Condições e tipos de colheitas, processamento, armazenamento e transporte dos produtos biológicos. Colheitas SOS

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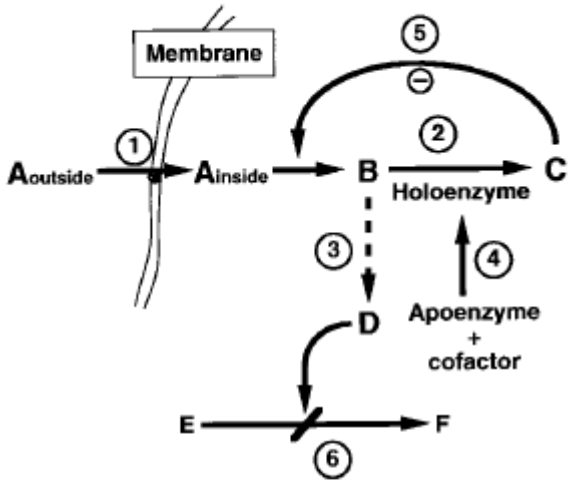
CENTRO HOSPITALAR DO PORTO

Consequências primárias do Erro Inato do Metabolismo

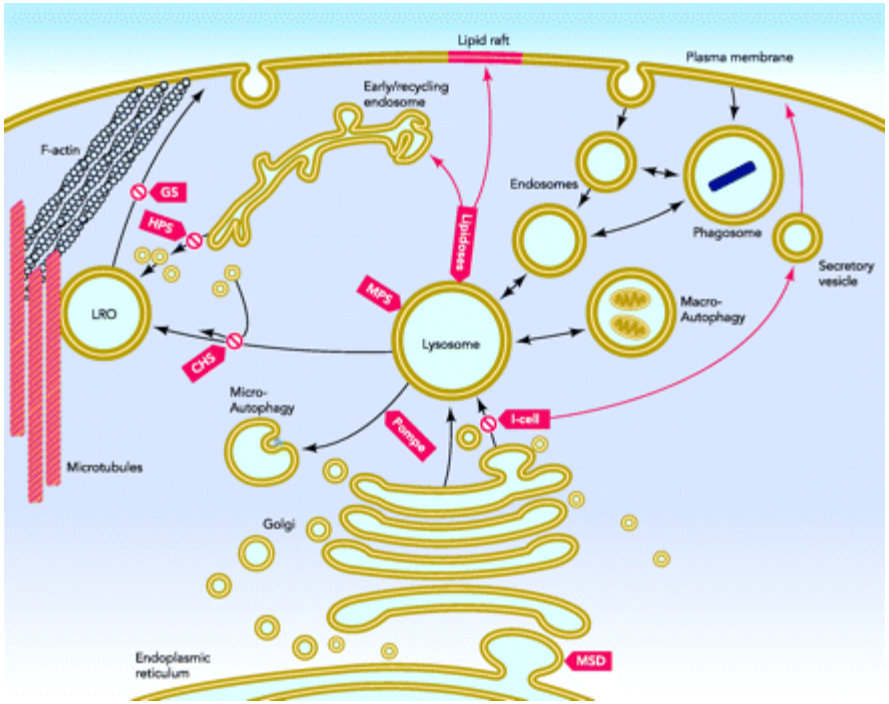
Disease results from point defects in metabolism



The primary consequences of inborn errors of metabolism.
The figure shows diagrammatically the various possible mutation-sensitive defects affecting the compartmentalization and metabolism of Compound A. **1**, transporter-mediated movement of A from one compartment to another; **2**, defect in the conversion of B to C; **3**, increased conversion of B to D caused by accumulation of B; **4**, defect in the interaction between an apoenzyme and an obligatory cofactor; **5**, decreased feedback inhibition of the conversion of A_{in} to B as a result of deficiency of C; and **6**, secondary inhibition of the conversion of E to F caused by accumulation of D.

Patofisiologia das DHM

1. Doenças de intoxicação provocada por precursores ou produtos derivados de pequenas moléculas (e: aminoacidopatias)
2. Défices energéticos – privam a célula de energia para o seu normal funcionamento- défices da oxidação dos ácidos gordos e mitocondriais
3. Doenças envolvendo moléculas complexas- Doenças de sobrecarga do Lisossoma, defeitos da síntese do colesterol, defeitos congénitos da glicosilação



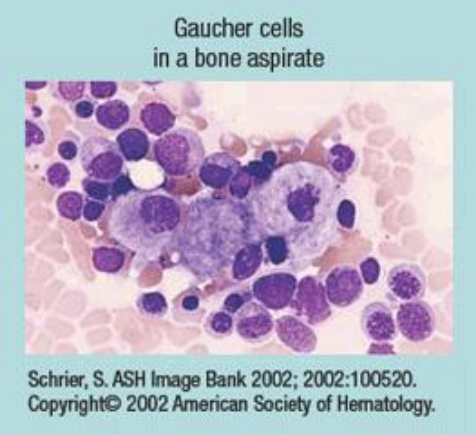
Categorias de apresentação clínica das DHM

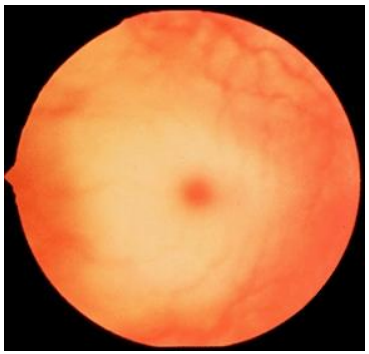
Table 9.1. Clinical differentiation of organelle disease and small molecule diseases

Feature	Organelle disease	Small molecule disease
Onset	Gradual	Often sudden, even catastrophic
Course	Slowly progressive	Characterized by relapses and remissions
Physical findings	Characteristic features	Nonspecific
Histopathology	Often reveals characteristic changes	Generally nonspecific changes
Response to supportive therapy	Poor	Brisk

Categorias de apresentação clínica das DHM

1. Sintomas precoces no período pré-natal ou neonatal
2. Apresentação tardia e descompensação recorrente como coma, ataxia, vómitos e acidose
3. Sintomas generalizados e progressivos que podem ser gastrointestinais, musculares ou neurológicos
4. Específicos e permanentes com órgãos alvo sugestivos de DHM como cardiopatia, hepatomegalia, deslocação do cristalino, etc

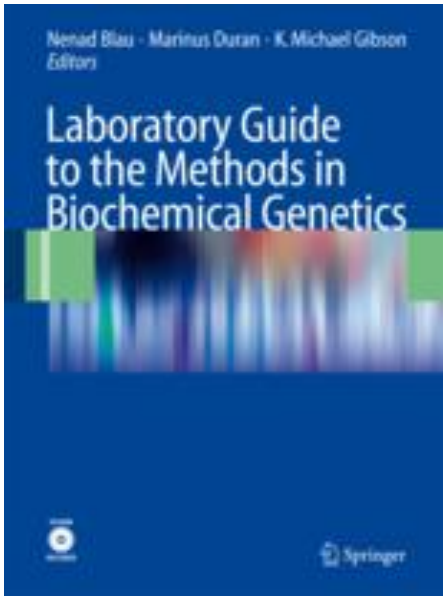




Mancha vermelho cereja na retina de doentes Tay Sachs

Diagnóstico das DHM

- Compostos acumulados ou deficientes
- Determinação da atividade da enzima deficitária
- Identificação de compostos que estando presentes não se apresentam na sua conformação normal
- Estudo molecular



Metabolic Profile over the Course of the Day

Indications

This first line of evaluation of intermediary metabolism may be performed following an initial or recurrent clinical incident associated with:

- a disturbance of intermediary metabolism in which the aetiology is unknown.
- The investigation is used in the metabolic/endocrine investigation of hypoglycaemia, hyperlactatemia, hyperketosis or hypoketosis
- in these situations, should always be undertaken before any provocative test that may lead to metabolic decompensation.
- The metabolic profile is also used for monitoring treatment in many disorders.

