8 were optimal), EDTA/EGTA pH 8 (1 out of 1 was optimal), Citrate pH 6 (1 out of 3 was optimal) and Target Retrieval Solution S1699 (Dako, 1 out of 1). In the optimal protocols Ov185:1 was typically used in the range of 1:30 – 1:200 depending on the sensitivity of the protocol applied.

With clone M11, the following HIER buffers were used in the optimal protocols: Citrate pH 6 (3 out of 3 were optimal) Tris-EDTA/EGTA pH 9 (2 out of 3 were optimal) and EDTA pH 9 (1 out of 1 was optimal). In the optimal protocols M11 was typically used in the range of 1:40 – 1:100 depending on the sensitivity of the protocol applied. Alternatively a ready-to-use (RTU) Ab was used.

With clone OC125 the following HIER buffers were used in the optimal protocols: Tris-EDTA/EGTA pH 9 (3 out of 10 were optimal) and Target Retrieval Solution S1699 (Dako, 1 out of 1 was optimal). In the optimal protocols OC125 was typically used in the range of 1:50 – 1:100 depending on the sensitivity of the protocol applied. With clone OC125 other HIER buffers such as CC1 (n=6) or citrate pH 6 (n=5) could not be used to obtain an optimal result in this assessment. Using CC1 all 6 laboratories had an insufficient staining. However, CC1 was frequently combined with OC125 as an RTU Ab (n=3). 3 out of 5 laboratories using citrate pH 6 had a good (but not optimal) staining while 2 had an insufficient staining, of which 1 used OC125 as an RTU Ab.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab
- Using clone OC125 as a RTU Ab (3 out of 4 were insufficient)
- Less successful primary Ab
- Inappropriate epitope retrieval (HIER in CC1 or citrate pH 6)

The prevalent feature of an insufficient staining was a too weak staining of the epithelium in the fallopian tube and a false negative staining of the mesothelioma. In general the majority of the laboratories were able to detect
CA125 in both ovarian serous adenocarcinomas. In the optimal protocols the membranes of both the normal epithelial cells of the fallopian tube and the membranes of the neoplastic cells in the mesothelioma distinctively were demonstrated. In the insufficient staining the epithelium of the fallopian tube was weakly labelled and the mesothelioma was negative. In general the mAb clone M11 labelled a higher proportion of both normal mesothelial cells and the neoplastic mesothelial cells compared to the clones Ov185:1 and OC125.

**Conclusion**

MAb clone **M11** (Dako, HistoCIS) seems to be the most sensitive marker for CA125. HIER seems to be the most appropriate pre-treatment for the clones **Ov185:1, M11** and **OC125**. The epithelium in the fallopian tube is a reliable positive control. The staining reaction should be strong, membranous.

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**Fig. 1a**  
Optimal staining for CA125 of the fallopian tube. The epithelial cells show a distinct membranous staining.

**Fig. 1b**  
Insufficient staining for CA125 of the fallopian tube. The epithelial cells only show a focal and weak staining.

**Fig. 2a**  
Optimal staining for CA125 of the ovarian serous carcinoma gr. 3. The neoplastic cells are strongly stained.

**Fig. 2b**  
Insufficient staining for CA125 of the ovarian serous carcinoma gr. 3. The neoplastic cells are weakly stained or unstained. Same field as Fig. 2a.
Fig. 3a
Optimal staining for CA125 of the colon adenocarcinoma. All cells are negative.

Fig. 3b
Staining for CA125 of the colon adenocarcinoma using an insufficient protocol. The neoplastic cells show a distinct staining probably due to endogenous biotin. Same field as in Fig 3a.

Fig. 4a
Optimal staining for CA125 of the mesothelioma using the mAb clone M11. Both the normal mesothelium and the neoplastic mesothelial cells show a distinct membranous staining.

Fig. 4b
Optimal staining for CA125 of the mesothelioma using the mAb clone OC125. Both the normal mesothelium and the neoplastic mesothelial cells show a distinct membranous staining. However fewer cells are demonstrated compared to the result obtained in Fig. 4a.

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