Biomarker Assay Development and Application, Advanced Topics

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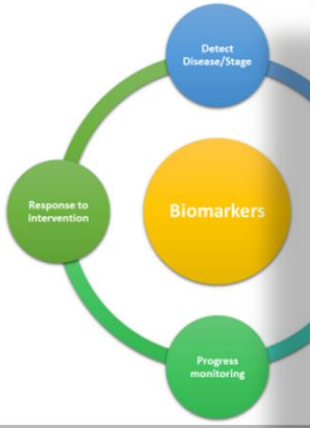
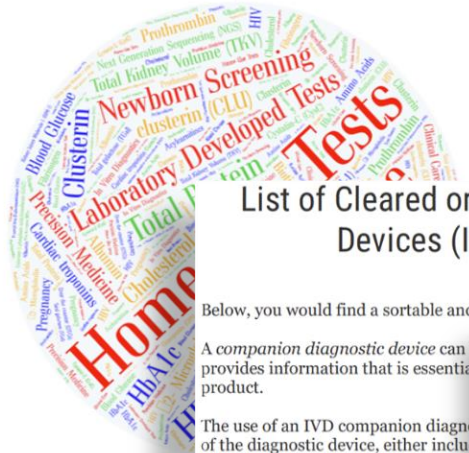
Biomarkers, Best Practices, and Regulatory Guidance

A Biomarker is a measurement, observation or indicator of normal or disease processes, their progression and response to intervention





Clinical Laboratory Improvement Amendments

CAP ACCREDITED Clinical Laboratory Improvement Amendments
COLLEGE of AMERICAN PATHOLOGISTS

List of Cleared or Approved Companion Devices (In Vitro and Imaging

Below, you would find a sortable and searchable table that lists all active

A *companion diagnostic device* can be in vitro provides information that is essential for product.

The use of an IVD companion diagnostic of the diagnostic device, either including products, a specific group of oncology th industry Developing and Labeling In vitro Oncology Therapeutic Products). In addi stipulated in the labeling of the therapeu equivalents and biosimilar equivalents o

Some devices have indication for a specif detailed information can be found in a se

For a list of all FDA cleared or approved

Please submit any questions to DICE@fd

Bioa
Method

Guidan
Technical Spe

For questions regarding thi
CDER at

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2019
Technical Specifications

ICH harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONIS
REQUIREMENTS FOR PHARMACEUTICAL

ICH HARMONISED GUIDE

BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS
M10

Final version
Adopted on 24 May 2022

Regulations: Good Clinical Practice and Clinical Trials

FDA Regulations Relating to Good Clinical Pract
Clinical Trials

Here are links to FDA regulations governing human subject pr
the conduct of clinical trials.

- Electronic Records; Electronic Signatures (21 CFR Part 11)
- Regulatory Hearing Before the Food and Drug Administration (21 C
- Protection of Human Subjects (Informed Consent) (21 CFR Part 50
- Financial Disclosure by Clinical Investigators (21 CFR Part 54)
- Institutional Review Boards (21 CFR Part 56)
- Good Laboratory Practice for Nonclinical Laboratory Studies (21 C
- Investigational New Drug Application (21 CFR Part 312)
- Applications for FDA Approval to Market a New Drug (21 CFR Part
- Bioavailability and Bioequivalence Requirements (21 CFR Part 320
- New Animal Drugs for Investigational Use (21 CFR Part 511)
- New Animal Drug Applications (21 CFR Part 514)
- Applications for FDA Approval of a Biologic License (21 CFR Part 6

INFORMATION SHEET

Institutional Review Boards Frequently Asked Questions

Guidance for Institutional Review Boards and Clinical Investigators

JANUARY 1998

Final

Issued by: Office of the Commissioner, Office of Clinical Policy and Programs, Office of Clinical Policy, Office of Good Clinical Practice