Investigações laboratoriais de rotina

Anemia (macrocytic)	Disturbances in cobalamin or folic acid metabolism or transport
Reticulocytosis	Glycolysis defects, disorders of the γ-glutamyl cycle
Vacuolized lymphocytes	Lysosomal storage disorders
↑ Alkaline phosphatase	Hypoparathyreoidism, bile acid synthesis defects
↓ Cholesterol	A-, hypobetalipoproteinemia, sterol synthesis defects, peroxisomal disorders
↑ Triglycerides	Glycogen storage disorders, lipoprotein disorders, e.g., lipoprotein lipase deficiency
↑ CK	Mitochondrial disorders, fatty acid oxidation defects, glycogen storage disease types II, III, and IV, glycolysis defects, muscle-AMP-deaminase deficiency, dystrophinopathies
↑ α-Fetoprotein	Ataxia telangiectasia, hepatorenal tyrosinemia
↓ Glucose in CSF	Mitochondrial disorders, glucose transport protein deficiency
↑ Uric acid	Glycogen storage disorders, disorders of purine metabolism, fatty acid oxidation defects, mitochondrial disorders
↓ Uric acid	Disorders of purine metabolism, molybdenum cofactor deficiency
↓ Creatinine	Creatine synthesis defect
↑ Iron, transferrin	Hemochromatosis, peroxisomal disorders
↓ Copper (in plasma)	Wilson disease, Menkes disease
↑ Copper (in plasma)	Peroxisomal disorders
↑ Copper (in urine and liver)	Wilson disease, peroxisomal disorders
↓ Ceruloplasmin	Wilson disease, Menkes disease, aceruloplasminemia
Hypothyroidism, hypoparathyreoidism	Mitochondrial disorders, CDG syndromes

AMP adenosine monophosphate; *CDG* congenital disorders of glycosylation; *CK* creatine kinase

- Achados inesperados na avaliação inicial exigem uma avaliação crítica.

- Particularmente em doentes com sintomas inexplicados e pouco comuns que possam indiciar um EHM e podem orientar para investigações específicas

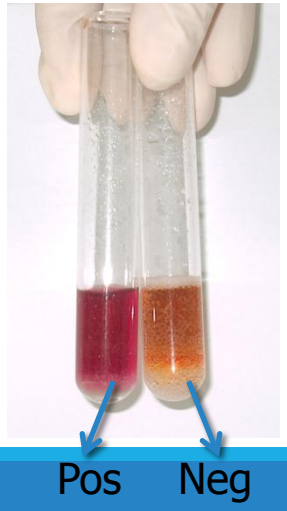
Testes simples - urina

Testes colorimétricos simples na urina: Pesq. substâncias redutoras na urina para diagnóstico da IHF, galactosemia

- Falsos positivos – antibioticoterapia

Prova de Brand identifica os grupos sulfidril (-SH) livres ou dissulfitos, como nas Homocistinúrias.

- Falsos negativos em urina acidificada.



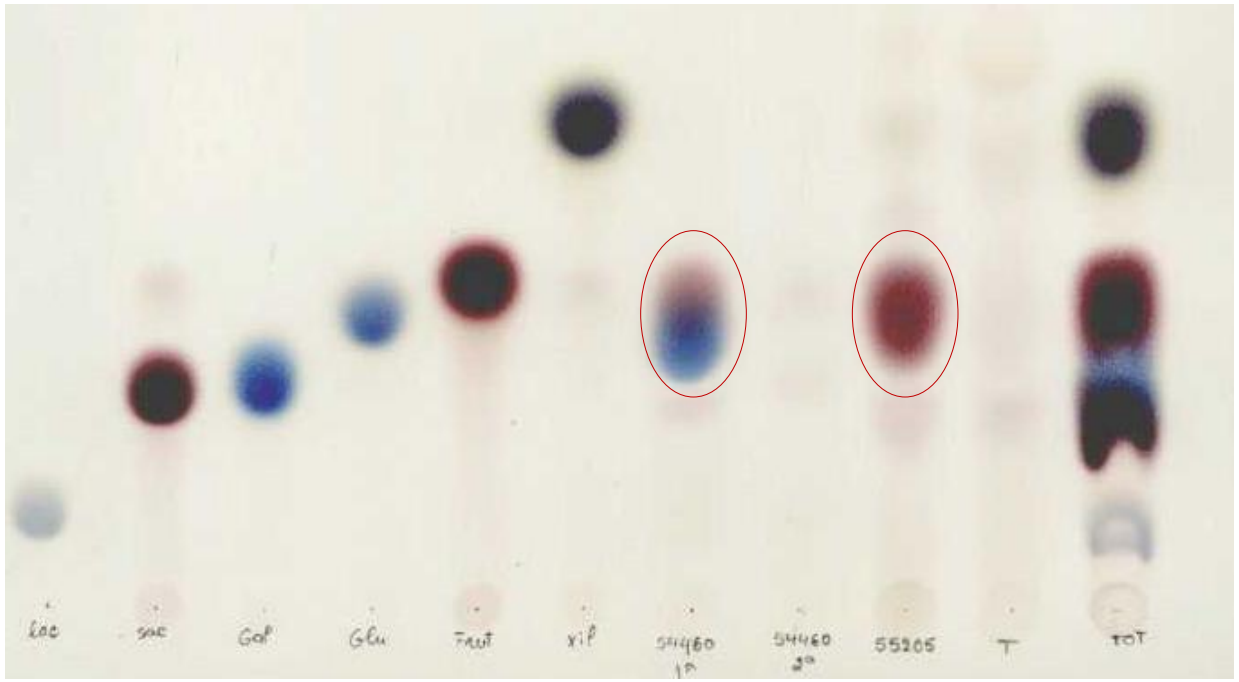
Reducing substances

Although the presence of nonglucose reducing substances in the urine of a sick

newborn may suggest galactosemia, their absence does not rule out the possibility.

Even short-term galactose restriction is usually sufficient to reverse the galactosuria of the disease. Because the enzyme defect is demonstrable in erythrocytes, but not in plasma, specimens of whole blood should be submitted for galactosemia screening, and the blood must be obtained before the infant receives any transfused blood.

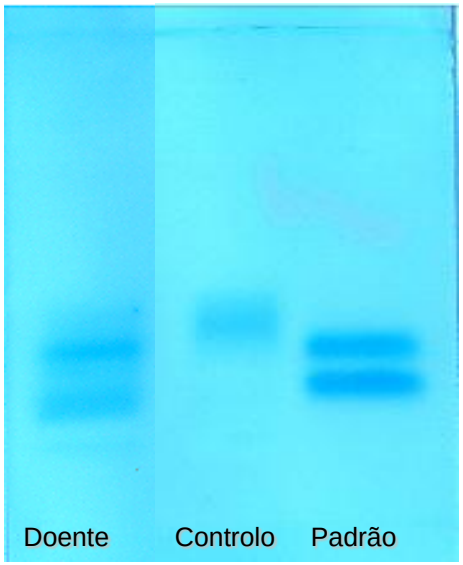
Técnicas simples – Cromatografia de açucares



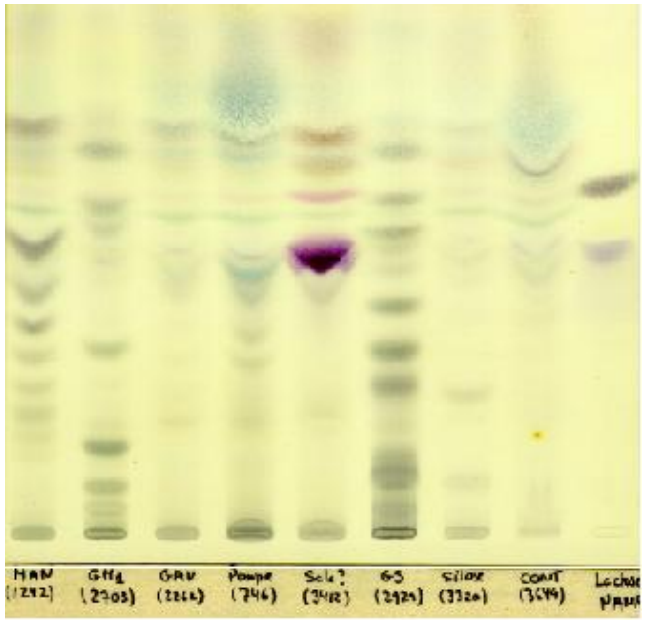
Ex: É fundamental a colheita em crise e, se possível, antes de serem tomadas medidas terapêuticas- IHF com e sem fluidoterapia

