Optimized, High-Throughput Antibody Microarray of Pathogens

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OUTLINE

• Microarrays- Historical (Gene expression on glass slides)
• Research Objective- optimize high-throughput multiplexed detection of pathogens with antibody microarray (limit of detection; speed; reagents)
• Results- Fluorescent Sandwich Immunoassay Microarray
• Conclusions- LOD ~2e5 cells/mL [2e6 cell/mL]; ~80 min [~2.5 hr]
• Future- Typing and subtyping of pathogens with microarrays?
Microarrays

• Traditionally- Gene Expression (also sequence & gene mutation)
• Substrates (glass, nitrocellulose, plastic, etc.)
• Microarray printers (contact vs. non-contact)
• Printed recognition elements (probes/targets or features); Conjugation chemistry
• Reporters/Labels- fluorescent targets/probes (also precipitating/colorimetric)
• Detection (typically fluorescence-laser scanning)
• Software based microarray analysis
Microarray Contact Printer and Laser Scanner

Genomic Solutions OmniGrid Accent Pro

Tecan LS-400 4 laser Array scanner
Research Objective:
Develop detection microarrays for the high-throughput screening of pathogens in foods.

(Specifically)

- Develop antibody based or protein microarrays for multiplexed detection of live, pathogenic bacteria, toxins, structural protein, metabolites, etc.
- Antibody microarray- fluorescent sandwich immunoassay for *E. coli* O157 and STX-1
Antibody Microarray Approach

- Passively adsorb capture antibodies on polystyrene surface
- Reporter (fluorescent) antibodies reacted after capture of analyte (“sandwich immunoassay”)
- Potential to screen for many (tens - hundreds) pathogens at once
- Rapid analytical time (< 90 min)
Fluorescent Immunoassay Schematic

- Reporter antibody
- Capture antibody
- Bacteria
- Bovine serum albumin
- Polystyrene substrate
96, 13x8 Subarray Microarray
96, 13x8 Subarray Microarray (inset)
Multiplex detection of *E. coli* O157:H7 (in the presence of $10^8$ cells/mL *Salmonella typhimurium*, and 100 mg/mL Chicken IgG)
Time Course of Bacterial Capture

(200 µm diameter spots of biotinylated capture antibody, exposed to $10^8$ cells/mL heat-killed bacteria)
Microarray Detection of Live *E. coli* O157:H7 (Variable reaction conditions)
Microarray Detection of *E. coli* O157:H7 (Variable conjugate reaction times)

- $y = 0.16x^{0.32}$, $r^2 = 0.89$
- $y = 0.052x^{0.42}$, $r^2 = 0.95$
- $y = 0.078x^{0.39}$, $r^2 = 0.94$
Microarray Detection of Heat-killed *E. coli* O157:H7 (Variable reaction conditions)
Microarray Detection of Live *E. coli* O157:H7 (Variable reaction conditions)
Microarray Detection of *E. coli* O157:H7 (Variable centrifugation times)
Microarray Detection of STX-1 (Variable reaction conditions)

\[ y = 2.29x^{0.39} \]

\[ r^2 = 0.94 \]
Mixed Culture in Ground Pork
(30°C, 24 h enrichment) - BLEB*

*very similar results with TSB or UPB
Conclusions

• Multiplex detection of *E. coli* O157:H7 cells and STX-1 toxin in multiwell plate format

• Total assay time (per 96 samples) - < 90 min (formerly ~2.5 hr)

• Limit of detection for bacteria - ~2e5 cells/mL (formerly ~2e6 cells/mL)
Future Research

- Detection of more pathogens and associated toxin
- Automation - Plate washers, robotic manipulation
- Typing and subtyping of pathogens with microarrays?