The following is a FDA regarding the protection answers are

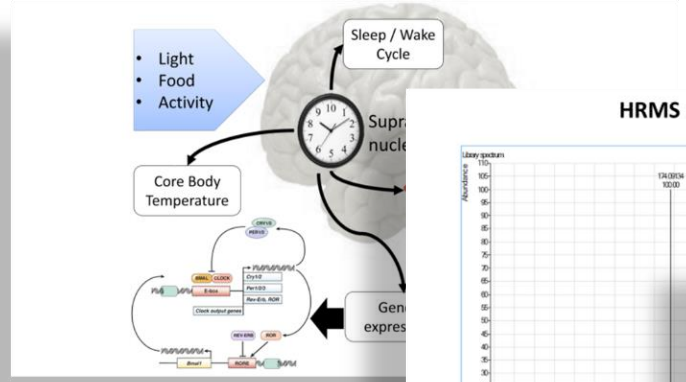
That's right Mara, but we need to be aware these topics exist and how they influence our biomarker work!

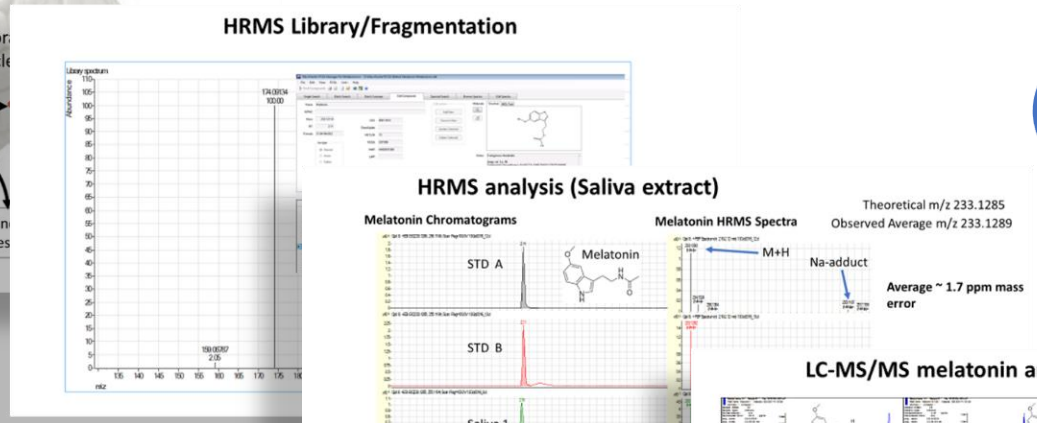
Wow, there is so much to consider!

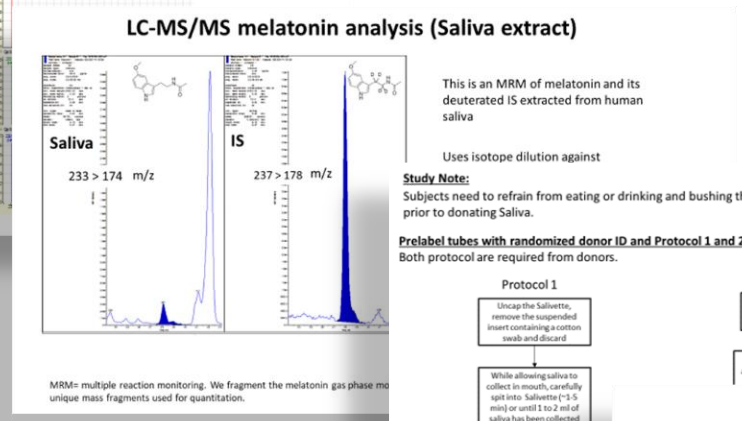
Is this why we are focusing primarily on case studies?

