The slide to be stained for mammaglobin comprised:
All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a mammaglobin staining as optimal included:

- A strong, distinct cytoplasmic reaction in scattered ductal epithelial cells and in the 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 neoplastic cells of the breast carcinoma no. 4.
- At least a weak to moderate cytoplasmic and focally a dot-like reaction in the majority of the neoplastic cells the breast carcinoma no. 5.
- No more than a weak background reaction in the vicinity of the positive cells (antigen diffusion).

23 laboratories participated in the assessment. 83 % achieved a sufficient mark. The results are summarized in Table 1.

### Table 1. Abs and scores for mammaglobin, run 25

<table>
<thead>
<tr>
<th>Concentrated Abs</th>
<th>N</th>
<th>Vendor</th>
<th>Optimal</th>
<th>Good</th>
<th>Borderl.</th>
<th>Poor</th>
<th>Suff.¹</th>
<th>Suff. OPS²</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb clone 304-1A5</td>
<td>14</td>
<td>Dako</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>83 %</td>
<td>100 %</td>
</tr>
<tr>
<td>3625</td>
<td>1</td>
<td>AnaSpec. Inc.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ready-To-Use Abs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mAb clone 304-1A5</td>
<td>2</td>
<td>Dako, IR074</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mAb clone 31A5</td>
<td>2</td>
<td>Ventana, 760-4623</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proportion</td>
<td></td>
<td></td>
<td>48 %</td>
<td>35 %</td>
<td>9 %</td>
<td>9 %</td>
<td>83 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

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 304-1A5: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (5/9)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (1/1) or EDTA/EGTA pH 8 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 out of 11 (100 %) laboratories produced a sufficient staining (optimal or good).

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

**Ready-To-Use Abs**

mAb clone 304-1A5, IR074, Dako: The protocols giving an optimal result were all based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) and an incubation time for 20 min in the primary Ab and EnVision Flex as the detection system. Using these protocol settings 2 out of 2 (100 %) laboratories produced an optimal staining.

rmAb clone 31A5, 760-4623, Ventana: The protocols giving an optimal result were based on HIER using Cell Conditioning 1, mild or standard and the incubation time for the primary Ab was 32 min. UltraView was used as detection system. Using these protocol settings 2 out of 2 (100 %) laboratories produced an optimal staining.
The frequent causes of insufficient staining were:
- Too low or too high concentration of the primary Ab
- Less successful primary Ab

In this assessment the prevalent feature of an insufficient staining was either a generally too weak staining or a false positive staining. The former was in particular observed in the breast carcinoma no. 5, while the two other breast carcinomas were demonstrated by virtually all participants. Also the number of positive cells in the normal breast was significantly reduced compared to the reaction in a sufficient staining. The false positive staining was characterized as both a nuclear staining in the breast carcinomas and as a cytoplasmic staining in many cell types as smooth muscle cells, fibroblasts etc.

Skin was found to be an appropriate control in which the epithelial cells of the eccrine sweat glands should show an as strong as possible cytoplasmic reaction, while other cells as e.g. smooth muscle cells, squamous epithelial 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 clone 304-1A5 and the rmAb clone 31A5 are both useful markers for mammaglobin. 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 mammaglobin using the mAb clone 304-1A5 optimally calibrated and with HIER.
Left: Skin: The majority of the epithelial cells of the eccrine sweat glands show a distinct cytoplasmic reaction.
Right: Breast: The apocrine metaplastic cells and few ductal epithelial cells show a distinct cytoplasmic reaction. Also compare with Figs. 2a & 3a – same protocol.

Fig. 1b
Insufficient staining for mammaglobin using the mAb clone 304-1A5 too diluted.
Left: Skin: Only scattered epithelial cells of the eccrine sweat glands show a weak cytoplasmic reaction.
Right: Breast: The epithelial cells are virtually negative and only extracellular mucus is demonstrated. Also compare with Figs. 2b & 3b – same protocol.
Fig. 2a
Optimal staining for mammaglobin of the breast ductal carcinoma no. 4 using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong and distinct cytoplasmic reaction. Also note a weak nuclear reaction is seen in some stromal cells – this was seen in the majority of the results and is probably due to absorption of antigen diffusion from the tumour cells.

Fig. 2b
Insufficient staining mammaglobin of the breast ductal carcinoma no. 4 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 mammaglobin 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.

Fig. 3b
Insufficient staining for mammaglobin of the breast carcinoma no. 5 using same protocol as in Fig. 1b & 2b. None or only a dubious reaction is seen in the neoplastic cells.

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