**Consortium for Functional Glycomics**

**Pathogen Array v1.0**

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Supervisors – James Paulson and Nahid Razi

<table>
<thead>
<tr>
<th>Objectives:</th>
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<tbody>
<tr>
<td>1. Print a minimum of 48 individual glycans isolated from Gram-negative bacteria in both Native and Native + Spacer forms</td>
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<tr>
<td>2. Compare printing and detection of the glycans in Native (N) and Native + Spacer (N+S) forms using rabbit serum and commercial monoclonal antibodies</td>
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Bacterial Polysaccharides

and

Sample Preparation
The Cell Envelope of Gram-Negative Bacteria

Removal of Lipid A and KDO II By Mild Acid Hydrolysis (Performed by Yuri Knirel)

Ketosidic linkage of KDO is very acid-labile.

Polysaccharides Prepared for Printing

Each bacterial polysaccharide contains the o-antigen linked to the full core (both outer core and inner core without lateral Kdo II). The Kdo I - lipid A bond and the Kdo II- Kdo I bond were both cleaved by mild acid hydrolysis.


Polysaccharides Suspension and Dilution

Native and Native+spacer derivatives were dissolved in aqueous 300mM Sodium Phosphate + 0.005% Tween-20 printing buffer to a stock concentration of 1mg/mL which was stored at -20ºC.

Stock solution were transferred to 96-well microplates at 0.5 mg/mL and serial dilutions were performed mechanically by a robot to obtain 5 concentrations of 1:2 dilutions ranging from 0.5 mg/mL to 0.03 mg/mL of carbohydrate dissolved in printing buffer.
Printing of Polysaccharides

**Slide H**: Schott Nexterion Slide H (Item No: 1070936).

Amine binding hydrogel slide comprised of a matrix scaffold with amine reactive groups
Detection Strategy

Printed bacterial polysaccharides were detected using a sandwich-type immunoglobulin reaction.

Monoclonal antibodies or diluted serum were dissolved/diluted in 1X PBS Buffer + 0.005% Tween-20 to a previously optimized concentration:
- **Monoclonal antibodies, mouse-IgG** – 10µg/mL
- **Rabbit serum** – 1:5000 dilution (via 1:100 + 1:50 dilution series)

1mL of the resulting dilution was applied to each array and incubated with agitation for 1hr at room temperature and 100% relative humidity.

Following primary incubation, slides were rinsed 3 times each in 1X PBS + 0.005%Tween, 1X PBS and ddH₂O, respectively.

Secondary antibodies were all diluted to 10µg/mL:
- monoclonal antibodies were detected using **anti-mouse-IgG-Alexa488 conjugate** (Invitrogen)
- serum was detected using **anti-rabbit-IgG-Biotin conjugate** (Jackson Biotech) + **Streptavidin-AF488** (2µg/mL – Invitrogen)

Following secondary incubation, slides are rinsed 3x3 times in 1X PBS + 0.005%Tween, 1X PBS and ddH₂O.

Slides were dried using nitrogen gas and scanned in a Perkin-Elmer Pro HT scanner at 80% Laser power and 60% Photo-Multiplier Tube transmission. Resulting images were analyzed in Imagene software for Relative Fluorescence values of bound secondary antibody
## Antibodies and Serum Samples

### Monoclonal Antibodies (mouse IgG)

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>Concentration used for detection</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-PO1 – PO17</td>
<td><em>P. aeruginosa</em> O3 (IATS2)</td>
<td>10ug/mL</td>
<td>Erfa Biotech (Montreal, Canada)</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> O2 (IATS18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> O4 (IATS4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> O1 – 17</td>
<td>1:5000 dilution</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1:5000 dilution</td>
<td>Dr. Gerald Pier</td>
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<tr>
<td></td>
<td></td>
<td>1:5000 dilution</td>
<td>Dr. Antonio Rozalski</td>
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<tr>
<td></td>
<td></td>
<td>1:5000 dilution</td>
<td>Zelinski Institute</td>
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</tbody>
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### Serum samples (raised in rabbit)

<table>
<thead>
<tr>
<th>Name</th>
<th>Specificity</th>
<th>Concentration used for detection</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3WB</td>
<td><em>P. aeruginosa</em> O3 (IATS2)</td>
<td>1:5000 dilution</td>
<td>Dr. Gerald Pier</td>
</tr>
<tr>
<td>T7WB</td>
<td><em>P. aeruginosa</em> O2 (IATS18)</td>
<td>1:5000 dilution</td>
<td>Dr. Antonio Rozalski</td>
</tr>
<tr>
<td>HABS4 WB</td>
<td><em>P. aeruginosa</em> O4 (IATS4)</td>
<td>1:5000 dilution</td>
<td>Zelinski Institute</td>
</tr>
<tr>
<td>Anti-ProvO5</td>
<td><em>Providencia alcalifaciens</em> O5</td>
<td>1:5000 dilution</td>
<td></td>
</tr>
<tr>
<td>Anti-ProvO14</td>
<td><em>P. rustigianii</em> O14</td>
<td>1:5000 dilution</td>
<td></td>
</tr>
<tr>
<td>Anti-ProvO19</td>
<td><em>P. alcalifaciens</em> O19</td>
<td>1:5000 dilution</td>
<td></td>
</tr>
<tr>
<td>Anti-ProvO22</td>
<td><em>P. alcalifaciens</em> O22</td>
<td>1:5000 dilution</td>
<td></td>
</tr>
<tr>
<td>Anti-ProvO44</td>
<td><em>P. stuartii</em> O44</td>
<td>1:5000 dilution</td>
<td></td>
</tr>
</tbody>
</table>