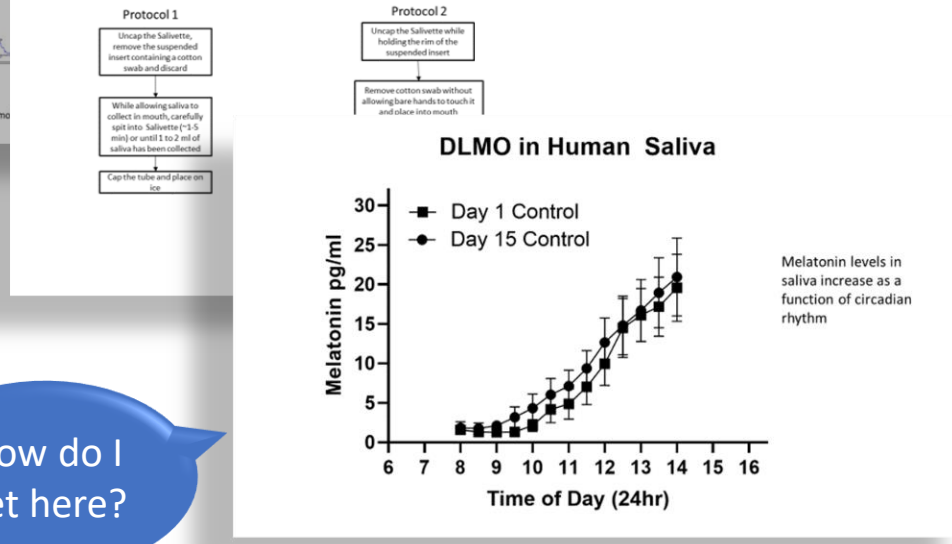
The case studies will help us explain how regulatory and general scientific topics relate to building and testing MS based biomarker assays.

Example Case Study: Quantitation of Melatonin in Human Saliva









Where do I start?

Hmmm, seems straightforward…

Why bother with HRMS? Is it really needed?

Is this method even valid?

Is saliva really a viable matrix for biomarker testing?

Can I use a surrogate saliva matrix?

How do I get here?

Example Case Study: Quantitation of Branched Chain Amino Acids and Keto-Acids

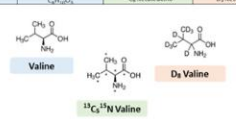
LC-MS BCAA/BCKA Assay Approach

Our validated LC-MS assay measures the concentrations of the three branched chain amino acids and their corresponding ketoacids in human plasma.

This assay uses the surrogate analyte approach to biomarker quantification, where isotopically labeled standards are spiked into authentic matrix (human plasma) to create calibration standards. A second set of isotopically labeled standards are used as the internal standards.

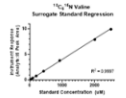
The surrogate analyte:IS area ratio is used to generate a calibration curve from each set of surrogate standards, and this regression is applied to the corresponding endogenous analyte:IS area ratio in the unknown samples to calculate the endogenous concentration values.

Endogenous Analyte		Surrogate Standard	Internal Standard
Valine	$C_5H_{11}NO_2$	$^{13}C_5{}^{15}N$ Valine	D_8 Valine
Isoleucine	$C_6H_{13}NO_2$	$^{13}C_6$ Isoleucine	D_{10} Isoleucine
Leucine	$C_6H_{13}NO_2$	$^{13}C_6$ Leucine	D_{10} Leucine
Ketovaline	$C_5H_8O_3$	$^{13}C_5$ Ketovaline	$^{13}C_5D_3$Ketovaline
Ketoisoleucine	$C_6H_{10}O_3$	$^{13}C_6$ Ketoisoleucine	D_8 Ketoisoleucine
Ketoleucine	$C_6H_{10}O_3$	$^{13}C_6$ Ketoleucine	D_3 Ketoleucine



Pfizer ECD – Precision Medicine LC/MS Biomarker Lab

Assay Performance Metrics: Inter-run QC Precision and Accuracy



Representative Surrogate Standard Calibration Curve

Analyte: Valine	Inter-run Precision %CV	Inter-run Accuracy %Bias	n
Low QC (75.0 µM)	4.1	-1.1	16
Mid QC (250 µM)	5.9	-2.0	16
High QC (1880 µM)	4.9	-5.9	16

Analyte: Ketovaline	Inter-run Precision %CV	Inter-run Accuracy %Bias	n
Low QC (3.0 µM)	3.3	-3.3	16
Mid QC (10.0 µM)	4.2	-2.6	16
High QC (75 µM)	5.1	-4.7	16

Analyte: Isoleucine	Inter-run Precision %CV	Inter-run Accuracy %Bias	n
Low QC (45.0 µM)	5.0	6.9	
Mid QC (150 µM)	6.2	7.3	
High QC (1130 µM)	5.0	-11.7	

Analyte: Leucine	Inter-run Precision %CV	Inter-run Accuracy %Bias
Low QC (45.0 µM)	6.8	8.2
Mid QC (150 µM)	5.1	5.3
High QC (1130 µM)	6.9	-8.0

Analyte: Ketoisoleucine	Inter-run Precision	Inter-run Accuracy

Pfizer ECD – Precision Medicine LC/MS Biomarker Lab

Does the surrogate analyte approach impact processing?

