Arrays In Wells: Multiplexing a Celiac Panel

Introduction

We developed a multiplexed, colorimetric immunoassay for Celiac Disease showing proof-of-principle for multiplexed immunoassays. Multiplexing, i.e. measuring several analytes simultaneously in one sample is a promising technique for making diagnostic testing faster and more affordable and saving valuable sample and is especially well-suited for the diagnosis of auto-immune diseases.

Materials & Methods

We used an array-in-well approach to simultaneously measure Gliadin and tissue transglutaminase (tTG) in each well of a standard 96-well plate. An antigen-down assay format was chosen where the antigens were spotted on the bottom of each well in triplicate. We assayed the arrays with serum from Celiac patients and normal human serum (NHS). An HRP-labeled anti-human antibody and a TMP substrate were used to detect auto-antibodies in patient serum. For read-out we used the Sensovation CLAIR$_{reflex}$ Colorimetric Array Imaging Reader. This instrument is able to read colorimetric microarrays on non-transparent surfaces like nitrocellulose or PVDF.

Results

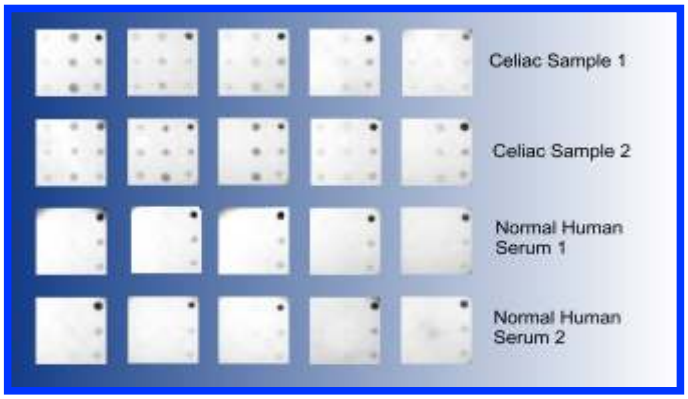

Fig.1 Multiplexed Assay in Wells
Rows 1 and 2 show the 3 x 3 microarrays assayed with serum from Celiac patients. Rows 3 and 4 show the same array assayed with NHS. Each array contains tTG (column 1) and Gliadin (column 2) in triplicate, as well as a positive, negative, and low positive control (column 3).

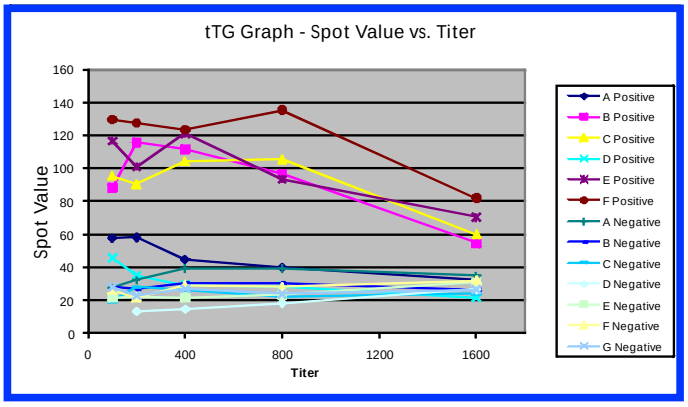

Fig.2 tTG Data
Spot values (shown for tTG) plotted against serial dilutions of Celiac patient and NHS. All Celiac sera show positive spots whereas NHS show very low values.
A titer of 1:800 was sufficient to distinguish between sera of Celiac patients and NHS. Additional experiments with other autoimmune markers showed no cross-reactivity.

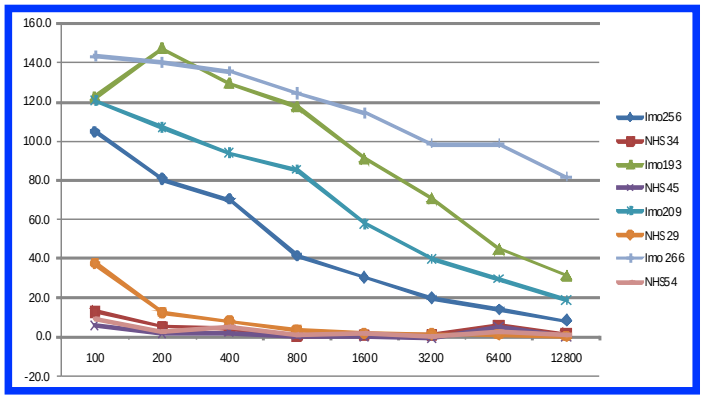

Fig.3 Gliadin Data
Gliadin spot values obtained with the CLAIR reader are plotted against serial dilutions of patient serum and NHS. Celiac samples are clearly distinguishable from NHS at a 1: 12,800 serum dilution.

Discussion

We successfully developed a multiplexed microarray for Celiac Disease showing proof-of-principle for multiplexed, antigen-down colorimetric assays.

- Sensitivity for the multiplexed assays is very good
- No cross-reaction with other autoimmune markers exists proving that the assay is specific
- The results were reproducible
- Read-out with the Sensovation CLAIR instrument is quick and easy.

Summary

We showed proof-of-principle for a multiplexed antigen-down assays for Celiac Disease establishing multiplexing as a promising way to diagnose autoimmune diseases while saving time, reagents and sample.

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CLAIR / FLAIR Array Imaging Reader Family

Sensovation provides a complete family of instruments for routine microarray analysis: FLAIR, CLAIR and CLAIRreflex. All instruments share the same design concept and the same basic hardware and software platform. The main difference between the different instruments is the detection method.

FLAIR - the fluorescent array imaging reader - uses fluorescence technology for sensitive and highly linear detection.

CLAIR - the colorimetric array imaging reader – is made for the analysis of colorimetric microarrays. It is the ideal instrument for cost-sensitive assays based on TMB or BCIP/NBT.

CLAIRreflex - is a variation of CLAIR, designed for the measurement of microarrays on non-transparent surfaces, for example for microarrays on nitrocellulose with BCIP/NBT or TMB as dyes.

The Power of Multiplexing

Your established assay ...

- ELISA
  - Autoimmune
  - Allergy
  - Infectious Disease
  - Respiratory Viruses
  - Borreliosis
- Genotyping
  - Infectious Disease
  - HPV

... with a twist: a whole array in each well

FLAIR / CLAIR Instrument Features

- **Compact and robust**
  With a footprint as small as a simple microplate photometer FLAIR and CLAIR offer full microarray scanner functionality.
- **Fully integrated system**
  FLAIR and CLAIR come with built-in processing power, an integrated touch screen, and an intuitive instrument control- and array-analysis software package.
- **Flexible analysis with immediate results**
  FLAIR and CLAIR enable to user to read and analyze all 96 microarrays in a SBS microplate in less than 3 minutes.
- **Affordable**
  FLAIR and CLAIR are available at a price point well below the price of conventional microarray scanners. As a fully integrated instrument it comes with everything needed for microarray detection and -analysis.
- **Designed for Routine Applications**
  The integrated instrument concept make FLAIR and CLAIR the ideal instrument for routine diagnostics applications, biochip analysis in clinical research, as well as biochemical analysis.
- **Unrivaled applications**
  FLAIR can readily be used for microarray analysis on slides or any other biochip format, not exceeding the dimensions of a 96-well SBS plate.
- **Automation-friendly**

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