Técnicas simples

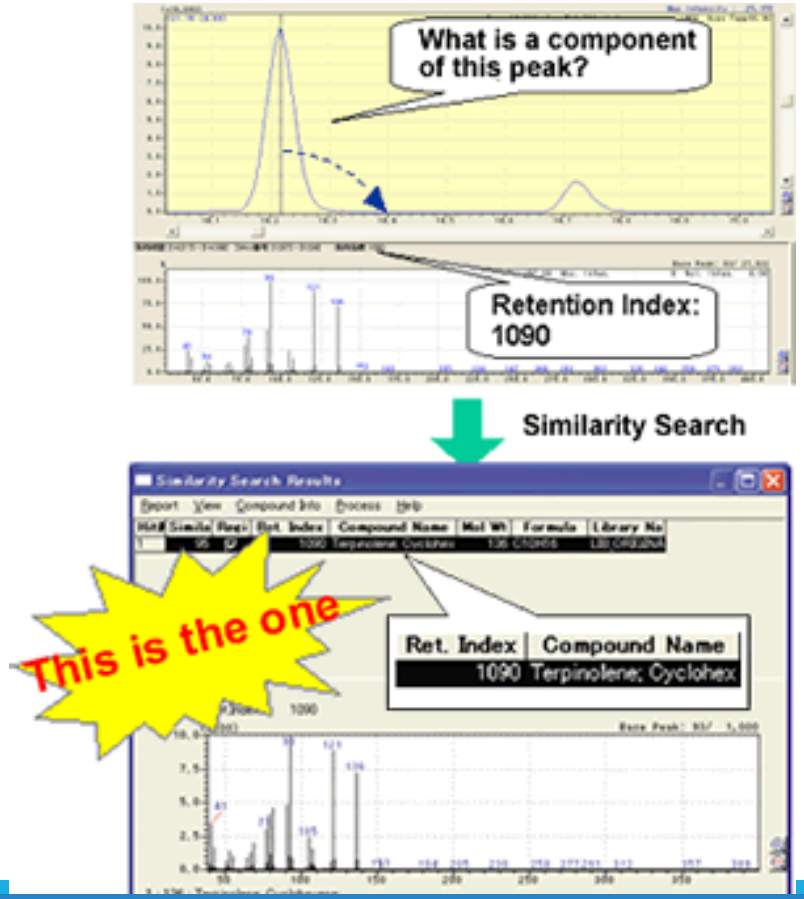
- Eletroforese de Glicosaminoglicanos na urina



- Cromatografia de oligossacaridos na urina



Equipamentos de um Laboratório de DHM



GC- MS aplicado a diferentes métodos:

- Identificação e doseamento de ácidos orgânicos
- Doseamento de metabolitos intermédios de vias metabólicas como colesterol e creatina

Outros métodos e equipamentos..

HPLC – ácido orótico, Neurotransmissores

Fluorímetro para doseamentos de enzimáticos de enzimas

do lisossoma

Espectrofotómetro, autoanalizador…..

Testes para investigação das DHM

Amónia, lactato, piruvato, carnitina

Aminoácidos, homocisteína total

Ácidos orgânicos, creatina, 7-DHC, neurotransmissores

Ácidos gordos de cadeia muito longa, ácido fitânico

Ensaios enzimáticos – lisossoma, CRM

Testes organolépticos – Cor e cheiro da urina

Table 3.5. Collection, processing and storage of blood, urine, and cerebrospinal fluid (CSF) for metabolic and endocrine investigation. The volumes of blood, urine, and CSF are subject to local practice, which must be taken into account

Blood

Hematology: 0.5 ml in EDTA tube
Blood gases: 0.5 ml on heparin-coated syringe (eject air bubble, cap syringe immediately)
Electrolytes, urea, creatinine, urate, total protein, liver function tests: 1–2 ml (centrifuge after clotting)
Glucose: 0.3 ml fluoride-heparin cup (dry heparin and fluoride salts, no solution)
Lactate/pyruvate and 3OHB/AcAc: 1 ml blood (no forcing), mix immediately with 0.5 ml perchloric acid (18% v/v, keep on ice, centrifuge under refrigeration, store supernatant at –20°C)
Ammonia: 0.5 ml in heparin-coated syringe on ice (eject air bubble, cap syringe immediately)
Amino acids: 1–2 ml in EDTA or heparin tube
Carnitine: 1–2 ml in EDTA tube on ice, centrifuge under refrigeration, store at –20°C
Free fatty acids: 0.3 ml in fluoride-heparin cup (dry heparin and fluoride salts, no solution)
Insulin: 1 ml in EDTA tube on ice, centrifuge under refrigeration, store at –20°C
Cortisol and ACTH: 1 ml in plastic, heparin-coated syringe (keep on ice, centrifuge under refrigeration in plastic tube, store at –20°C)
Growth hormone: 1 ml (centrifuge under refrigeration after clotting, store at –20°C)
Glucagon: 3 ml in heparin tube (centrifuge under refrigeration, store in plastic vial at –20°C)

Urine

pH, amino acids, organic acids, ketone bodies, lactate, reducing substances: 5 ml (at least), freeze at –20°C

Cerebrospinal fluid

Cells, protein, glucose: 0.5 ml in plastic tube
Lactate/pyruvate: 1 ml, add to 0.5 ml perchloric acid (18% v/v, keep on ice), centrifuge under refrigeration, store supernatant at –20°C
Amino acids: 0.5 ml in plastic tube
Culture: 1 ml in sterile tube

AcAc, acetoacetate; *ACTH*, adrenocorticotrophic hormone; *EDTA*, ethylenediaminetetraacetic acid; *3OHB*, 3-hydroxybutyrate.

Factores pré-analíticos - Amónia

Punção venosa – Colheita? Amostra hemolisada?

Transporte – o sangue deve chegar ao laboratório no prazo de 2 horas, caso não seja possível, o sangue deve ser centrifugado o plasma deve ser separado e congelado até ser efectuado o ensaio

Jejum

Colheita com anticoagulante inadequado – contaminantes

Hiperamonémia secundária

Doença hepática severa

Diminuição do fluxo sanguíneo ao fígado diminui a capacidade de metabolizar a amónia - cardiopatias

Síndrome de Reye

Falência renal

Metabolismo Proteico-Amónia

Amónia é um composto produzido pelas bactérias intestinais e pelas células do corpo durantes a digestão das proteínas

Produto de degradação transmitido normalmente para o fígado, onde é convertido em ureia e glutamina.

Se o Ciclo da Ureia não garante uma completa degradação da amónia - acumulação no sangue e atravessa a barreira hemato-encefálica- neurotóxica

Nenhum outro teste laboratorial sustitui este doseamento, nem nenhum outro indicia a necessidade de o efectuar – a suspeita clínica desempenha um papel primordial

Plasma lactate and pyruvate

Plasma lactate and pyruvate levels are generally analyzed enzymatically.

same precautions required for accurate measurement of plasma ammonium apply to measurements of plasma lactate.

The use of a tourniquet and delayed sample handling are common causes of spurious elevations of plasma lactate.

Levels in arterial blood and CSF are generally more reliable than venous plasma lactates.

The measurement of pyruvate is cumbersome, and the results are seriously prone to error. Pyruvate levels in blood are always at least an order of magnitude lower than lactate levels, and pyruvate in blood is unstable.

