

Arrays In Wells: Multiplexing a Respiratory Virus Panel

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Introduction

The trend towards personalized medicine requires better, more, and more frequent diagnostic testing. Multiplexed analysis, i.e. measuring many analytes simultaneously in one sample is a promising technique for making diagnostic testing faster and more affordable. Here we show the results of a multiplexed respiratory virus assay. A panel of 88 clinical samples was analyzed using an 8 x 8 multiplexed array in each well of a 96-well plate.

Materials & Methods

We used an array-in-well approach to simultaneously measure seven different respiratory viruses in each well of a standard 96-well plate. A sandwich assay format with an HRP-labeled primary detection antibody was chosen. Read-out of the in-well-array was performed with the Sensovation CLAIR instrument.

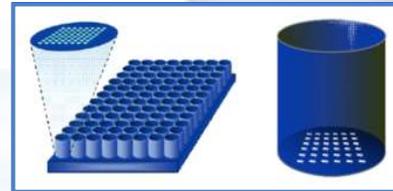


Fig.1 Array in Well Technology: Microarrays are spotted on the bottom of the wells of a 96-well plate = Multiplexed ELISA Assay in Wells.



Fig.2 CLAIR, Sensovation's colorimetric Array Imaging Reader for measurement of Microarrays in 96-Well Plates.

Results

We assayed the multiplexed respiratory virus array with panel of 88 clinical samples and used CLAIR for colorimetric read-out and analysis. We had previously analyzed the sample panel with time-resolved fluoroimmunoassays (TR-FIA) and found 44 samples to be antigen-negative and 44 antigen-positive.

Each capture antibody was spotted 6 times (3 high-density, 3 low-density spots) to increase the dynamic range of the assay. The array layout is shown in Fig. 3, selected array images for all 7 respiratory viruses in Fig. 4.

Fig. 3 Array-In-Well Layout of the antigens in each well of a 96-well plate. Viruses spotted: Adenovirus (Adeno), Influenza A and B (Inf A, Inf B), Respiratory Syncytial Virus (RSV), Parainfluenza 1, 2, 3 (PIV 1, PIV 2, PIV 3).

Anti-mouse IgG	Adeno high	Adeno high	Adeno high	Adeno low	Adeno low	Adeno low	Anti-mouse IgG
BLANK	RSV high	RSV high	RSV high	RSV low	RSV low	RSV low	BLANK
BLANK	InfA high	InfA high	InfA high	InfA low	InfA low	InfA low	BLANK
BLANK	InfB high	InfB high	InfB high	InfB low	InfB low	InfB low	BLANK
BLANK	PIV1 high	PIV1 high	PIV1 high	PIV1 low	PIV1 low	PIV1 low	BLANK
BLANK	PIV2 high	PIV2 high	PIV2 high	PIV2 low	PIV2 low	PIV2 low	BLANK
BLANK	PIV3 high	PIV3 high	PIV3 high	PIV3 low	PIV3 low	PIV3 low	BLANK
Anti-mouse IgG	Mouse IgG high	Mouse IgG high	Mouse IgG high	Mouse IgG low	Mouse IgG low	Mouse IgG low	Anti-mouse IgG

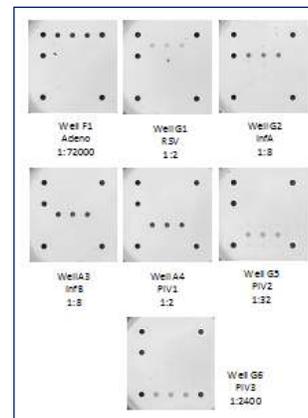


Fig. 4 Selected Array Images Examples of images obtained with antigen positive clinical samples. Positive controls are located in all four corners and also serve as reference spots for grid alignment during read-out.

Discussion

Colorimetric measurements with CLAIR compared well to the TR-FIA method.

- CLAIR/colorimetry characterized 77 of 88 samples correctly
- All mischaracterized samples were PIV 3 samples which had degraded over time (TR-FIA was performed several weeks prior to this study). Antigen concentration at CLAIR measurement is unknown and could well be below cut-off.

Summary

- The array-in-well method allows for multiplexing of seven respiratory viruses
- Detection and data analysis with CLAIR was fast and easy
- Colorimetric and fluorescent measurements are comparable in terms of sensitivity and linear range
- Colorimetric assays and equipment are less complex and less costly than those for fluorescent detection
- Colorimetric assays are ideal for routine testing in clinical labs, veterinary, and food testing

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 the 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 makes 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