



Newborn Screening with LC/MSMS

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Genetic Screening

Does LC/MSMS Newborn Screening Reveal Metabolic Diseases around the world?

Inborn Errors of Metabolism (IEMs)

- ▶ Newborn Screening (NBS) with LC/MSMS for IEMs uncover genetic diseases involving disorders of metabolism detected with extremely sensitive methods
- ▶ The majority are due to defects of single genes that code for enzymes which are required in different phases of metabolism
- ▶ Affected enzyme is not functional and thus blocks or alters the metabolic pathway which may cause build-up of harmful metabolites and/or lack of substances that would be produced via normal pathway
- ▶ Newborn Screening with MSMS is currently focused on disorders of amino acid (AA) and acylcarnitine (AC) metabolism
- ▶ Abnormal conditions result in elevated or decreased concentrations of amino acids, free carnitine and acylcarnitines in blood
- ▶ Large number of disorders are treatable and damage can be prevented if treatment is started early enough \Rightarrow highly suitable class of disorders for population screening

Commonly studied organic acid disorders

Organic acid disorders	Analytes	Caused by	Sign & Symptoms	Treatment	Approx. incidence
Propionic acidemia (PA)	C3	Defect in propionyl CoA carboxylase (PCC) enzyme	Protein intolerance, vomiting, ketosis, failure to thrive, lethargy	Diet: protein restriction and supplement of carnitine	1:35 000 to 1:75 000
Methylmalonic acidemia (MMA)	C3, C4DC	Defect in activity of methylmalonyl CoA mutase	Lethargy, failure to thrive, vomiting, dehydration, respiratory distress, hypotonia, hepatomegaly	Diet: low-protein regimen and/or restriction of isoleucine, valine and threonine.	1:50 000 to 1:100 000 (U.S.)
Malonic aciduria (MA)	C5-OH	Defect in malonyl CoA decarboxylase enzyme	Dystonia, hypertonicity, acidosis	Acute treatment with administration of electrolytes and glucose	-
Isovaleric acidemia (IVA)	C5	Defect in Isovaleryl CoA dehydrogenase enzyme	Vomiting, lack of appetite, listlessness, lethargy, neuromuscular irritability	Diet: Restriction of leucine and supplementation of carnitine and glycine	1:200 000

Commonly studied fatty acid oxidation disorders

Fatty acid oxidation	Analytes	Caused by	Signs & symptoms	Treatment	Approx. incidence
Short chain acyl CoA dehydrogenase deficiency (SCAD)	C4	Defect in enzyme that catalyzes β -oxidation of fatty acyl CoA compounds of chain length 4 to 6 carbons.	Metabolic acidosis, neuromuscular manifestations	Diet: Restriction of dietary fat, supplementation of carnitine	1:40 000 – 1: 100 000
Medium chain acyl-CoA dehydrogenase deficiency (MCAD)	C6, C8, C10, C10:1	Defect in enzyme that catalyzes β -oxidation of fatty acyl CoA compounds of chain length 6 to 12 carbons.	Hypoketotic hypoglycemia, lethargy, coma	Diet: avoidance of fasting	1:10 000
Long chain L-3-hydroxyacyl CoA dehydrogenase deficiency (LCHAD)	C16:OH, C18:OH	Defect in L-3-hydroxyacyl CoA dehydrogenase enzyme	Hypoketotic hypoglycemia, death	Diet: avoidance of fasting,	-
Very long chain acyl-CoA dehydrogenase deficiency (VLCAD)	C14:1, C14:2, C16:1, C18:1	Defect in enzyme that catalyzes β -oxidation of fatty acyl CoA compounds of chain length 14 to 20 carbons.	Hypoketotic hypoglycemia, lethargy, seizures	Diet: avoidance of fasting,	>1 : 75 000?

- ▶ SA IEM incidences
 - Some incidences were presented by George & Co. yesterday
 - Need to get an understanding of this by doing a pilot study in SA

How does the extreme sensitivity of MSMS assist in identifying these diseases ?

- ▶ **What is needed in the lab & Clinical area?**
 - High Quality, specificity and multiplex assays for different endocrinology markers
 - Sensitive methods for low concentration of analytes
 - Ready validated & easy to use assays
 - Stabilized methods
 - Standardization of the results
 - Quick turnaround of results

- ▶ **Why not to use the existing IAs?**
 - Lower sensitivity than MSMS

- ▶ **Why not to use HPLC ?**
 - Not adequate separation of all the different analytes
 - Not sensitive enough

What is Mass Spectrometry?

