B-036

Systematic Evaluation of Antibody-Antigen Interactions for Suitability in Immunoassays Using Parallel Microarray ELISA

M. Coolen, K. M. van Ham, T. J. Schulte, G. Schilders. *Future Diagnostics, Wijchen, Netherlands*

micro-SCY

SCAN ME

Background

Each immunoassay has unique specifications that are largely affected by the used capture & detection molecules / antibodies:

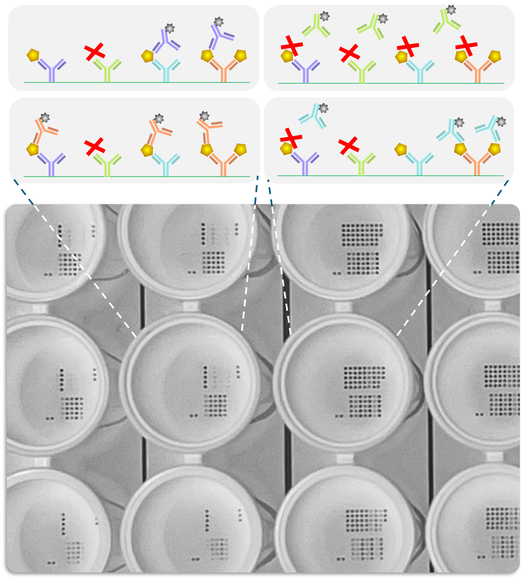
- specificity
- sensitivity
- dynamic range
- robustness
- speed of interaction
- strength of interaction
- matrix compatibility
- …

Direct testing of all capture & detection molecule combinations and parameters in the final assay configuration is often not possible: time-, material- & budget-constraints. Theoretical $K_{on}/K_{off}/K_D$ determinations are costly and have only limited translatability to the end format.

Needed:

Method to systematically compare capture & detection molecule options for their desired specifications and under realistic assay conditions

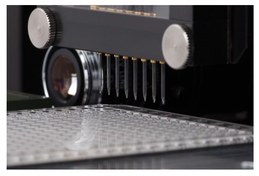
Approach

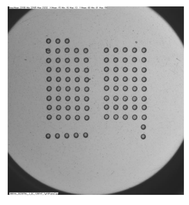


Parallel microarray ELISA

Method

nano-liter dispensing





microarray ELISA inside each well

> 121 spots/well

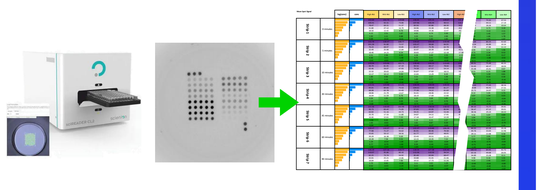
> 11,000 data points per plate

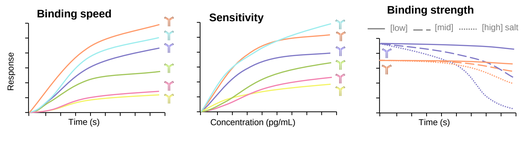
ELISA-based toolbox to address required specifications