Lactate to pyruvate ratios in plasma should, therefore, be interpreted with care, especially if the lactate concentration is not elevated.

Análise quantitativa e qualitativa de Aminoácidos

Plasma / Soro

- a concentração de AA no soro é ↑↑ do que no plasma
  - libertação dos AA das células durante a coagulação

Urina

- Um dos fluídos fisiológicos mais complexos
  - 175 compostos que reagem com a ninidrina!
  - Num adulto normal, o conteúdo em AA durante 24h é constante e aparentemente independente da dieta

LCR

- A concentração dos AA é < à do plasma: 5-15%
  - Antigamente era necessário concentrar por liofilização
  - Evitar contaminação de sangue

Análise quantitativa e qualitativa de Aminoácidos

Líquido amniótico

- A conc. de AA é semelhante à do plasma
- Deve ser isento de sangue

Humor vítreo

- É particularmente útil na colheita *post-mortem* por ser mais estável

Embora a análise quantitativa dos aminoácidos possa apresentar ligeiros desvios em um, ou mais aminoácidos não implica necessariamente um defeito no metabolismo dos aminoácidos.

Factores que afectam a concentração dos aminoácidos

Idade – taurina, prolina,hidroxiprolina, glicina

Variações fisiológicas – ritmo sarquidiano, prematuridade, diabetes materno, gravidez, exercício prolongado

Estado nutricional- obesidade

Patologia associada – infecção, hiperinsulinismo

Medicação administrada – valproato, ampicilina/amoxicilina

Intoxicações

Condições pré-analíticas com alterações no perfil de Aminoácidos

Factor/Condition	Source	Amino acid(s) affected	Value	
Contamination, bacterial	U	Ala, Gly, Pro	↑	H
Contamination, bacterial	U	Trp, aromatic amino acids, Ser	↓	L
Contamination, fecal	U	Pro, Glu, Leu, Ile, Val, OH-pro-line	↑	H
Contamination, protein	U	Cys	↓	L
Contamination, RBC	U	Orn	↑	H
Contamination, unwashed skin	B	most amino acids	↑	H
Contamination, WBC	U	Tau	↑	H
Contamination, WBC	B	Asp, Glu, Tau	↑	H
Hemolysis	B	Asp, Glu, Gly, Orn	↑	H
Hemolysis	B	Arg, Gln	↓	L
Serum vs. plasma	B	Serum Tau >plasma Tau		
Serum vs. plasma	B	serum homocysteine >plasma homocysteine		
Storage	U	Glu, Asp, GABA	↑	H
Storage	U	Gln, Asn, phosphoethanol-amine	↓	L
Storage	B	Gln, Cys, homocyst(e)ine	↓	L
Storage	B	Glu	↑	H
Tube artifact, thrombin	B	Gly	↑	H
Tube artifact, EDTA	B	Ninhydrin positive artifact		
Tube artifact, metasulfite	B	S-sulfocysteine	↑	H
Unspun blood left at rm. temp.	B	Orn, total homocysteine	↑	H
Unspun blood left at rm. temp.	B	Arg, Cys, homocystine	↓	L

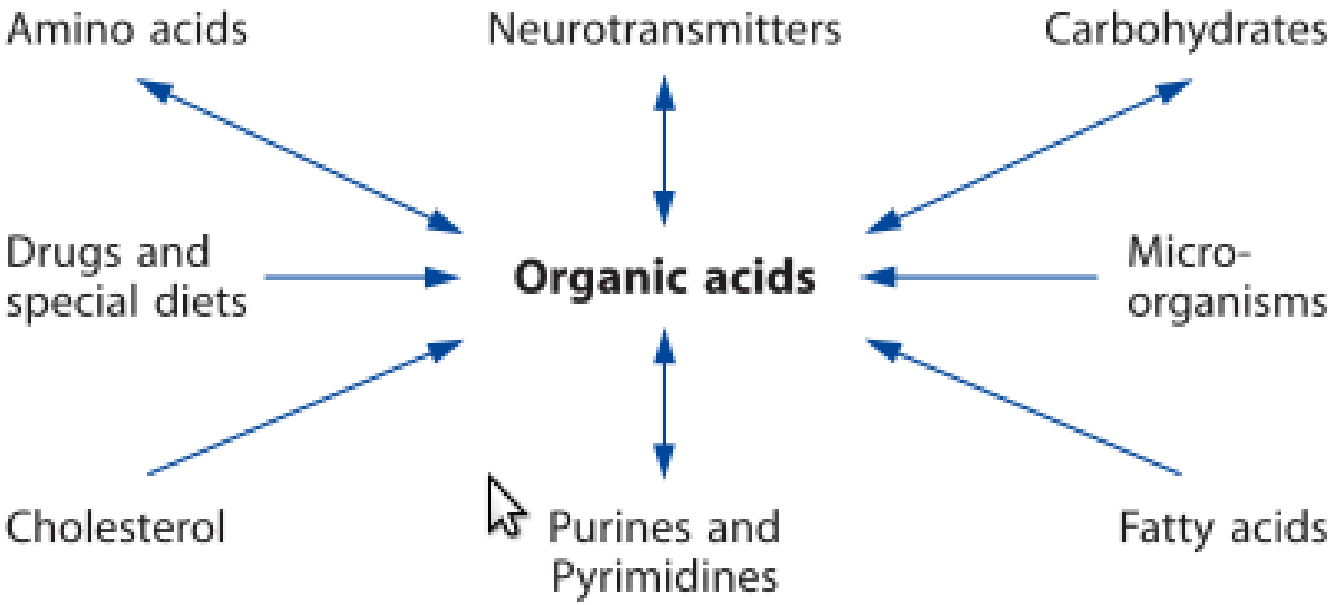
Analysis of acylcarnitines

Analysis of acylcarnitines and selected amino acids in very small amounts of blood, such as the dried blood spots used for screening for phenylketonuria (PKU), by tandem MS–MS

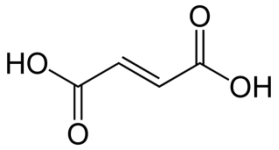
is becoming more widely available as more tertiarycare pediatric institutions adopt this relatively new technology.

This is a particularly fast and powerful tool for the investigation of a wide range of disorders of organic acid, fatty acid and amino acid metabolism.

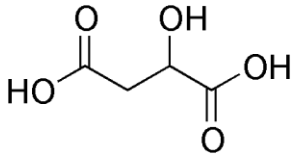
Ácidos orgânicos no metabolismo intermediário de pequenas moléculas



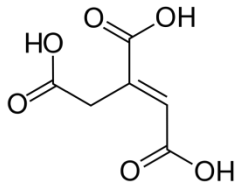
Que compostos?



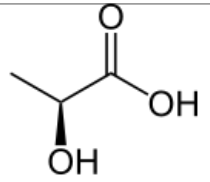
Ácido fumárico



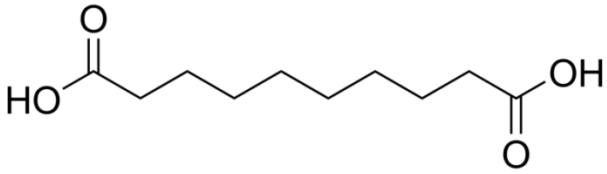
Ácido málico



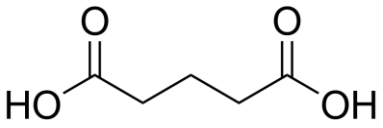
Ácido aconítico



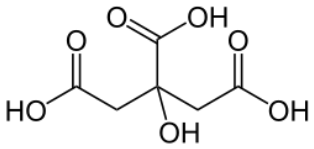
Ácido láctico



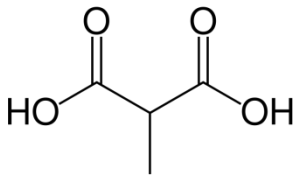
Ácido sebácico



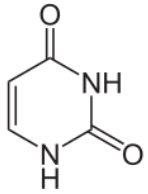
Ácido glutárico



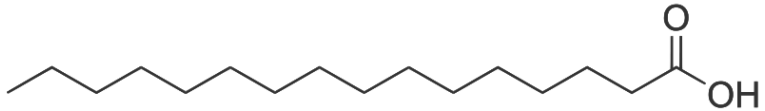
Ácido cítrico



Ácido metilmalónico



Uracilo



Ácido palmítico

Condições pré-analíticas

Primeira urina da manhã – mais concentrada, congelada – amostra de eleição

Informação sobre o estado clínico do doente – crise/após a crise ausência de crises