- ▶ An analytical technique in which:
 - Ions are produced from neutral molecules
 - Ions are separated according to their mass-to-charge (m/z) ratio
 - Ions are detected and recorded as a plot of ion abundance vs. m/z (mass spectrum)
 - Which allows you to achieve:
 - Max sens = extreme low detection of analyte available
 - Max spec = no cross reactions with other carbons
 - Multiplexing = multiple analytes at the same time

The Scale of Measurement

1 μM

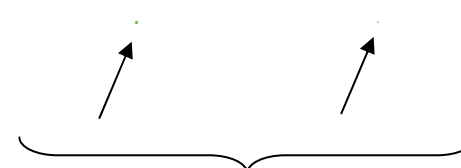
Amino Acids
Acylcarnitines

0.01 μM

•
Organic Acids
(C5DC)

0.001 μM

0.0001 μM



Vitamin D
Steroids

Uniqueness of MSMS Reagent Kits & MSMS Kits offering

► *NeoBase Non-derivatized MSMS kit*

- Internal Standards for amino acids (11) ketone (1), free carnitine (1) and acylcarnitines (12)
- DBS controls for 22 analytes (low and high levels)
- Amount of disorders that can be screened with AAAC measurements ~30
- One kit contains reagents for 960 assays
- Includes plates, plate covers, barcodes etc. needed for the assay
- Lot specific Quality Control certificate is included in each kit

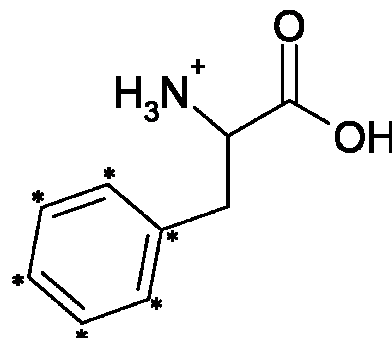
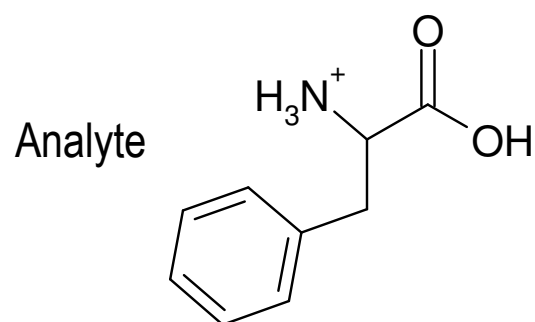
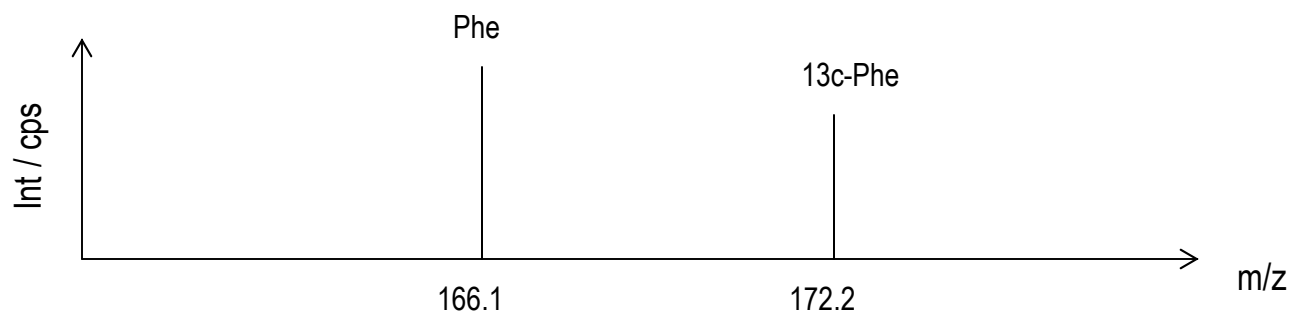


Stable Isotope Dilution MSMS

- ▶ Each dried blood spot sample is extracted with solution containing stable isotope labeled internal standards
- ▶ After extraction, mixture contains naturally occurring analytes (amino acids and acylcarnitines) as well as isotope labeled internal standards
- ▶ Mixture is injected to tandem mass spectrometer and intensity of signals corresponding to analytes and internal standards are obtained

Phenylalanine vs. Phenylalanine IS

Schematic mass spectrum



Isotopically labeled *
Internal Standard (IS)