How can I approach isomers?

How do we determine the quantitation range?

Sample Preparation Scheme

Aliquot 30 ul K2 EDTA plasma to 96-well block

Add 270 ul internal standard solution in methanol

Vortex 5 min and incubate at -20°C for 1 hr

Centrifuge for 20 min at 1643 g

Transfer 100 ul of supernatant to new 96-well block and dry under N_2 for ~ 30 min

Reconstitute in 150 ul 0.2% acetic acid in water, vortex for 5 min

Centrifuge for 15 min at 1643 g

Inject 2.5 ul for analysis

Total Time Required: ~4 - 5 hr for a batch of <150 samples

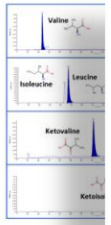
Pfizer ECD – Precision Medicine LC/MS Biomarker Lab

LC/MS Conditions

Shimadzu Nexera LC
MPA: 0.2% Acetic Acid in water
MPB: Acetonitrile
Column: Waters Acquity HSS T3 2.1x100 mm
12 minute total LC method

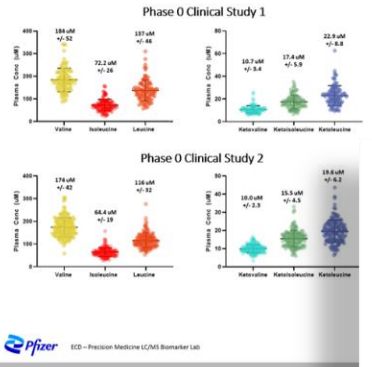
Sciex Triple Quad 5500/6500
Ionization mode: Negative
MS Method: Parent-to-parent MRM

This assay requires chromatographic separation of two sets



Data Processing Workflow

Rationale for Clinical BCAA/BCKA Quantitation Range



Quantification Limits (uM)	Initial Assay Range	Updated Assay Range
Valine	25.0-2500	10.0-1000
Isoleucine	15.0-1500	4.00-400
Leucine	15.0-1500	6.00-600
Ketovaline	1.00-100	0.750-75.0
Ketoisoleucine	1.00-100	0.750-75.0
Ketoleucine	1.00-100	1.00-100

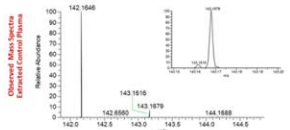
- Based on data from Phase 0 studies, we amended the validation to include lower detection limits for several of the analytes while still

Pfizer ECD – Precision Medicine LC/MS Biomarker Lab

Getting more interesting…

What about isotope envelopes?

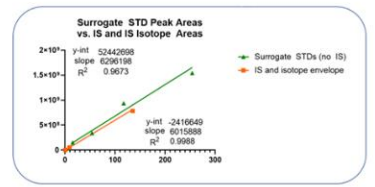
Evaluation of Isotope Envelopes and Potential Use in Quantitation



Endogenous Analyte		Surrogate Standard	Internal Standard
Leucine	$C_6H_{13}NO_2$	$^{13}C_6$ Leucine	D_{10} Leucine

Besides the isotopologues generated from the endogenous leucine, we also can identify isotopologues from the isotopically labeled standards.

The D-10 labeled internal standard used in this assay is particularly helpful because it will still have the natural abundance of the ^{13}C and ^{15}N isotopes.



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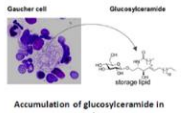
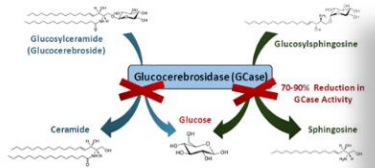
Example Case Study: Quantitation of Glucocerebrosidase Activity from Dried Blood

Gaucher Disease – Type 1 Disease Overview

- Autosomal recessive genetic disorder caused by a deficiency of the lysosomal enzyme, **glucocerebrosidase**, or **GCase**.
- Most common lysosomal storage disorder (~1/50,000, up to 1/800 in Ashkenazi Jewish populations)



Accumulation of glucosylceramide in macrophages



How is this relevant to disease?