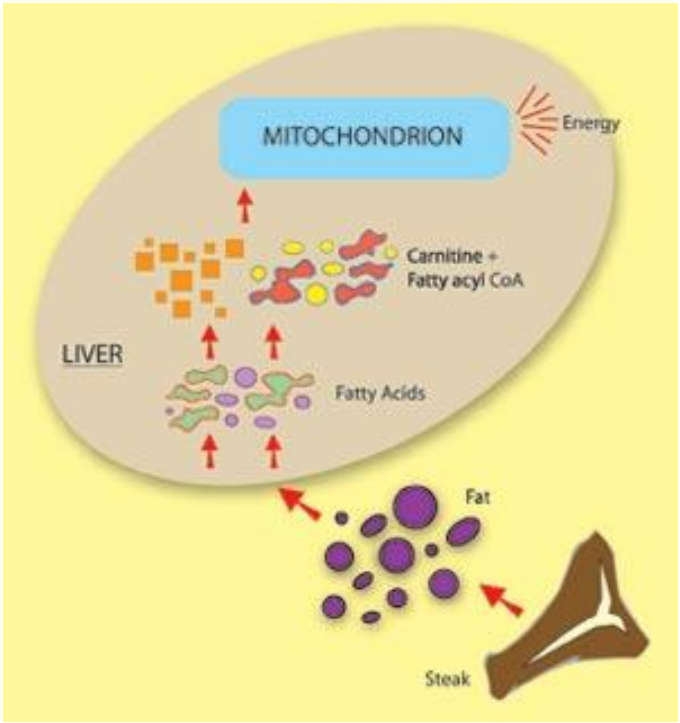
Informação sobre alterações dietéticas

Terapêutica instituida

Artefactos na análise de ácidos orgânicos

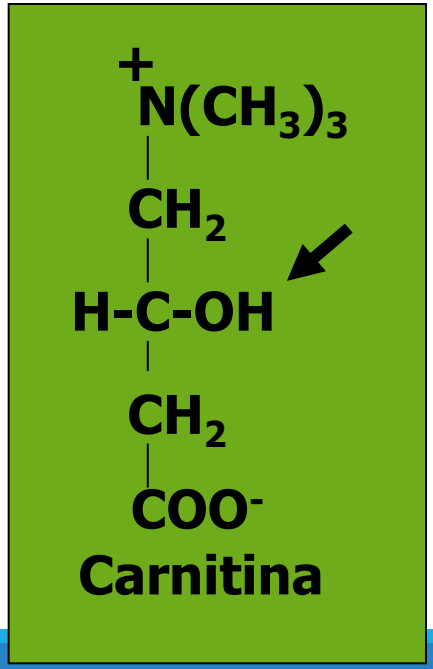
Condition	Organic acid(s) involved	Value (↑/↓)
Dietary origin	Adipic acid	↑
	Furoic acid	↑
	Tartaric acid	↑
	N-Acetyltyrosine	↑
	N-Acetyltryptophane	↑
MCT feeding	Sebacic → suberic → adipic acid	↑
	7-Hydroxyoctanoic acid;	↑
	octanoylglucuronide	↑
Nutramigen feeding	5-Oxoproline	↑
Pregestimil feeding	di-(2-ethylhexane)phthalate	↑
Medications	Cyclohexanediol	↑
i.v. Solutions	Propanediol	↑
L-DOPA	Vanillinlactic acid	↑
Paracetamol (acetaminophen)	5-Oxoproline	↑
Valproic acid	Numerous metabolites (including dicarboxylic acids)	↑
Acetylsalicylic acid	2-Hydroxyhippuric acid	↑
Ethosuximide	Numerous metabolites	↑
Phenytoin	Numerous metabolites	↑
Primidone	Numerous metabolites	↑
Ethylene glycol poisoning	Ethylene glycol	↑
	Glycolic acid, oxalic acid	↑
	Benzoic acid	↑
	Hippuric acid	↓
	Glutaric acid	↑
Bacterial contamination	3-Hydroxypropionic acid	↑
	4-Hydroxyphenylacetic acid	↑
	D-Lactic acid	↑
	2-Oxoglutaric acid	↑
	Phenylpropionylglycine	↑
	Succinic acid	↑
	Uracil	↑

L- CARNITINA



L-carnitina é derivado do aminoácido lisina. O seu nome é derivado de ter sido isolada da carne (*carnus*).

Em determinadas condições as necessidades de L-carnitine podem exceder a capacidade individual de síntese – micronutriente essencial



Amostras para doseamento da carnitina

Soro para determinação da carnitinina livre

Urina para determinação da carnitina livre e total

Sangue em cartão para realizar perfil de acilcarnitinas

- Indicação da terapêutica é fundamental – inibição da hidrólise para o doseamento da carnitina total na (Carnitina livre>carnitina total!)
- Suplementação com carnitina

Analysis of acylcarnitines

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Cholestanol

description / indication

Cerebrotendinous xanthomatosis

Method GC/GCMS

material **plasma** amount 500 µl conditions dry ice material **serum** amount 500ul conditions dry ice

Creatine and guanidinoacetic acid

description / indication

Creatine synthesis and transport defects

Method LC tandem MS/GC-MS

material **plasma** amount 250 µl conditions dry ice material **urine** amount 1 ml, preferably 24-hour urine conditions dry ice

Diagnóstico de Doenças Lisossomais de Sobrecarga em DBS

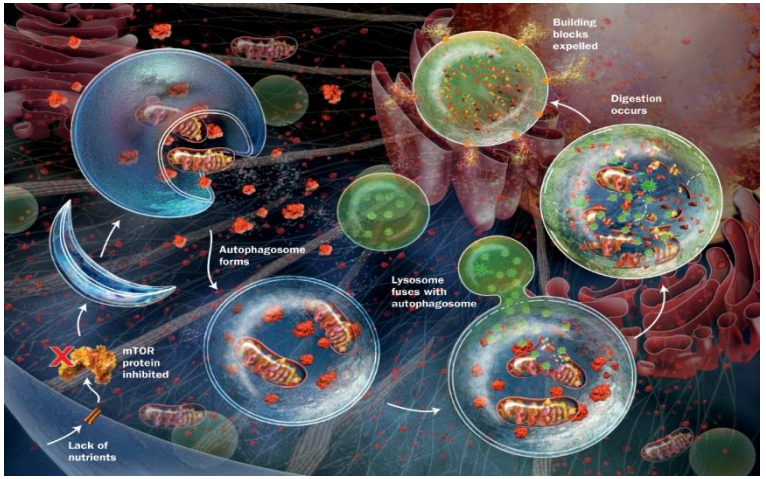
❖Atendendo à sobreposição da clínica, o diagnóstico laboratorial é fundamental.

❖A abordagem laboratorial consiste em:

- •Doseamento de metabolitos;
- •Actividade enzimática;
- •Genética molecular.

❖A existência de tratamento para 7 DLS
Impulsionou a procura de métodos de rastreio rápidos e acessíveis que visem o aumento da taxa de detecção.