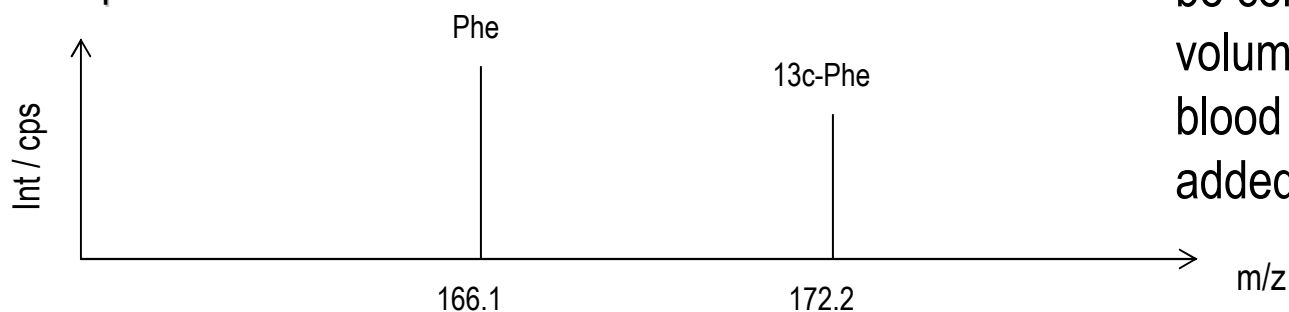
Due to difference in molecular weight the naturally occurring analytes can be separated from the isotopically labeled internal standards

Principle for the calculation of analyte concentration

- ▶ The amount of the internal standard in each sample is known since the concentration of each IS in the added extraction solution is known
- ▶ The concentration of the analyte is obtained by simple comparison of Intensity of Analyte vs. Intensity of the Internal Standard

$$\frac{\text{Analyte intensity}}{\text{IS intensity}} \times \text{IS Conc} \times \frac{\text{Extraction vol}}{\text{Blood vol}} = \text{Analyte conc}$$

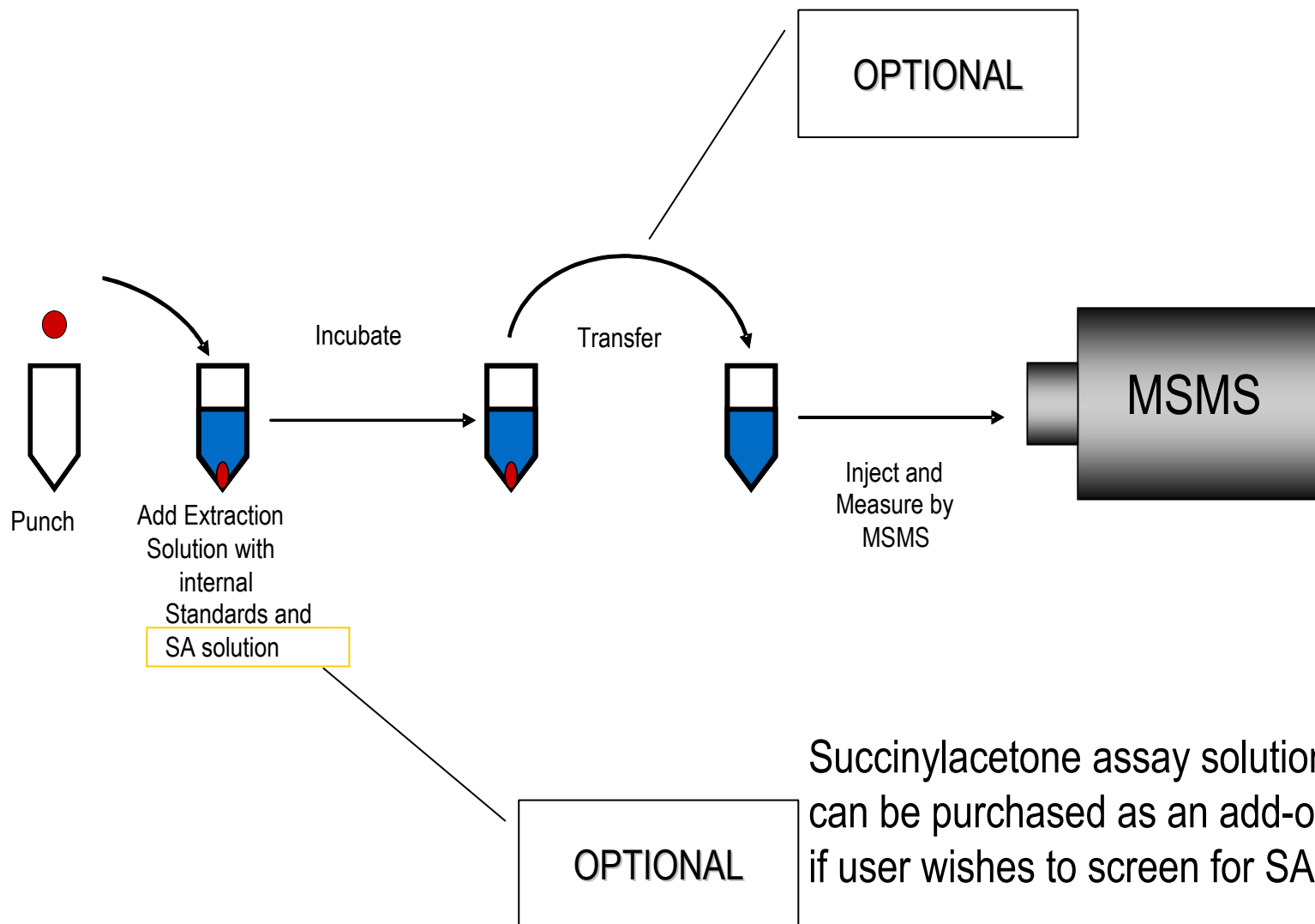
Example:



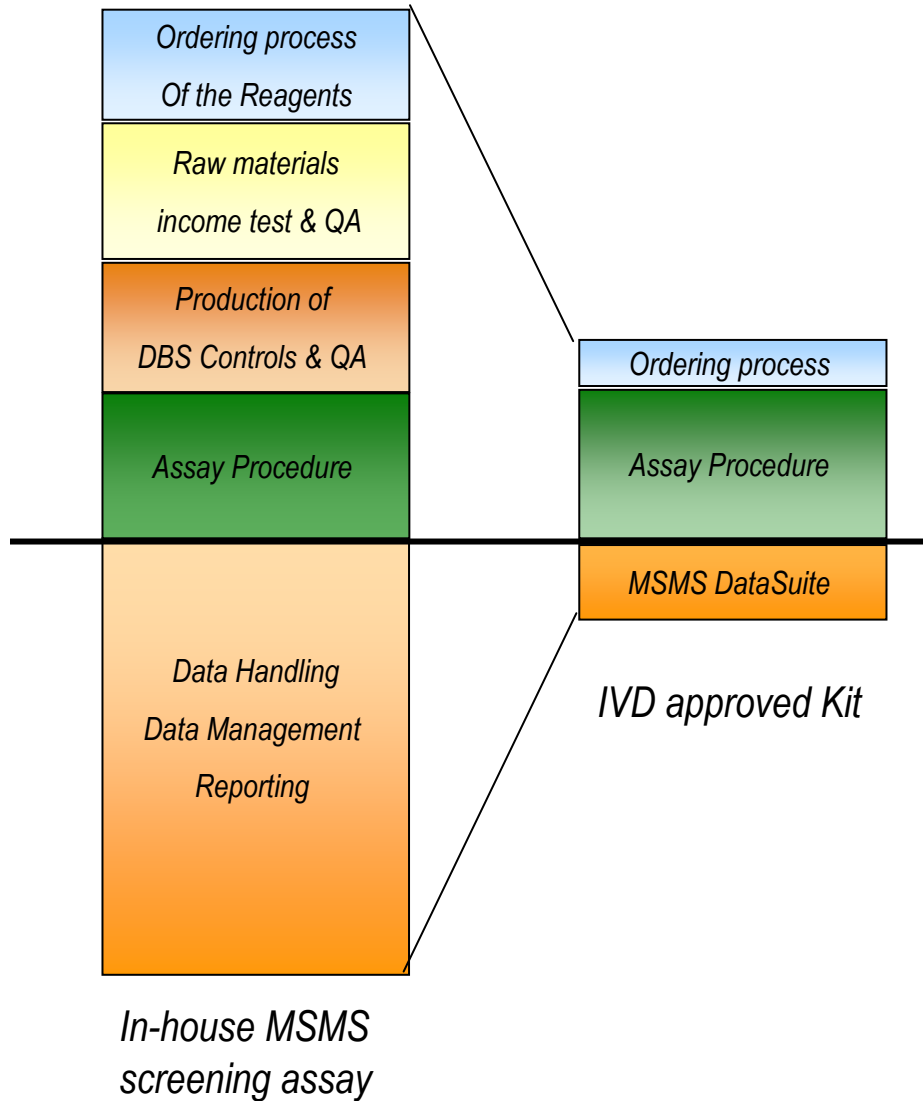
← Calculation has to be corrected with the volume difference between blood in the sample and added extraction solution

$$\frac{100\ 000}{65\ 000} = \frac{100\ \mu\text{l}}{3\ \mu\text{l}} = 256\ \mu\text{M} = \text{Concentration of analyte}$$