- Time courses
- Serial dilutions
- Interferent spikes
- Buffer challenges
- …

Parallel microarray ELISA

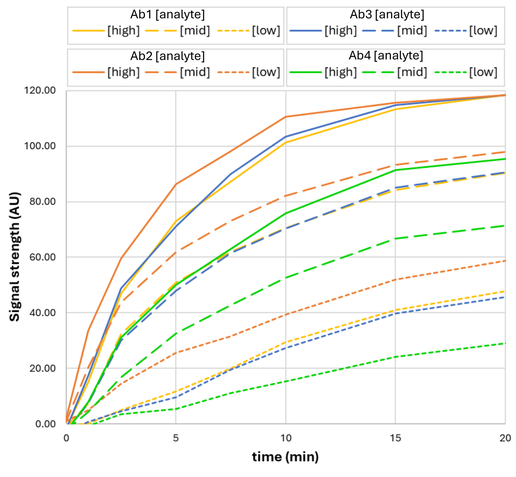
Automated data interpretation



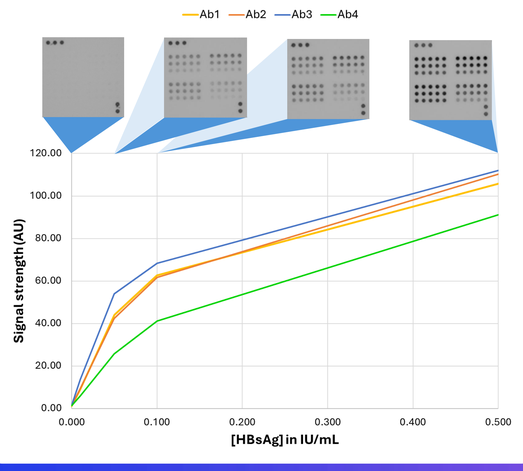


Results: Kinetics & Sensitivity

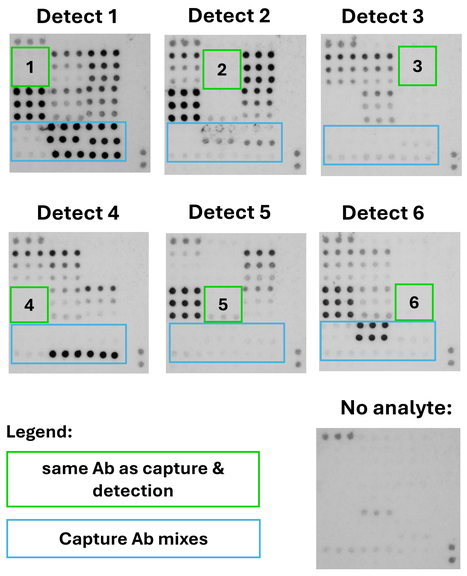
HBsAg: Capture Ab binding kinetics



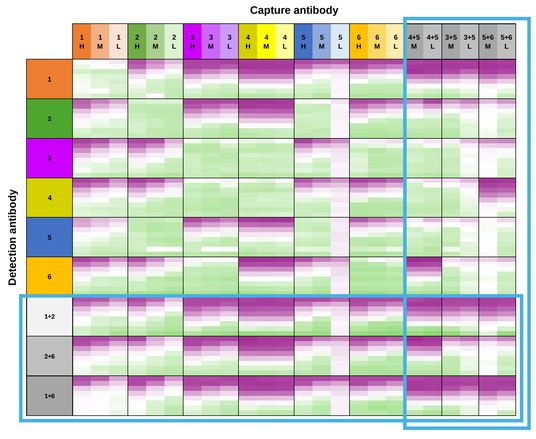
HBsAg: Capture Ab sensitivity



Results: Antibody pairs



Capture Abs each spotted in a 3x3 grid at 3 concentrations. Different detection Abs per well. Green numbered boxes indicate same Ab as capture & detection. Blue box shows capture Ab mixes.

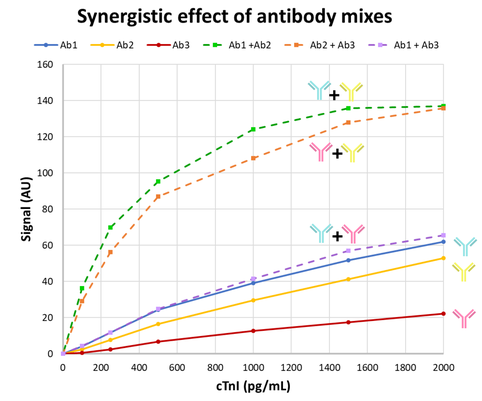


Heatmap of signal intensities from all options from analyte dilutions. High = purple; low = green. Blue boxes indicate antibody mixes.

Other observations:

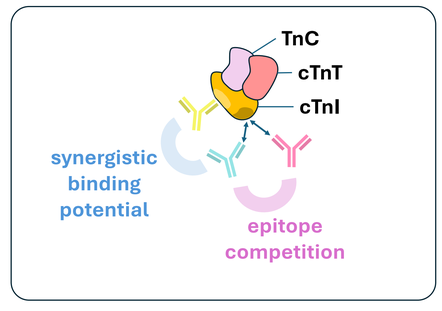
- Marked performance differences in different sample matrices
- Sample cohort screen to identify Ab pair with best clinical performance (unique epitope/conformation/modifications)
- Sensitivity down to 0.01 IU/mL (~40 pg/mL)
- Serotype reactivity confirmation (Adw/Ayw)

Results: Synergistic capture



Strong synergistic effect observed when specific monoclonal antibodies were combined in the capture mix, resulting in a ~6-fold sensitivity enhancement compared to their separate performances.

Elevated binding potential and strength for antibodies with non-overlapping epitopes:



Note: Synergistic performance also observable at detection site of assay

Conclusions

- **Parallel microarray ELISA evaluation of antibody-antigen interactions & antibody pairs is a robust and reliable method for identifying the best antibodies for an immunoassay.**
- **It allows for a systematic and quantitative screen of dozens of antibody options within 1-2 weeks under real immunoassay conditions using nanoliter spot volumes.**
- **This method has shown to be highly translatable and is customizable to accommodate diverse assay specifications.**

ISO 13485:2016

provincie Gelderland The Netherlands

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