More information on the sources can be found at:

- Dr. Gerald Pier: [http://www.channing.harvard.edu/pier.htm](http://www.channing.harvard.edu/pier.htm)
Image Analysis

Example image of detection of rabbit serum at low dilution (1:50)

Non-specific binding of carbohydrates by serum has been demonstrated to be very common and must be highly diluted to show specificity to one particular pathogen

Each half contains 6 replicates of each carbohydrate printed at 5 concentrations in a 9x9 grid for a total of 480 subspecies (48 sugars x 2 derivatives x 5 concentrations)

The total array contains 3440 individual spots (2880 sugar spots + biotin marker spots)
Example subarray showing detection of all printed sugars (Native BPS @ 0.5 mg/mL).

Each subarray is 9x9 with 72 spotted sugars – 12 subspecies at a single concentration printed in 6 replicates – and 4 biotin spots printed at 100uM as grid markers.

5 spotting locations are left blank.
Non-specific detection of all sugars with low dilution pooled anti-sera

T3WB + P. alcalafaciens O19 pooled rabbit serum
1:50 serum dilution detected with anti-rabbit-IgG-Biotin (10ug/mL) + StrepAF488 (2ug/mL)
Detection of *Pseudomonas sp* by commercial monoclonal antibodies and experimental serum

**Monoclonal antibodies**: obtained from Erfa Biotech (Montreal, Canada) with individual specificity directed against the IATS-types 1-17 (All mouse IgG).

**Anti-*Pseudomonas* serum**: from Dr. Gerald Pier raised in rabbit with the following specificities:

**T3WB**: Anti IATS 2 antisera
T3WB (stands for T3 Whole Bug antiserum): Raised to boiled cells of the Fisher-Devlin Immunotype 3 (T3) bacteria. Corresponds to Lanyi/Bergan O(2a)2c and IATS 2.

**T7WB**: Anti IATS 18 antisera
T7WB (stands for T3 Whole Bug antiserum): Raised to boiled cells of the Fisher-Devlin Immunotype 7 (T7) bacteria. Corresponds to Lanyi/Bergan O(2a)2d2f and IATS 18.

**Habs 4 WB**: Anti IATS 4 antisera
Raised to boiled cells of IATS prototype strain Habs4, corresponds to Lanyi/Bergan O4a,4b.
Detection of *Pseudomonas aeruginosa* O2 by **T3WB Serum**

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)

**Fluorescence**

- **Native BPS**
- **Native+Spacer BPS**

**Bacterial Polysaccharide**

- PS041 - *E. coli* O180
- PS005 - *Pseudomonas aeruginosa* O2a, 2c (IATS 2, FI 3)
- PS056 - *P. aeruginosa* O2 2a,2b (IATS 16)
- PS057 – *P. aeruginosa* O2 2a,2b,2e
- PS058 - *P. aeruginosa* O2 2a,2d
- PS059 – *P. aeruginosa* O2 (FI 7)
Detection of *Pseudomonas aeruginosa* O2 by T7WB Serum

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)
Detection of *Pseudomonas aeruginosa* O2 by monoclonal anti-PO5

10ug/mL mouse-IgG detected w/ anti-mouse-IgG-AF488 (10ug/mL)
Detection of *Pseudomonas aeruginosa* O2 by monoclonal anti-PO16

10µg/mL mouse-IgG detected w/ anti-mouse-IgG-AF488 (10µg/mL)

PS056 - *P. aeruginosa* O2 2a,2b (IATS 16)

\[ \rightarrow 4\)-D-ManNAc3NAmA-(β1→4)-D-ManNAc3NAcA-(β1→3)-D-FucNAc-(β1→ \]

PS005 – *P. aeruginosa* O2a, 2c (IATS 2, FI 3)

\[ \rightarrow 4\)-D-ManNAc3NAmA-(β1→4)-L-GulNAc3NAcA-(α1→3)-D-FucNAc-(β1→ \]

PS057 – *P. aeruginosa* O2 2a,2b,2e

\[ \rightarrow 4\)-D-ManNAc3NAmA-(β1→4)-D-ManNAc3NAcA-(β1→3)-D-FucNAc4Ac-(β1→ \]
Detection of *Pseudomonas aeruginosa* O4 by HABS4 Serum

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)

PS063 – *P. aeruginosa* O4 4a,4c

→2)-L-Rha-(α1→3)-L-FucNAc-(α1→3)-L-FucNAc-(α1→3)-D-FucNAc-(α1→

Bacterial Polysaccharide Fluorescence
Detection of *Pseudomonas aeruginosa* O11 by **monoclonal** anti-PO11