NeoBase non-derivatized kit – Assay Procedure



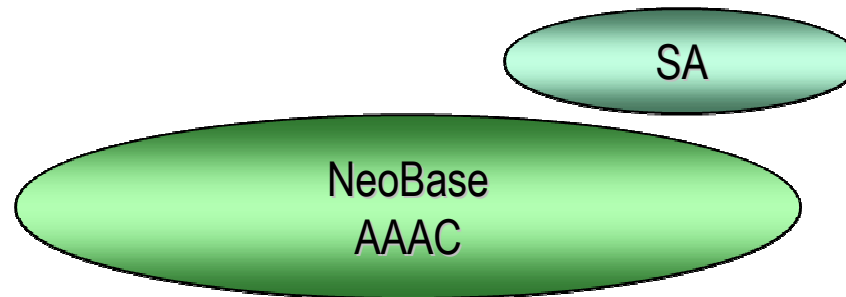
Why should anyone use commercial kits?



- ▶ Lot specific Quality Control certificate is included PerkinElmer MSMS kits manufacturing meets strict quality system requirements (FDA QSR, ISO13485, ISO9001, GMP)
- ▶ All components of the kit (IS, DBS controls, solvents, plates etc.) are qualified and quality controlled
- ▶ High and low DBS controls set at critical decision levels
- ▶ Stability of the reagents proven
- ▶ SpecimenGate MSMS DataSuite for efficient data management
- ▶ Reduced workload
- ▶ Reliability

NeoBase kit can be enhanced with Succinylacetone

- ▶ NeoBase Succinylacetone assay solution
 - Contains NeoBase Succinylacetone Assay Solution (1 bottle, 2.8 ml)
 - Solution is an add-on to the NeoBase kit if user wishes to use succinylacetone in their screening program



Measurable Analytes with NeoBase & SA combination

Amino acids

Alanine	Ala
Arginine	Arg
Citrulline	Cit
Glycine	Gly
Leucine/Isoleucine/Hydroxyproline*	Leu/Ile/Pro-OH
Methionine	Met
Ornithine	Orn
Phenylalanine	Phe
Proline	Pro
Tyrosine	Tyr
Valine	Val

Carnitines

Free carnitine	C0
Acetylcarnitine	C2
Propionylcarnitine	C3
Malonylcarnitine / 3-Hydroxy-butyrylcarnitine*	C3DC/ C4OH
Butyrylcarnitine	C4
Methylmalonyl / 3-Hydroxy-isovalerylcarnitine*	C4DC/C5OH
Isovalerylcarnitine	C5
Tiglylcarnitine	C5:1
Glutarylcarnitine / 3-Hydroxy-hexanoylcarnitine*	C5DC/C6OH
Hexanoylcarnitine	C6

