

# Comparison of Colorimetric and Fluorescence Detection Using a Multiplexed Respiratory Virus Assay

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## Introduction

Microarrays are becoming increasingly more popular not just for R&D applications but also in diagnostics. Commonly, microarray systems use fluorescence labeling which is detected using a laser scanner. These Laser scanners are fairly expensive and ill-suited for routine diagnostic applications.

In contrast, colorimetry is a well-established, affordable method and a feasible alternative to fluorescence. In a colorimetric system the microarray spots are visualized through the precipitation of TMB dye created in an enzymatic reaction. For this investigation, we converted an existing fluorescent multiplexed sandwich ELISA to a colorimetric format and compared the results.

## Materials & Methods

We performed multiplexed array-in-well sandwich ELISA assays and compared two labeling methods: fluorescent detection with a directly labeled capture antibody and colorimetric detection using a HRP-labeled capture antibody. The protocol of the colorimetric assay was modified to enable the formation of a blue precipitate at the reaction site instead of a soluble dye. Fluorescence was detected using the Tecan LS 400, for colorimetric detection we used the Sensovation CLAIR reader.

Our model system was Adenovirus hexon protein in serial dilutions (156 ng/ml to 0.0009 ng/ml) plus a zero control. Each well contained a 3 x 3 array of microspots with the Adeno hexon protein in triplicate and six positive controls.

## Results

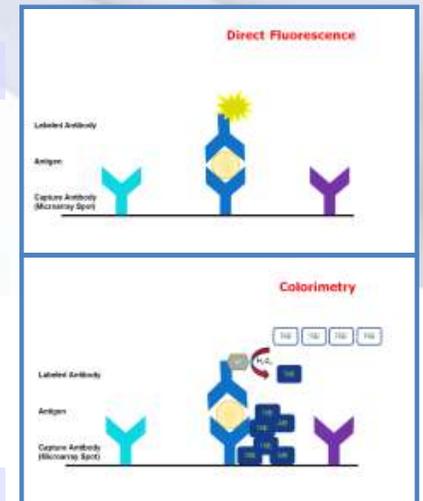
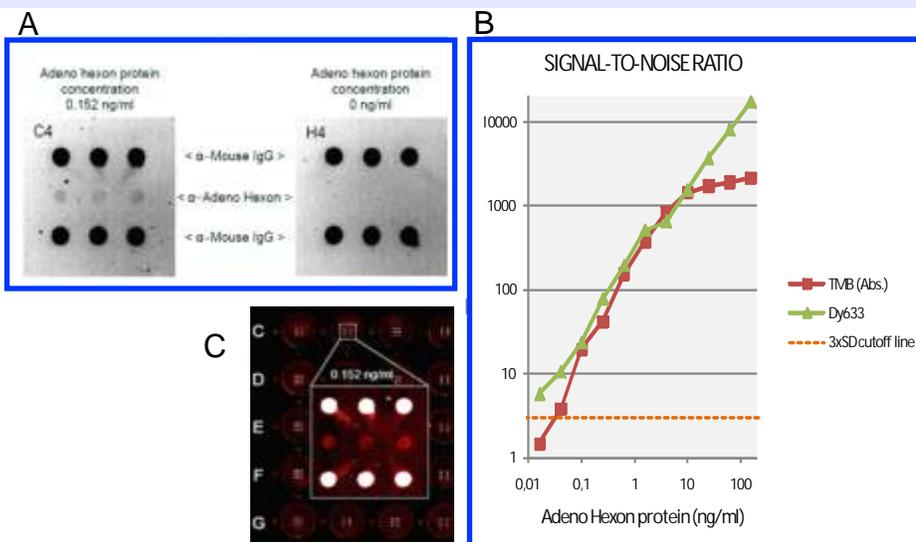


Fig. 1 Labeling Technologies Comparison of fluorescent and colorimetric detection

Figure 2 Multiplexed Array-in-Well Multiplexed arrays detected with colorimetric (A) and fluorescent read out (B) are compared. The top and bottom rows contain positive controls, the middle row the Adeno hexon protein in triplicate. (C) shows signal-to-noise ratios for the two assay and readout methods in response to antigen concentration. The dotted line represents the detection limit at 3xSD. Detection limit for the colorimetric/ TMB assay is approx. 0.041 ng/ml, for fluorescence/Dy633 approx. 0.03 ng/ml.

## Discussion

Colorimetric detection with CLAIR, a compact routine microarray reader, compares very well to the fluorescent detection using a high-end laser scanner.

## Summary

The comparison of labeling method and detection technology shows:

- The colorimetric assay is marginally less sensitive than the fluorescent assay
- Colorimetry has good linearity for a 100-fold dynamic range
- CLAIR compared very well to fluorescent read out on the Tecan LS 400

## 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 ...

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  - 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