Disclosures: None

Learning Objectives
After this presentation the participants should be able to:
• Explain workflow for MALDI-TOF/MS PGx testing.
• Create a practical laboratory preparation plan to set up MALDI-TOF/MS PGx tests.
• Identify the challenges of implementing MALDI-TOF/MS in PGx testing.
• Create a method validation plan
Outline (3 Segments)

- **Segment I:**
  - Pharmacogenetics testing (PGx) and personalized medicine.
  - Basics of MALDI-TOF/MS.
  - Workflow/Protocol for PGx testing by MALDI-TOF/MS.

- **Segment II:** Discuss the challenges of implementing MALDI-TOF/MS PGx tests.
  - Technical difficulties.
  - Cost.
  - Personnel.

- **Segment III:** Discuss method validation for MALDI-TOF/MS PGx testing.
  - Specimen requirements.
  - Accuracy (Method comparison).
  - Reproducibility.
  - Carryover.

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**Segment I**

- Pharmacogenetics testing and personalized medicine.
  - Basics of MALDI-TOF/MS.
  - Workflow for PGx testing by MALDI-TOF/MS.

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**Case 1**

- A 5.5-year-old boy (21 kg).
  - Underwent dental extractions under general anesthesia without complications.
  - Discharged with tramadol prescription for pain relief.
  - Received 1 oral 20 mg dose of tramadol (1 mg/kg) in the afternoon at home.
  - Found comatose a few hours later.
Case 1: (Cont’d)

- Admitted to the PICU with
  - Pin-point pupils
  - Minimal respirations
  - Low oxygen saturation (48%)
  - Abnormal arterial blood gases

- Treated with naloxone -- improved dramatically with normalizing consciousness, pupils and respiration.

Classical opioid overdose presentations!

Case 1 (Cont’d)

Major In Vivo Metabolic Pathways for Tramadol

200 × more potent at opioid binding site

Inactive

Case 1 (Cont’d)

Urinary Tramadol Result

- Tramadol: 38.0 µg/mL
- M1: 24.0 µg/mL ([H])
- M2: 4.6 µg/mL
- Ratio (tramadol/[M1])=1.58
  (significantly decreased)

Genotyping

- CYP2D6*2/*2/CYP2D6*2 (Ultra rapid Metabolizer)

Conclusion: Accidental overdose by excess production of M1
Concept of Personalized Medicine

- 4Rs:
  - Right patient; Right drug; Right dose; Right time

- Pre-therapeutic pharmacogenetics testing can personalize drug selection to prevent accidental overdose or therapeutic failure

  > 160 FDA approved drugs contain PGx biomarkers information in drug labeling.

PGx Service Implementation Process

CDS: Clinical decision support
KPI: Key performance indicator
**PGx Platforms**

Examples of FDA-cleared Tests

<table>
<thead>
<tr>
<th>Gene</th>
<th>Trade name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>xTag CYP2D6 Kit v3</td>
<td>Roche</td>
</tr>
<tr>
<td></td>
<td>PyroDiag</td>
<td>Roche</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>INFINITI CYP2C19</td>
<td>AutoGenomics</td>
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<tr>
<td></td>
<td>Assay</td>
<td>Roche</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>INFINITI 2C9 &amp;</td>
<td>AutoGenomics</td>
</tr>
<tr>
<td></td>
<td>VKORC1 Multiplex Assay for Warfarin</td>
<td>Molecular Systems</td>
</tr>
</tbody>
</table>

**Mass Spectrometry Variant Detection**

Agena Bioscience **MassArray**

*The only platform able to detect and discriminate the alleles is MALDI TOF.*

**Conventional Methods vs. Mass Spec**

<table>
<thead>
<tr>
<th>Conventional Methods</th>
<th>Mass Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Measurement</td>
<td>• Measurement</td>
</tr>
<tr>
<td>• Not assess the amplicon; detect probes/fluorescence labels</td>
<td>• Directly measure the amplicon</td>
</tr>
<tr>
<td>• Sensitivity</td>
<td>• Sensitivity</td>
</tr>
<tr>
<td>• Sanger sequencing – discriminate mutations with a sensitivity of ~25%</td>
<td>• Discrimination between heterogeneous alleles: 1-10%</td>
</tr>
<tr>
<td>• Multiplexing capabilities</td>
<td>• Multiplexing capabilities</td>
</tr>
<tr>
<td>• Sanger: no multiplexing</td>
<td>• Multiplexed capabilities</td>
</tr>
</tbody>
</table>

Segment I

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- Workflow for PGx testing by MALDI-TOF/MS.

### Schematic Diagram of MALDI-TOF/MS

**MALDI-Laser Ionization Mechanism**

- Analytes mixed with matrix
- A Laser beam (e.g., 337 nm from a Nitrogen Laser)
- Plate (High voltage)
- Plume (ions & neutrals)
- Laser
- Time of Flight Tube
- Ion Source
- Plume (ions & neutrals)
- Only ions to MS
MALDI Sample Plates

Traditional MALDI Plates
- 100 Wells
- Spot 0.3-1 µL of the sample solution on each well

Nano MALDI Plates/Chips
- 384 Wells
- 96 Wells
- Spot 2 µL of the sample solution each well

Common UV MALDI Matrices for Nucleotides Analysis

- 2,5-dihydroxy benzoic acid (DHB)
  - Phosphopeptides & glycoproteins

- Alpha-cyano-4-hydroxycinnamic acid (HCCA or CHCA):
  - Peptides and proteins from 0.7 to 20 kDa

- 3-Hydroxypicolinic acid (3-HPA):
  - Useful for the analysis of DNA/RNA between 1-30 kDa

Sample Preparation Methods

- Dried Droplet Method

  Prepare a matrix solution (a saturated solution of 3-HPA in 50:50 acetonitrile:0.1%TFA in water containing ammonium hydrogen citrate)

  Deposited 0.5 µL of the matrix on the plate and allow to dry
  Deposit 0.5 µL sample solution and allow to dry
TOF Basics

- Total flight path length = 0.5-2 meters
- How do we measure the mass?
  - Use standards to calibrate the mass and time scale.

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MALDI-TOF/MS SNP Analysis

- Step 1: Genomic DNA isolation
- Step 2: Multiplexed PCRs
- Step 3: neutralize unincorporated dNTPs by shrimp alkaline phosphatase (SAP) treatment
- Step 4: single base extension into SNP site (also known as iPLEX extension reactions)
- Step 5: sample cleanup and plate preparation
- Step 6: Acquire data with MALDI-TOF

http://agenabio.com
How does single base extension work?

- Target DNA: 3' NNNTCAGTACCG5'
- Primer: AGTCATGGC
- Polymerase: ddNTP
- Extended Primer: AGTCATGGC
- Target DNA: 3' NNNTCAGTACCG5'
- Mass Spec

How does single base extension work?

- DNA elongation
  - 3'-OH group of dNTPs is essential for polymerase-mediated strand elongation in a PCR.

A Representative MS Spectra

Dr. Roberta Melis, ARUP lab