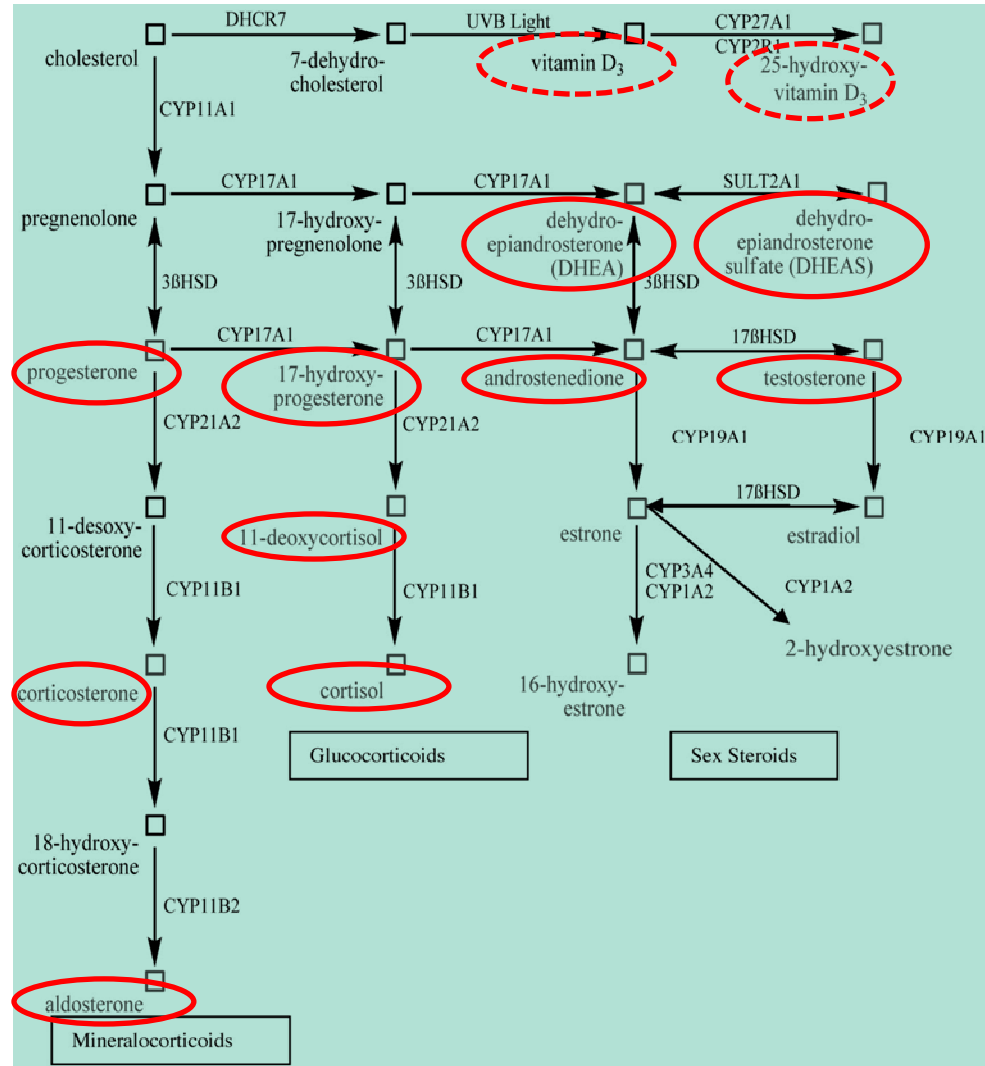
Adipylcarnitine	C6DC
Octanoylcarnitine	C8
Octenoylcarnitine	C8:1
Decanoylcarnitine	C10
Decenoylcarnitine	C10:1
Decadienoylcarnitine	C10:2
Dodecanoylcarnitine	C12
Dodecenoylcarnitine	C12:1
Tetradecanoylcarnitine (Myristoylcarnitine)	C14
Tetradecenoylcarnitine	C14:1
Tetradecadienoylcarnitine	C14:2
3-Hydroxy-tetradecanoylcarnitine	C14OH
Hexadecanoylcarnitine (palmitoylcarnitine)	C16
Hexadecenoylcarnitine	C16:1
3-Hydroxy-hexadecanoylcarnitine	C16OH
3-Hydroxy-hexadecenoylcarnitine	C16:1OH
Octadecanoylcarnitine (Stearoylcarnitine)	C18
Octadecenoylcarnitine (Oleylcarnitine)	C18:1
Octadecadienoylcarnitine (Linoleylcarnitine)	C18:2
3-Hydroxy-octadecanoylcarnitine	C18OH
3-Hydroxy-octadecenoylcarnitine	C18:1OH