10ug/mL mouse-IgG detected w/ anti-mouse-IgG-AF488 (10ug/mL)
Detection of *Providencia sp* by experimental serum

Anti-*Providencia* serum from Dr. Antonio Rozalski (Zelinski Inst.) raised in rabbit by inoculation with:

*Providencia alcalafaciens* O5  
*Providencia alcalafaciens* O19  
*Providencia alcalafaciens* O21  
*Providencia rustigianni* O14  
*Providencia stuartii* O44
Providencia alcalifaciens O5 detection by rabbit anti-serum

1:5000 dilution detected w/ anti-rabbit-IgG-B (10ug/mL) + Strep-AF488 (.04ug/mL)

- PS140 - Providencia alcalifaciens O5
  →3)-α-D-Galp-(1→3)-β-D-Galp-(1→3)-β-D-GlcNAc-(1→4)-β-D-Quip3NAc-(1→

- PS076 - Pseudomonas aeruginosa O14
Detection of *Providencia alcalafaciens* O19 by rabbit anti-serum

1:5000 dilution detected w/ anti-rabbit-IgG-B (10ug/mL) + Strep-AF488 (.04ug/mL)
Detection of *Providencia alcalifaciens* O21 by rabbit **anti-serum**

1:5000 dilution detected w/ anti-rabbit-IgG-B (10ug/mL) + Strep-AF488 (.04ug/mL)

**PS145 - Providencia alcalifaciens O21**

$\rightarrow 4\alpha\rightarrow D\text{-GalpNAc}-(1\rightarrow 4\alpha\rightarrow D\text{-GalpNAc}-(1\rightarrow 3\beta\rightarrow D\text{-GalpNAc}-(1\rightarrow 3\alpha\rightarrow D\text{-GalpA}-(1\rightarrow$

**PS135 - Providencia stuartii O44**

$\rightarrow 3\alpha\rightarrow L\text{-Fucp}-(1\rightarrow 3\alpha\rightarrow D\text{-Glcp}-(1\rightarrow 4\alpha\rightarrow L\text{-Quip}-(1\rightarrow 3\alpha\rightarrow D\text{-GlcpNAc}-(1\rightarrow 4\alpha\rightarrow D\text{-GalpNAc}-(1\rightarrow$
Detection of *Providencia rustigianii* O14 by rabbit anti-serum

1:5000 dilution detected w/ anti-rabbit-IgG-B (10ug/mL) + Strep-AF488 (.04ug/mL)

PS153 - *Providencia rustigianii* O14

\[ \alpha-D\text{-GalpNAc-(1}\rightarrow{3})\alpha-D\text{-GlcNAc-(1}\rightarrow{3})\alpha-D\text{-GalpA-(1}\rightarrow{6})2S, 8S-AlaLys \]

PS042 - *Proteus mirabilis* O3a, 3c (G1)

PS076 - *Pseudomonas aeruginosa* O14
Detection of *Providencia stuartii* O44 detected with rabbit anti-serum

1:5000 dilution detected w/ anti-rabbit-IgG-B (10ug/mL) + Strep-AF488 (.04ug/mL)
Glycan binding is amine-dependant

Printing via Absorption or Covalent Bond Formation?
**Slide H: Standard vs. Pre-Blocked Detection**

In order to ensure that the attachment of polysaccharides was chemically dependant on the reactive surface coating of the slides, standard SlideH protocol was compared to slides which had blocked reactive groups by pre-blocking slides prior to printing with 50mM ethanolamine in 50mM borate buffer for 1hr and drying according to the standard post-printing blocking protocol.

**anti-IATS2 mouse monoclonal IgG (10ug/mL)**
detected w/ anti-mouse-IgG-AF488 (10ug/mL)
Conclusion:
The reactive chemistry greatly increases the amount of detectable bound antigen by monoclonal antibodies suggesting that covalent attachment is occurring and the reaction is amine-dependant
Serum Detection is decreased by addition of the target glycan in solution

Assays were performed to determine if addition of the detected glycan in the incubation solution would inhibit said detection via competition/blocking of the detecting serum.

**Detection of Pseudomonas aeruginosa O4 by HABS4 Serum**

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)
**Detection of Pseudomonas aeruginosa O4 by HABS4 Serum + 10ug/mL PS063**

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)

**Detection of Pseudomonas aeruginosa O4 by HABS4 Serum + 50ug/mL PS063**

1:5000 dilution of rabbit serum detected w/ anti-rabbit-IgG-Biotin (10ug/mL) + Streptavidin-AF488 (.04ug/mL)
Conclusions

• There is excellent specificity obtained for reactivity against related glycans using either monoclonal antibodies or antisera.

• The concern about amine groups in the inner core being immunodominant and causing cross-reactivity seems to be moot.

• The majority of the glycans (97+%) print well on the chips, and the printing is amine dependent (e.g. pre-blocking the chips there is vastly reduced printing).

• For most glycans adding a linker has no effect, but for one, there is no printing unless a linker is added (BPS4 – data not shown).