❖Desde 2009 que a UBG tem vindo a implementar o estudo de actividade enzimática em amostras de DBS, estando neste momento disponível o diagnóstico por esta metodologia



- **Hunter (mucopolissacaridose tipo II)**
- **Maroteaux-Lamy (mucopolissacaridose tipo VI)**
- **Doença de Hurler-Scheie (mucopolissacaridose tipo I)**
- **Morquio B (mucopolissacaridose tipo IV B)**
- **Sly (mucopolissacaridose tipo VII)**
- **Fabry**
- **Gaucher**
- **Pompe (glicogenose tipo II)**
- **Gangliosidose GM1**
- **Gangliosidose GM2**

Diagnóstico Bioquímico e Molecular

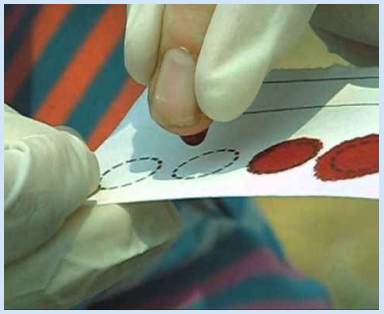
- Análise da atividade enzimática e da excreção de substratos
- Painel de mutações frequentes e sequenciação do gene

Estudo	Bioquímico	Genotipico
Gaucher	Glucocerebrosidase	Mutações frequentes Sequenciação gene
Fabry	Alfa-galactosidase A	Sequenciação gene
MPS I (Hurler-Scheie)	Alfa-iduronidase Gags	Mutações frequentes Sequenciação gene
MPS II (Hunter)	Sulfo-iduronato-sulfatase Gags	Sequenciação gene
MPS VI (Maroteaux-Lamy)	Arilsulfatase B Gags	Sequenciação gene
GSD type II (Pompe)	Alfa-glucosidase Oligossacaridos	Sequenciação gene
Niemann-Pick type C	Complexos colesterol-filipina	Mutações frequentes Sequenciação gene

Dried Blood Spot (DBS)

❖A colheita de sangue em papel de filtro já se revelou útil em muitas aplicações.

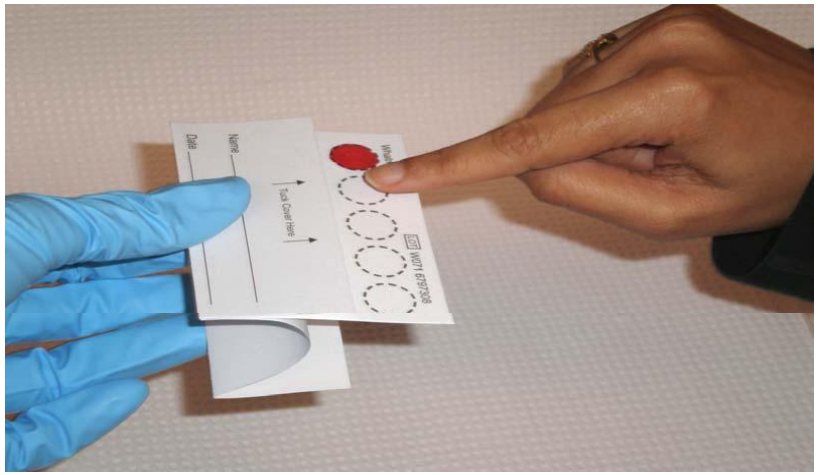
❖Permite o doseamento de substâncias, extracção de DNA e também estudos de actividade enzimática, dado que muitas enzimas mantém a sua funcionalidade nestas circunstâncias.



❖São várias as vantagens que contribuíram para a crescente procura de optimização de análises neste produto:

- **Pequena quantidade de sangue necessário – umas gotas – facilmente obtido ao nível capilar;**
- Estabilidade, mesmo em condições ambientais normais;
- Facilidade de transporte.

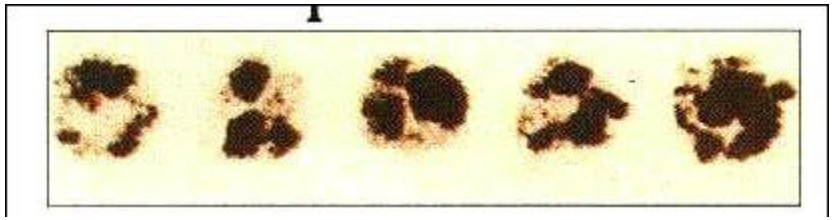
Ensaios enzimáticos em DBS (Dried Blood Spot)



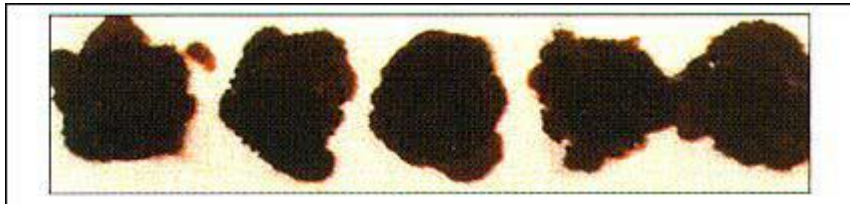
Disponíveis para algumas patologias

- Fabry
- Pompe
- MPS I
- Gaucher

Amostra insuficiente



Amostra colhida por fricção do calcanhar no cartão



Mucopolysaccharides electrophoresis

description / indication

2D-electrophoresis of mucopolysaccharides for the differentiation of mucopolysaccharidoses

Method 2D-Electrophoresis

material **urine** amount 2,5 ml

random specimen or 24-hour collection conditions dry ice

Oligosaccharides

description / indication

Oligosaccharidoses

Method TLC

material **urine** amount 1 ml, random specimen or 24-hour collection

conditions dry ice

Peroxisomal disorders (PD)

Peroxisomal disorders (PD) are a group of genetic diseases with single or multiple peroxisomal functions impairment and are subdivided into two major categories:

Peroxisome biogenesis disorders (PBD) - disorders in which a failure to form normal peroxisomes leads to multiple metabolic abnormalities

- Rhizomelic Chondrodysplasia Punctate (RCDP) type 1
- Zellweger spectrum disorders (ZSD)
  - Zellweger syndrome (ZS)
  - Neonatal Adrenoleukodystrophy (NALD)
  - Infantile Refsum Disease (IRD)

Peroxisomal disorders due to single enzyme deficiencies

- ✓ X-linked Adrenoleukodystrophy (X-ALD)
- ✓ Acyl-CoA oxidase deficiency (ACOX)
- ✓ D-bifunctional protein deficiency (D-BP)
- ✓ 2-Methylacyl-CoA Racemase deficiency (AMACRD)
- ✓ RCDP type 2 and 3
- ✓ Refsum Disease (RD)

Most PD are autosomal recessive disorders and first symptoms appear during prenatal period or until the first year of life. Other PD, such as RD, X-ALD and the AMACR, may arise in adulthood.

Peroxisomal disorders (PD)

Peroxisomes play a crucial role in metabolic pathways, such as alfa and beta-oxidation of very-long-chain fatty acids (VLCFA) and bile acids and plasmalogens biosynthesis.

PD diagnosis algorithm relies on the data obtained in these parameters evaluation.