Ketones

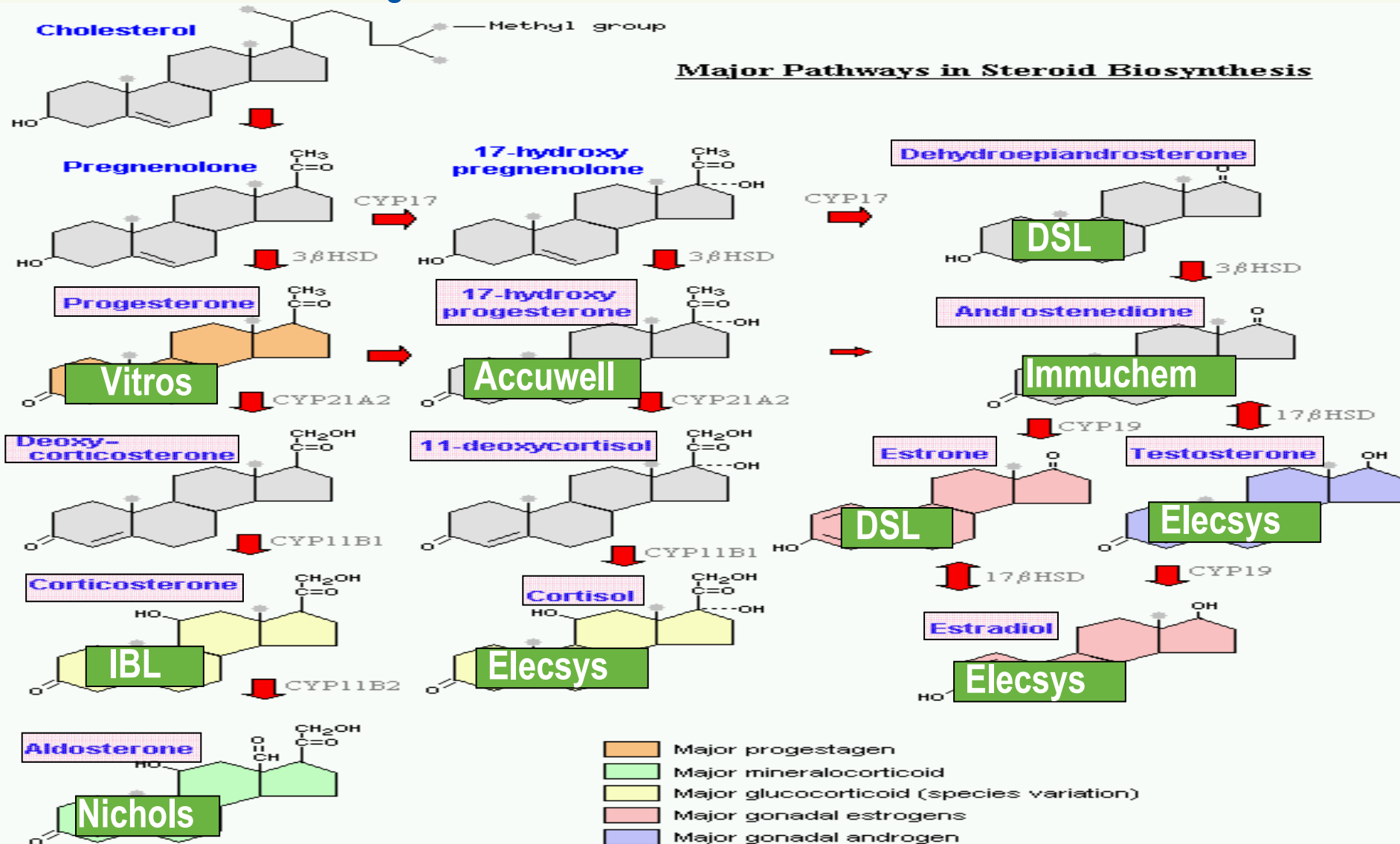
Succinylacetone	SA
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Steroid analytes

- ▶ **UNIQUE product - multiplexing with unique analyte panel**
 - a kit including all analyte specific standards, controls and calibrators