LC-MS Methods

LC Conditions

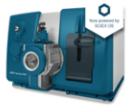
Column:	Water Aquity UPLC BEH C8 2.1 x 50
Mobile Phase A:	0.1% Formic Acid in 60:40 Acetonitrile:Water
Mobile Phase B:	0.1% Formic Acid in 80:20 Isopropanol:Acetonitrile
Flow Rate:	0.4 mL/minute
Gradient:	

MS Conditions

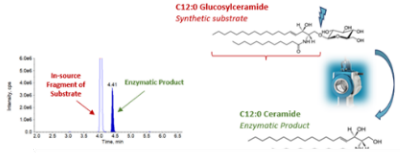
Analyte	Q1 Mass	Q3 Mass	DP (volts)	CE (volts)
C12:0 Ceramide	482.4	264.3	40	35
C14:0 Ceramide	510.4	264.3	40	45
C12:0 Glucosylceramide	644.5	264.3	40	35

Source Parameters: ISV at 4000V; Temp. at 400C; CUR at 20; GS1 and GS2 at 40


Shimadzu Nexera UPLC

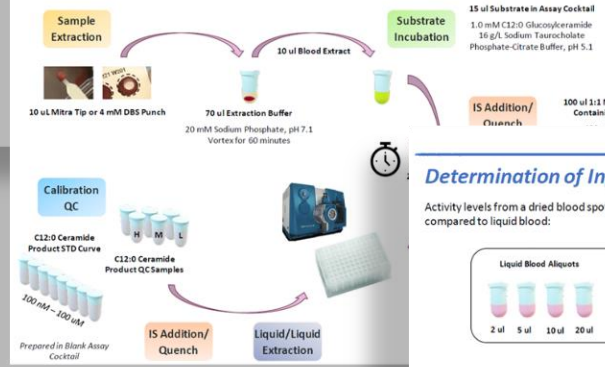

Sciex 6500+

Chromatography was required to separate the in-source fragment of substrate to product (loss of glucose).



Where do I begin?

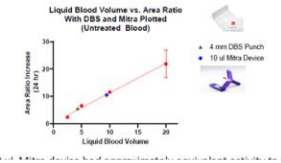
Sample Preparation Scheme – DBS and Mitra Tips



How much blood is in a DBS punch?

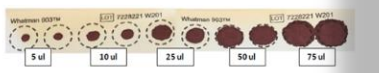
Determination of Input Volume

Activity levels from a dried blood spot or 10 ul Mitra device were compared to liquid blood:



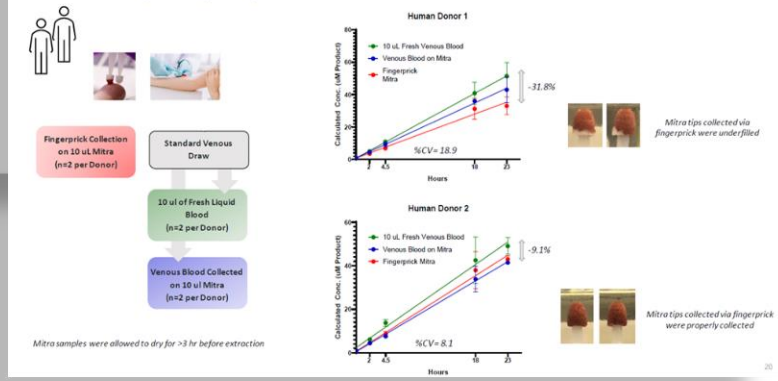
Evaluation of DBS Spot Volumes

Prepare DBS at volumes of 5, 10, 25, 50 and 75 ul (n=2)



4 mM punches (n=2) were extracted and assayed for enzymatic activity.

GCase Activity in Capillary Blood vs. Venous Draws - Two Human Donors



Phew, this can get complicated!

How could we implement this clinically?

That's it? But there is so much more to these……..

Pavan, did you see how much they crammed into a few slides?

I did and I can't wait to take this course!