Aim
To evaluate the clinical performance of three commercially available ELISA kits for determination of Chromogranin A.

Background
Chromogranin A (CgA) is a member of the granin family of neuroendocrine secretory proteins, i.e., it is located in secretory vesicles of neurons and endocrine cells. CgA is the most important serological marker for neuroendocrine tumors (NETs) and has been known as such for several decades. Lately CgA has been proven useful also for treatment monitoring and assessing disease progression. European and American guidelines recommend CgA as the general diagnostic marker for diagnosis as well as for prognosis and follow up of NETs.

Materials and Methods
A panel with 75 heparin plasma samples were tested for levels of CgA. 43 samples were from patients diagnosed with various NETs and 32 from blood donors. The samples were analyzed using the NEOLISA™ CgA (Euro Diagnostica, Sweden), Chromogranin A (Dako, Denmark) and Chromo™ (Cisbio, France) according to each manufacturer’s instructions for use.

The detection antibodies vary between the assays as those from Euro Diagnostica and Cisbio captures the CgA protein between two monoclonal antibodies, whereas the Dako assay uses two polyclonal antibodies. All three assays are sandwich ELISAs and all antibodies are unique to the assays.

The clinical performance of the assays was determined in terms of sensitivity, specificity and positive and negative predictive values (PPV and NPV, respectively) at the manufacturer’s recommended reference interval (cut-off). In order to generate data for direct comparison of the assays, ROC curve analyses as well as data at stratified specficity were calculated.

Results

Sensitivity and Specificity at supplier cut-off

<table>
<thead>
<tr>
<th>Test</th>
<th>Area</th>
<th>95% CI</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED</td>
<td>0.94</td>
<td>0.89 to 1.00</td>
<td>0.027</td>
</tr>
<tr>
<td>Dako</td>
<td>0.96</td>
<td>0.93 to 1.00</td>
<td>0.017</td>
</tr>
<tr>
<td>Cisbio</td>
<td>0.89</td>
<td>0.81 to 0.97</td>
<td>0.039</td>
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Table 1. Sensitivity and specificity calculated at the manufacturer’s reference intervals (cut-off) show that the Dako assay had the highest sensitivity, while the Euro Diagnostica (ED) assay had the highest specificity.

Sensitivity at stratified specificity (97%) (Fig. 2)

<table>
<thead>
<tr>
<th>Test</th>
<th>Area</th>
<th>95% CI</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED</td>
<td>0.90</td>
<td>0.82 to 0.98</td>
<td>0.058</td>
</tr>
<tr>
<td>Dako</td>
<td>0.88</td>
<td>0.79 to 0.96</td>
<td>0.065</td>
</tr>
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Table 2. When sensitivity was calculated at stratified specificity (97%) the Euro Diagnostica (ED) and Dako assays both showed 77% sensitivity which was slightly superior to the Cisbio assay with 63% sensitivity.

Discussion & Conclusions

• In our study, the assays from Euro Diagnostica and Dako tended to have somewhat higher clinical performance than the Cisbio assay, in terms of discrimination of NETs samples from blood donors.

• At stratified specificity (97%) the sensitivity, PPV and NPV results were equal for the Euro Diagnostica and Dako CgA kits.

• CgA levels, as measured with the different ELISAs, correlate well with each other, even though the detection antibodies are of different origin.

References

1. Ramage K et al, Guidelines for the management of gastrointestinal neuroendocrine (including carcinoid) tumours (NETs). Gut 2012;61:3-32
6. false positive rate (1-Sensitivity)
7. Figure 1. ROC analyses ROC plots comparing samples from blood donors and NET patients showed area under curve (AUC) to be slightly higher for Euro Diagnostica (ED) (0.94) and Dako (0.96) as compared to Cisbio (0.89).
8. Figure 2. Correlations between all three CgA ELISAs were very good. Dako vs. Euro Diagnostica R=0.980 and Cisbio vs. Euro Diagnostica R=0.9043. Cisbio vs. Dako R=0.937. One extremely high sample was excluded from the calculations.