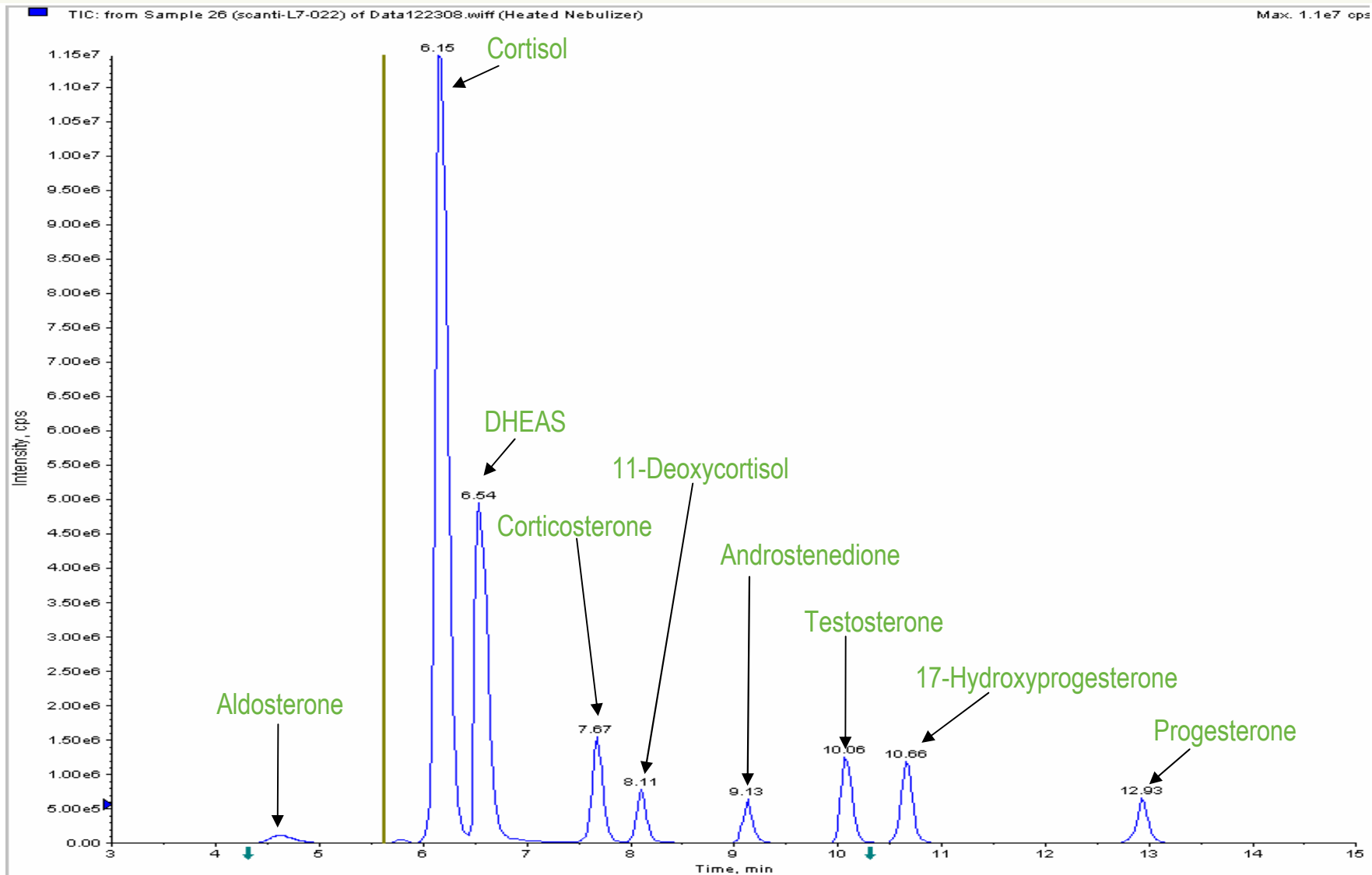
Steroid Hormones Biogenesis



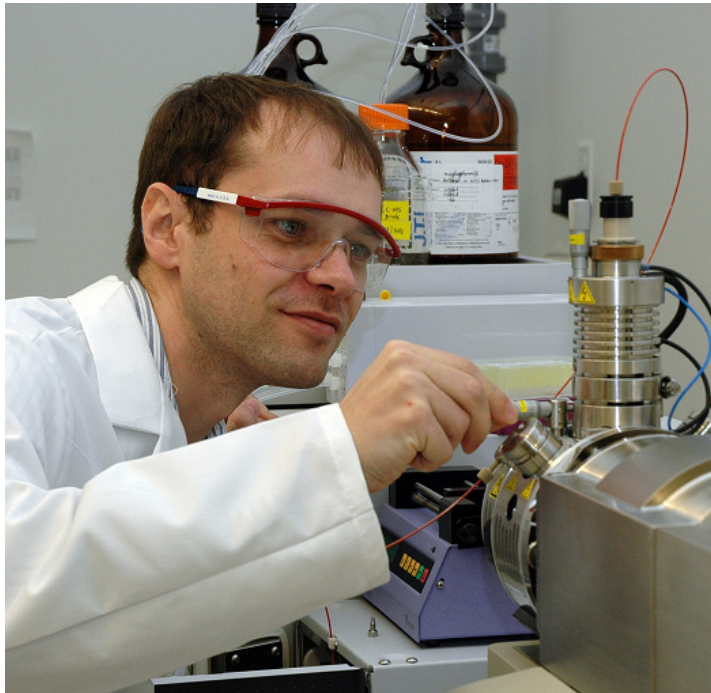
Test Consolidation

- ▶ MSMS benefits and value proposition in Clinical Lab specifically in areas of
 - Endocrine
 - steroids
 - Structural
 - VIT D

Multiples Steroid Analysis



■ MSMS a New Gold Standard!



- Sensitivity
- Specificity
- Speed
- Universal
- Small to large scale molecule detection
- Qualitative
- Quantitative
- Robust
- **Truer analytical results**
- One instrument -> multiple assays
- Better patient care
- **Cost and time efficient**

For further info, contacts

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THANK YOU!