Diseases	ZSDs (ZS,NALD,IRD)	ACOX1 deficiency	D-BP deficiency	RCDP type 1	RCDP type 2	RCDP type 3	X-ALD	RD	SCPx	AMACRD
Plasma										
β-oxidation										
Very-long chain fatty acids	↑	↑	↑	N	N	N	↑	N	N	N
Di- and trihydroxycholestanoic acid	↑	N	N-↑	N	N	N	N	N	↑	↑
Pristanic acid	N-↑	N	N-↑	N	N	N	N	N	↑	↑
α-oxidation										
Phytanic acid	N-↑	N	N-↑	N-↑	N	N	N	↑	↑	N-↑
Erythrocytes										
Etherphospholipid biosynthesis										
Plasmalogens levels	↓	N	N	↓	↓	↓	N	N	N	N
Fibroblasts										
Enzyme	–	ACOX1	D-BP	–	DHAPT	ADHAPS	ALDP	PhyH	SCPx	AMACR
DHAPAT activity	↓	N	N	↓	↓	↓	N	N	N	N
Mutated gene	PEX1,2,3,5,6,10, 12,13,14,16,19,26	*ACOX1*	*HSD17B4*	*PEX7*	*GNPAT*	*AGPS*	*ABCD1*	*PAHX*	*SCP2*	*AMACR*

PD- Take home messages

To achieve a peroxisomal disease diagnosis it needs several:

Biological samples to collect

- ✓Peripheral blood to obtain plasma, eritrocyte and DNA
- ✓Skin biopsy for fibroblast cell line establishment and RNA extraction

Biochemical parameters to request

- ✓plasma VLCFA, bile acids, phytanic and pristanic acids
- ✓fibroblast DHAPT enzymatic activity
- ✓Gene (s) sequencing

Clinical parameters to ascertain between

- ✓ Zellweger spectrum disorders (ranging from classical ZS to IRD)
- ✓ X-ALD phenotypic variants (ranging from the severe lethal childhood cerebral form to a Addison-only form without neurological dysfunction)

Essential fatty acids (PUFAs)

description / indication

Nutritional studies

Method GC/GCMS

material

erythrocytes amount 1 ml of whole blood conditions room temperature specifics arrival within 24 hours material

plasma amount 500 µl conditions dry ice protected from light

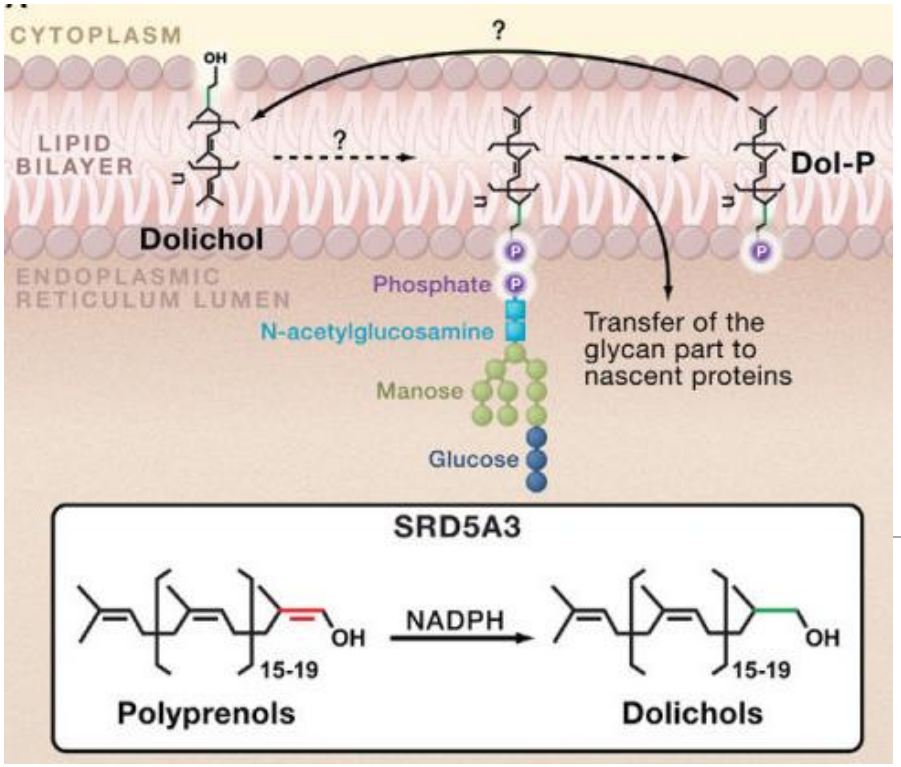
CDGs- Défices congénitos da glicosilação

Amostra – Soro
Transporte – Refrigerado a 4ºC
NUNCA- plasma obtido de sangue colhido em EDTA

- ✓ Taxa de excreção
- ✓ Biossíntese e "folding" das proteínas
- ✓ Actividade de enzimas, hormonas e citoquinas
- ✓ Funcionamento de receptores celulares
- ✓ Reconhecimento de anticorpos
- ✓ Transportadores, factores da coagulação

Uma enzima def.→várias proteínas afectadas

Congenital Disorder of Glycosylation
The relevance of transferrin glycosylation



➢Although most SRD5A3 deficient patients had defective glycosylation, there have been some reports in which abnormal glycosylation evaluation in serum might be less evident with aging, and Transferrin IsoEletric Focusing (IEF) might even become normal, especially in adults.

The authors present 2 patients, infantile and late infantile in which transferrin IEF was crucial for the diagnosis.

Protocol for collecting CSF samples for analyses of biogenic amine neurotransmitter metabolites, pterines, 5-methyl-tetrahydrofolate (5-MTHF), amino acids, GABA and pipecolic acid.

General considerations

Preferably collect CSF samples between 8 and 10 h in the morning, before any medication is taken.

Always mention the actual medication on the request form.

Collect the CSF samples in small clean tubes without additives.

Due to the ventriculo-lumbar concentration gradient for homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), lumbar CSF samples should be taken in fractions in order to analyze the right fraction for these neurotransmitter metabolites.

Protocol for collecting CSF samples for analyses of biogenic amine neurotransmitter metabolites, pterines, 5-methyl-tetrahydrofolate (5-MTHF), amino acids, GABA and pipecolic acid.

Sample collection and storage For newborns up to six months of age: collect a sample of 1-2 ml. For ages over six months collect fractions in separate **numbered tubes** according to the following scheme:

Age Fraction 1(# ml)

Fraction 2 (# ml)

Fraction 3 (# ml)

< 2 y -2, 1, 1_____2 – 16 y- 4, 2, 2_____> 16 y -8, 4, 4

In case of blood contamination immediately centrifuge samples prior to freezing and transfer the clear supernatants to labeled clean tubes

Freeze samples immediately at -80°C and store until shipment.

Because of the light sensitivity of the pterins, wrap all tubes in aluminum foil.

Shipment Send the frozen samples on sufficient dry ice, together with a completed laboratory form

Table 7.2. Initial laboratory investigation of suspected inherited metabolic disease presenting in the newborn period

Blood
Hemoglobin, white blood count, platelets
Blood gases and plasma electrolytes (calculate anion gap)
Glucose
Ammonium
Lactate
Calcium and magnesium
Liver function tests, including albumin and prothrombin and partial thromboplastin times
QUANTITATIVE Amino acid analysis
Carnitine, total and free
Galactosemia screening test
Blood or plasma acylcarnitine analysis (tandem MS–MS)
Plasma for storage at − 20°C: 2–5 ml
Urine
Ketones (Ames Acetest)
Reducing substances (Ames Clinitest)
Ketoacids (DNPH)
Sulfites (Merck Sulfitest)
Organic acids (GC–MS)
Urine for storage at − 20°C: 10–20 ml

Abbreviations: DNPH, dinitrophenylhydrazine; GC–MS, gas–chromatography-mass spectrometry; tandem MS–MS, tandem mass spectrometry–mass spectrometry (see Chapter 9).

Table 1.3. Protocol for emergency investigations

	Immediate investigations	Storage of samples
Urine	Smell (distinctive odor) Look (distinctive color) Acetone (Acetest, Ames) Reducing substances (Clinitest, Ames) Keto acids (DNPH) pH (pHstix Merck) Sulfitest (Merck) Electrolytes (Na, K), urea, creatinine Uric acid	Urine collection: collect fresh sample and put it in the refrigerator Freezing: freeze samples collected before and after treatment at –20°C, and collect an aliquot 24 h after treatment. Do not use them without having expert metabolic advice Metabolic investigations: OAC, AAC, orotic acid, porphyrins
Blood	Blood cell count Electrolytes (search for anion gap) Glucose, calcium Blood gases (pH, pCO_2, HCO_3^-, pO_2) Uric acid Prothrombin time Transaminases (and other liver tests) Ammonia Lactate, pyruvate 3-hydroxybutyrate, acetoacetate Free fatty acids	Plasma (5 ml) heparinized at –20°C Blood on filter paper: 2 spots (as »Guthrie« test) Whole blood (10-15 ml) collected on EDTA and frozen (for molecular biology studies) Major metabolic investigations: total homocysteine, AAC, acylcarnitines (tandem MS), OAC, porphyrins, neurotransmitters (HPLC, tandem MS)
Miscellaneous	Lumbar puncture Chest X-ray Cardiac echography, ECG Cerebral ultrasound, EEG	Skin biopsy (fibroblast culture) CSF (1 ml), frozen (neurotransmitters, AA) Postmortem: liver, muscle biopsies (Chap. 3)

AA, amino acid; *AAC*, amino acid chromatography; *CSF*, cerebrospinal fluid; *DNPH*, dinitrophenylhydrazine; *ECG*, electrocardiogram; *EDTA*, ethylenediaminetetra-acetic acid; *EEG*, electroencephalogram; *MS*, mass spectrometry; *HPLC*, high performance liquid chromatography; *OAC*, organic acid chromatography.

