# Microarray immunoassay development to specifically detect autoantibodies in small intestine neuroendocrine tumor patients


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## Conclusion

**Our findings by using the novel ImmunoCAP ISAC multiplexing platform support the notion that autoantibodies might be used as circulating biomarkers to be used in SI-NET diagnostics**

## Aim

To investigate protein specific autoantibodies as potential circulating clinical serum biomarkers to be used in diagnostics

## Methods

The ImmunoCAP ISAC platform is a multiplexing technology (Phadia-AB, Uppsala, Sweden) that uses blood to diagnose allergy. We extended its capacity to develop a novel diagnostic assay to detect autoantibodies towards SI-NET at different stage of disease. We used clinical patient serum and fluorescently-labeled secondary antibodies, specific for different human immunoglobulin isotypes.

The novel method is briefly explained in Figure 1 and 2.

**SI-NET associated antigens for the auto-antibody detection assay**

| Antigen | Antigen |
|---|---|
| Neuron-specific enolase (ENO2) | CTGF |
| Tph-1 | Tachykinin 1 (TAC1) |
| IGF1 | VMAT1 |
| Notch1 | IA-2 |
| Notch2 | CDX-2 |
| Notch3 | Survivin (BIRC5) |
| NF KappaB1 (NFKB1) | CXCR4 |
| C-reactive protein (CRP) | IGF-1R |
| Chromogranin A (CHGA) | HER-2 (ErbB2, neu) |
| Chromogranin B (CHGB) | ATP8B1 |
| Chromogranin C (CHGC) | MAML3 |
| PNMA2 | GHS-R |
| CXCL14 | IL-1a |

**ISAC-chip**


**Figure 1 SI-NET associated antigens and the chip, which was used in the analysis.**

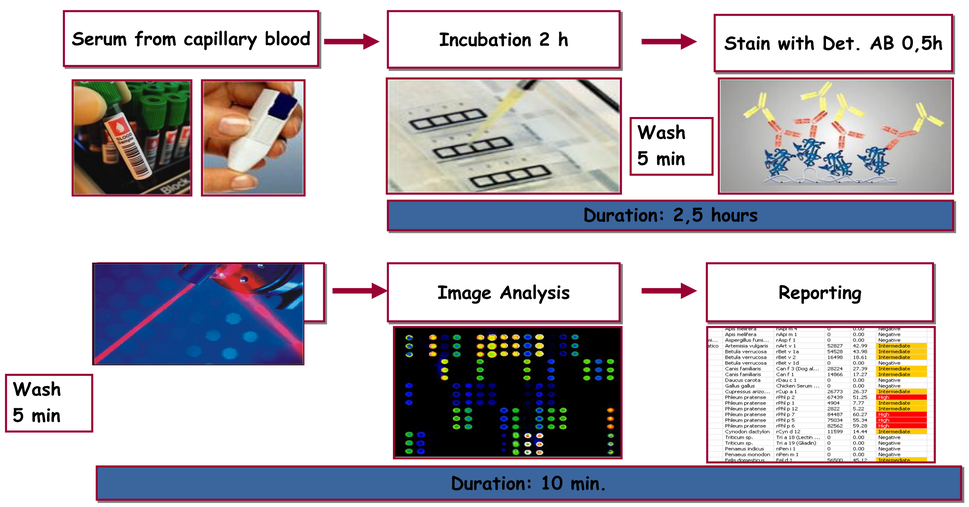


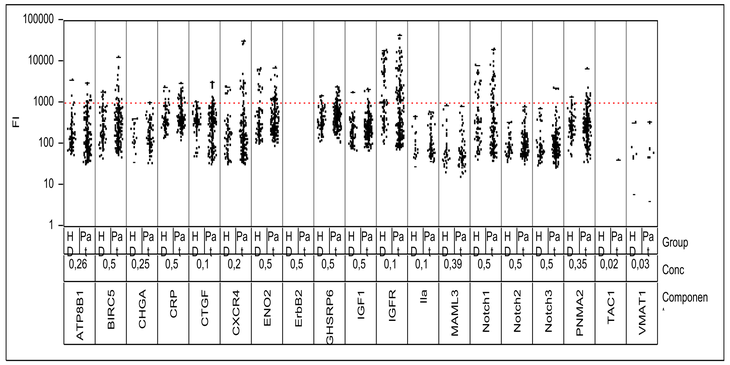

**Figure 2 Summary of experimental laboratory work**

## Background

Small intestine neuroendocrine tumor and carcinoma (SI-NET and SI-NEC) are rare malignancies. However, most patients develop metastases before diagnosis and there is no cure for this disease stage. The unmet identification of primary SI-NET require novel specific biomarkers.

## Results

ImmunoCAP ISAC is as a suitable platform to detect SI-NEC patients' autoantibody. The capacity to measure differences by a randomized and blinded study of 120 samples (30 healthy donors and 90 SI-NET patients, at different stage of disease) is relevant. We observed specific detection ability of IgG1 and IgG4 for several tumor associated antigens that we spotted on our novel chips. Moreover, some of these observations were unique for the SI-NET patient group. The most relevant results are shown in Figure 3 and 4



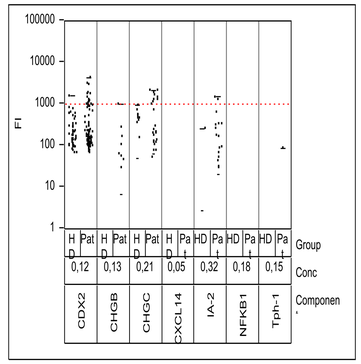




**Figure 3 Assay evaluation results displaying IgG1 specific uptake of protein components.** Fluorescent intensity (FI) for the set of tumor antigen components (logarithmic scale). Spotted protein concentrations in mg/ml are indicated. Sample groups are 30 healthy donors (HD) and 90 SI-NET patients (Pat). The red dotted line represents a desirable signal intensity at FI > 1000.

| Protein | IgG1 | IgG4 |
|---|---|---|
| ENO2 | | X |
| Tph-1 | X | X |
| IGF1 | | X |
| Notch1 | | |
| Notch2 | | |
| Notch3 | | |
| NFKB1 | X | - |
| CRP | | X |
| CHGA | | X |
| CHGB | X | X |
| CHGC | | X |
| PNMA2 | | X |
| CXCL14 | | X |
| CTGF | | - |
| TAC1 | X | - |
| VMAT1 | | - |
| IA-2 | | - |
| CDX-2 | | X |
| BIRC5 | | X |
| CXCR4 | | |
| IGF-1R | | |
| ErbB2 | - | - |
| ATP8B1 | | - |
| MAML3 | | - |
| GHS-R | | X |
| IL-1a | | |

**Figure 4 Results from the novel SI-NET autoantibody detection assay.** Unique antibody presence within the SI-NET patient group towards protein components is indicated by X. The dash represents cases were no signal was observed the 2 groups: healthy donors (HD) and Patients (Pat). The ratio represents: FI mean (Pat) / FI mean (HD).