A Multiplex Panel for Simultaneously Profiling Toxicant Levels and Biological Response Indicators in Serum Samples

Emily J. Triplett, Jared A. Carter, Preston C. Keller, Christopher C. Striemer, Benjamin L. Miller

Abstract
A central need of human toxicology and environmental exposure studies is the detection of environmental pollutants, and the human body’s response to those pollutants. Currently, accomplishing these tasks requires multiple analytical methodologies which increases the required sample volume, processing time, and cost per data point. We have used Arrayed Reflectometric Imaging (AIR™), a label-free microarray technology that relies on the target binding-induced perturbation of an antireflective coating on the surface of a silicon chip, to create a multiplex antibody array for the detection of human inflammatory markers and small molecule pollutants simultaneously using the same assay sensor. In this initial demonstration of the technique, three protein biomarkers and three pollutants are detected by the same sensor utilizing both direct detection for protein biomarkers and a competitive assay format for the surveillance of small molecule pollutants. The chip is able to provide quantitative information about protein concentrations, with lower limits of detection in picograms per mL and high dynamic range. Simultaneous use of direct and competitive detection further expands the versatility of AIR™.

How Do We Enable Detection of Hundreds of Proteins?
Suppress background illumination... reveal hidden detail.

The Process
Simple workflow and rapid turn-around.

Applying Sample to Chip - Add Reagents (optional)
Rinse, Dry, Acquire Image Fully automated, walk-away operation
Analyze Data Image to first results: 1 min

96 Well Plate
Singular Well

Each 96 well plate tests up to 400 analytes per well
Up to 400 unique analytes detected over thousands of spots in each well

How it Works
A laser beam is reflected off the chip surface and imaged with a digital camera in a fraction of a second, producing an array of bright and dark spots.

Representative Dual-Detection Arrays
Control
Standard Level 1

Methods
Assay sensors were fabricated with an array of Keyhole Limpet Hemocyanin (KLH)-coupled toxicants (benzo[α]pyrene (BaP), bisphenol A (BPA), and acrolein (Acrl)) and inflammatory protein antibodies (anti-CRP, anti-IL-29, and anti-MCP-2). A titration series of each inflammatory protein and small molecule toxicant was prepared in a background of 5% porcine serum doped with antibodies to each of the small molecules. Target solutions were incubated for one hour prior to sensor exposure.

Novel Dual-Mode Detection Principle
Small Molecules (Competitive Inhibition)

Inflammatory Proteins (Direct Detection)

Small Molecule Pollutant Response

Conclusions
This work demonstrates the first known integration of competitive inhibition and direct detection formats on a single assay platform. This quantitative method allowed for the successful detection of benzo[α]pyrene (LLOD: 0.64 nM), bisphenol A (LLOD: 3.2 nM), and acrolein (LLOD: 0.13 nM), well within the nanomolar range of physiological relevance. The simultaneous picogram per mL detection of a subset of inflammatory cytokines (IL-29 LLOD: 12.8 pg/mL, CRP LLOD: 12.8 pg/mL, and MCP-2 LLOD: 2.56 pg/mL) demonstrates both the versatility and the sensitivity of this method. The dual detection format further diversifies the multiplexing capabilities of the AIR™ platform without compromising its reliably low limits of detection.

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Analyte LLOD (pg/mL) LLOQ (pg/mL) Relevant range (plasma) (nM)
Benz[a]pyrene 0.64 3.2 0.95 – 5.2
Acrolein 0.13 16 0.13 – 16.7
Bisphenol A 3.2 3.2 2.8 – 12.7

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