Saskatchewan Health Authority

2018 MSACL Practical Training Track

Getting Started With Pharmacogenetics Testing and Mass Spectrometry

Fang Wu, PhD, DABCC
Clinical Biochemist & Assistant Professor
Department of Pathology & Lab Medicine
Saskatoon Health Region & University of Saskatchewan

University of Saskatchewan

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

Segment I

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

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 (1mg/kg) in the afternoon at home.
- Found comatose a few hours later

http://i.istockimg.com/file_thumbview_approve/58143380/3/stock-illustration-58143380-sad-child-vector-illustration.jpg

Orliaguet et.al., PEDIATRICS, 2015

Case 1: (Cont'd)

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

Classical opioid overdose presentations!

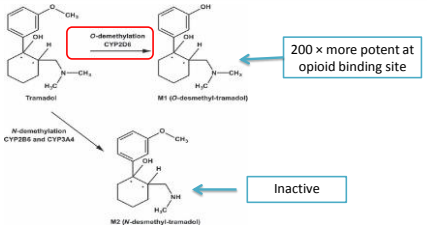
- Treated with naloxone -- improved dramatically with normalizing consciousness, pupils and respiration.

http://i.istockimg.com/file_thumbview_approve/58143380/3/stock-illustration-58143380-sad-child-vector-illustration.jpg

Orliaguet et.al., PEDIATRICS, 2015

Case 1 (Cont'd)

Major In Vivo Metabolic Pathways for Tramadol



200 × more potent at opioid binding site

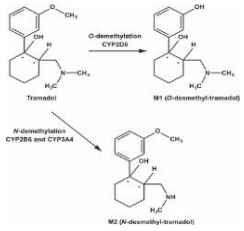
Inactive

http://i.istockimg.com/file_thumbview_approve/58143380/3/stock-illustration-58143380-sad-child-vector-illustration.jpg

Orliaguet et.al., PEDIATRICS, 2015

Case 1 (Cont'd)

Urinary Tramadol Result
• Tramadol: 38.0 ug/mL • M1: 24.0 ug/mL (H) • M2: 4.6 ug/mL • Ratio(tramadol/[M1])=1.58 (significantly decreased)
Genotyping
• CYP2D6*2X2/CYP2D6*2 (Ultra rapid Metabolizer)



Conclusion: Accidental overdose by excess production of M1

Orliaguet et.al., PEDIATRICS, 2015

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.

https://www.fda.gov/downloads/scienceresearch/specialtopics/personalizedmedicine/

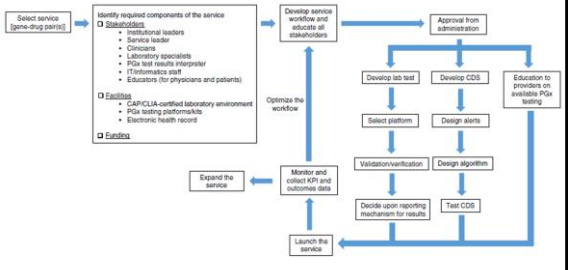
PGx Service Implementation Process

?

CDS: Clinical decision support
KPI: Key performance indicator

Clin Transl Sci (2016) 9, 233–245;

PGx Service Implementation Process



Clin Transl Sci (2016) 9, 233–245;

PGx Platforms

Sanger sequencing
Detection: Fluorescence and capillary electrophoresis

Pyrosequencing
Detection: Light

RT-PCR
Detection: Fluorescence; Taqman probes

Mass Spec
Detection: M/Z

GeneChip Array

Examples of FDA-cleared Tests

Gene	Trade name	Manufacturer
CYP2D6	xTAG CYP2D6 Kit v3 Roche AmpliChip CYP450 microarray	Luminex Roche Molecular Systems
CYP2C19	INFINITI CYP2C19 Assay Roche Amplichip	AutoGenomics Roche Molecular Systems
CYP2C9 and VKORC1	INFINITI 2C9 & VKORC1 Multiplex Assay for Warfarin	AutoGenomics

Alan Wu & Kiang-Teck J. Yeo Pharmacogenomic testing in current clinical practice

Mass Spectrometry Variant Detection

Agena Bioscience MassArray

Home-Brew PGx Assays with MALDI-TOF

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

https://www.omicsonline.org/open-access/early-outline-evaluation-of-genotyping-costs-of-pharmacogenomics-2155-0645.1000123.php?aid=23971