Protocolo Peri-mortem

Sangue	Punção intracardíaca	15mL (5mL se RN)	Células –10 mL em EDTA, à tEMPEratura ambiente. Plasma – 3-4 mL (min 1 mL) em heparinato de lítio; contactar laboratório para o plasma ser separado dentro de 20 min e posteriormente congelado a -80 ºC. Doseamento de aminoácidos, ácidos gordos de cadeia muito longa, carnitina, CDT, 7-DHC (…). DNA – tubo de EDTA, sem centrifugar. Conservado a +4ºC, durante 48h. 2-3 cartões de Guthrie – 6 a 9 preenchimentos (úteis pela facilidade de armazenamento e transporte – PCR de DNA) podendo ser utilizado para perfil de acilcarnitinas
Urina	Sonda/Punção vesical	10mL (min 2mL)	Glicosúria, cetonúria, proteinúria e Sulfitest®, pesquisa substâncias redutoras. Aminoácidos, ácidos orgânicos, ácido orótico, (…). Congelar o restante a -20ºC, sem conservantes.
Lcr	Punção lombar	3mL	(Bacteriologia – 1mL) Dois tubos, com 1mL cada, para congelar a -70ºC. Para efectuar doseamentos de aminoácidos, neurotransmissores, folatos e biopterinas, deve estar indicado a fracção (1, 2 ou 3) dada a variação rostro-caudal dos compostos a dosear. Um dos tubos deve ser envolvido em folha de alumínio, para ficar ao abrigo da luz (ver protocolo de colheita de Neurotransmissores)
Pele	Possível nas 6h que sucedem o óbito. Desinfecção cutânea com iodopovidona. Colheita de fragmento com 3x2mm de lado e pelo menos 1mm de profundidade.		Colocação em meio de cultura ou soro fisiológico estéril, à temperatura ambiente. Não congelar. A cultura de fibroblastos deverá ser realizada, se possível, 24h após a colheita.
Músculo	Obter dentro de 2 horas após a morte. Colheita de 3 cubos com 5mm de aresta (recto abdominal). Essencial se houver suspeita de um defeito na cadeia respiratória mitocondrial.		1 fragmento colhido para tubo seco – sem conservante – para congelamento a -70ºC; transporte em azoto líquido no prazo máximo de 24-48 h para o laboratório. Fragmentos para microscopia óptica e microscopia electrónica – a colheita e o transporte deverão ser realizados de acordo com o protocolo do respectivo laboratório.
Fígado	Obter dentro de 2 horas após a morte. Colheita de 5 fragmentos com 20x5mm.		2 a 3 fragmentos colhidos para tubo seco – sem conservante, para congelamento a -80ºC, transporte em azoto líquido no prazo máximo de 24-48 h para o laboratório. Fragmentos para microscopia óptica e microscopia electrónica - a colheita e o transporte deverão ser realizados de acordo com o protocolo do respectivo laboratório.

Deverá ser ainda fotografado e/ou radiografado tudo o que seja considerado relevante.

Postmortem Protocol Body Fluids for Chemical Investigations

Plasma from the centrifuged blood sample

urine (~10 ml),

cerebrospinal fluid (~4 ml) are immediately frozen at –20°C

If no urine can be obtained by suprapubic puncture or catheterization, the bladder may be filled with 20 ml of saline solution and diluted urine may be harvested.

Alternatively, vitreous humor can also be collected (by intraocular puncture) and frozen. This liquid is comparable to blood plasma with respects to its solubility for organic acids.

Recently, bile, readily available at autopsy, has been found to be useful material for the post-mortem assay of acylcarnitines .

Many biochemical parameters are impossible to interpret post-mortem due to rapid tissue lysis. These include lactate, ammonia, carnitine (total and free), and amino acids, all of which rapidly increase without any specific significance. In contrast, the acylcarnitine-ester profile, determined from dried blood spots or from bile, may be highly diagnostic for many disorders of fatty-acid oxidation and for organic acidurias.

Considerações finais

Estreita relação com o laboratório é fundamental

História clínica familiar e pessoal de relevo

Situação metabólica à data da colheita

Terapêutica instituida, transfusões...

Recolha de instruções detalhadas sobre a colheita a efectuar – ex: colheita de líquor, músculo

Considerações finais…

"Access to comprehensive routine laboratory testing is indispensable to the establishment of the diagnosis of any suspected inherited metabolic condition, and the clinical biochemist is an extremely important collaborator whose allegiance should be cultivated carefully."

A Clinical Guide to Inherited Metabolic Diseases. Second Edition

Joe T. R. Clarke

MD, PhD, FRCP(C), FCCMG

Department of Pediatrics, University of Toronto and

Division of Clinical and Metabolic Genetics

The Hospital for Sick Children

Sequenciação massiva
NGS-Next Generation Sequencing

Painel	Grupos de patologias	Nº de genes
1 – Hipoglicemia	Hiperinsulinismo, glicogenoses, gluconeogénese, beta-oxidação de ácidos gordos, cetogénese, cetólise, metabolismo de monossacáridos , outros	55
2 - CDG	Défices congénitos da glicosilação	30
3 – AA/AO/AC	Metabolismo de aminoácidos, ácidos orgânicos e acilcarnitinas	93
4b – Cadeia respiratória	Defeitos da cadeia respiratória mitocondrial e outras doenças que os mimetizam	150
5 – Moléculas complexas	Doenças lisossomais, peroxissomais e metabolismo do colesterol e ácidos biliares	78
6 – Leucodistrofias, distonias e ataxias	Leucidistrofias cavitárias, hipomielinizantes e outras, neurodegeneração com acumulação cerebral de ferro e distonias	54
7 – Epilepsia	Encefalopatias epilépticas congénitas	73
8a - Miopatias	Miopatias não metabólicas	53
8b – Miopatias metabólicas		48
8c - Hipotonia		51
9 – Hiperlaxitude articular		46
12 – RAS/MAPK	Síndromes associados a defeitos na via RAS/MAPK	10
13 – Morfogénese cerebral	Defeitos congénitos da morfogénese cerebral	31
14 - Ciliopatias		46
15 – Atraso mental ligado ao X	Sindrómico e não sindrómico	55
17 - Ataxias	Não inclui as associadas a expansões de trinucleótidos	45

Sequenciação massiva
NGS-Next Generation Sequencing

Os painéis estão agrupados em pacotes (designados "Combi"), por motivos de optimização técnica e económica, de acordo com os grandes grupos de apresentação clínica.

É possível solicitar apenas um painel individual desde que se consiga reunir um conjunto de pelo menos 16 casos (no âmbito de projectos, por exemplo). Nesta situação pode ser conseguida uma redução dos preços.

Combi	Paineis incluídos	Tamanho	Nº de genes	Preço
1 – DNMP1	5+6+7+17	0.89 Mb	**233**	1500€
2 – DNMP2	12+13+14+15+16	0.94 Mb	**206**	1500€
3 - MTB	1+2+3+4+10	0.86 Mb	**342**	1500€
4 - MPT	8A+8B+8C+9	1.165 Mb	**180**	1500€