Clin Transl Sci (2016) 9, 233–245

Conventional Methods vs. Mass Spec

Conventional Methods

- Measurement
  - Not assess the amplicon; detect probes/fluorescence labels
- Sensitivity
  - Sanger sequencing – discriminate mutations with a sensitivity of ~25%
- Multiplexing capabilities
  - Sanger: no multiplexing

Mass Spec

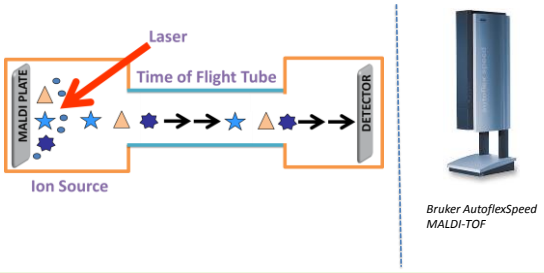
- Measurement
  - Directly measure the amplicon
- Sensitivity
  - Discrimination between heterogeneous alleles: 1-10%
- Multiplexing capabilities
  - Multiplexed capabilities

(Arcila et al., J. Mol. Diagn. 2011; 13 (1): 64-73; Fan et al., Clin. Chem. Lab. Med. 2008; 46 (3): 299-305)

Segment I

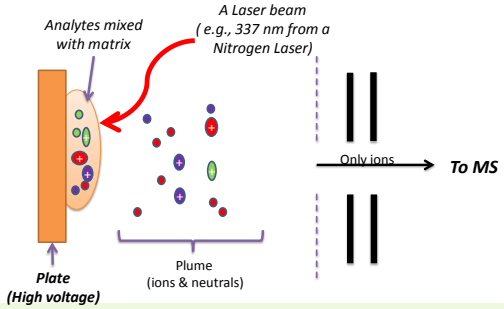
- Pharmacogenetics testing and personalized medicine.
- Basics of MALDI-TOF/MS.
- Workflow for PGx testing by MALDI-TOF/MS.

Schematic Diagram of MALDI-TOF/MS



Bruker AutoflexSpeed MALDI-TOF

MALDI-Laser Ionization Mechanism



MALDI Sample Plates

Traditional MALDI Plates

Image adapted from wikipedia

100 Wells

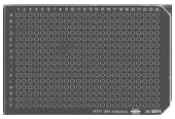
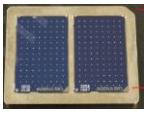


Image adapted from ms-textbook.com

384 Wells

Spot 0.3-1 uL of the sample solution on each well

Nano MALDI Plates/Chips



96 Wells

Spot 2 nL 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 kD

Roles of MALDI Matrix

- Analyte entrapment
- Analyte isolation
- Vaporization
- Ionization

https://en.wikipedia.org/wiki/3-Hydroxypicolinic_acid#/media/File:3_hydroxypicolinic_acid.svg

Sample Preparation Methods

- Dried Droplet Method

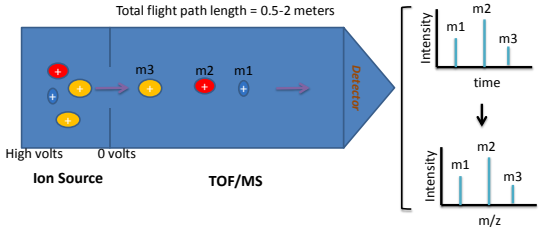
Prepare a matrix solution
(a saturated solution of 3-HPA in 50:50 acetonitrile : 0.1%TFA in water containing ammonium hydrogen citrate)

↓

Deposited 0.5 Ul of the matrix on the plate and allow to dry
Deposit 0.5 ul sample solution and allow to dry

J Am Soc Mass Spectrom 2003, 14, 992–1002

TOF Basics



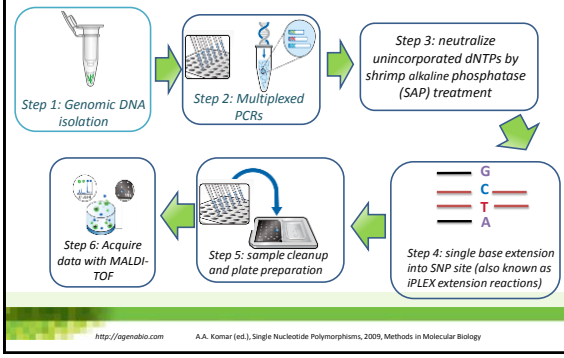
How do we measure the mass?

- Use standards to calibrate the mass and time scale.

Segment I

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

MALDI-TOF/MS *SNP Analysis*



How does single base extension work?

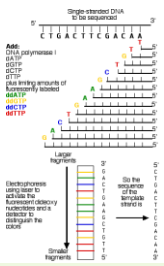
Primer
AGTCATGGC
Target DNA 3' NNNTCAGTACCG**T**GCATANNN 5'

Polymerase ***ddNTP***

Extended Primer
AGTCATGGC**A**
Target DNA 3' NNNTCAGTACCG**T**GCATANNN 5'

Mass Spec

Sanger Sequencing

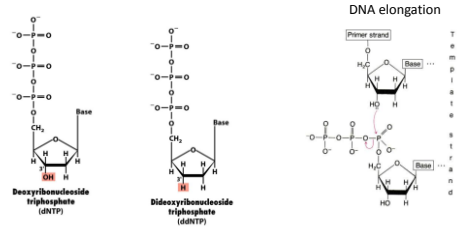


Clinical Chemistry 53, No. 1, 2007 Nucleic Acids Research, 2000, Vol 28, No 12

https://www.jenabioscience.com/molecular-biology/dna-sequencing-sanger-method/dideoxynucleotides-ddntps

How does single base extension work?

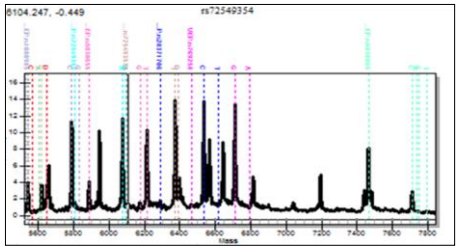
DNA elongation



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

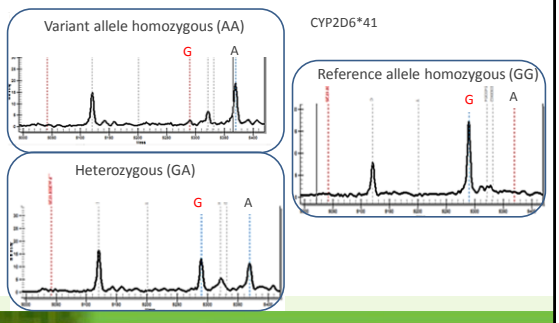
http://www.cas.miamioh.edu/~wilsonkg/gene07/chap12/fig11p22.jpg

A Representative MS Spectra



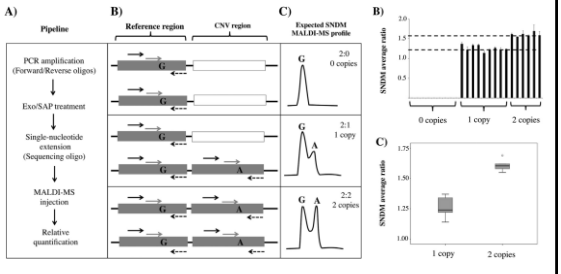
Dr. Roberta Melis, ARUP lab

A Representative MS Spectra



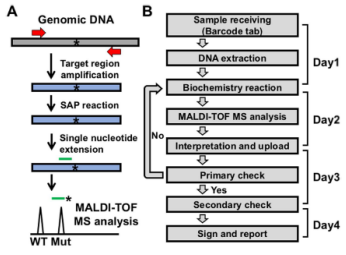
Data was collected by Dr. Roberta Melis

Copy Number Variant Detection with MALDI-TOF



Molecular and Cellular Probes 29 (2015) 517e521

Turnaround Time(TAT)



Su, K.-Y, et.al, Implementation and quality control of lung cancer EGFR Genetic Testing by MALDI-TOF mass spectrometry in Taiwan clinical practice. Sci. Rep. 6,30944