Importance of laboratory Technology in Inborn Errors of metabolism Diagnosis

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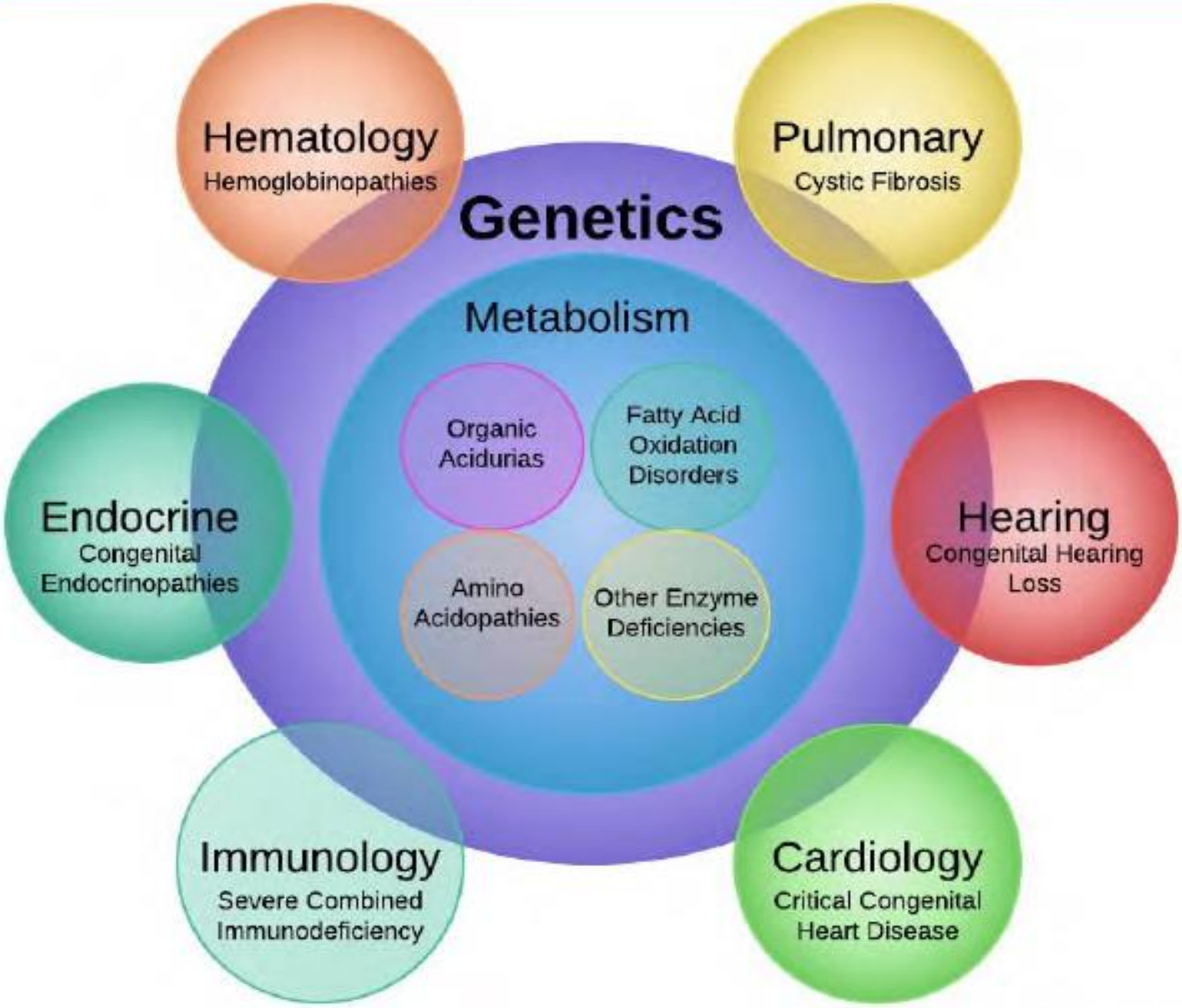
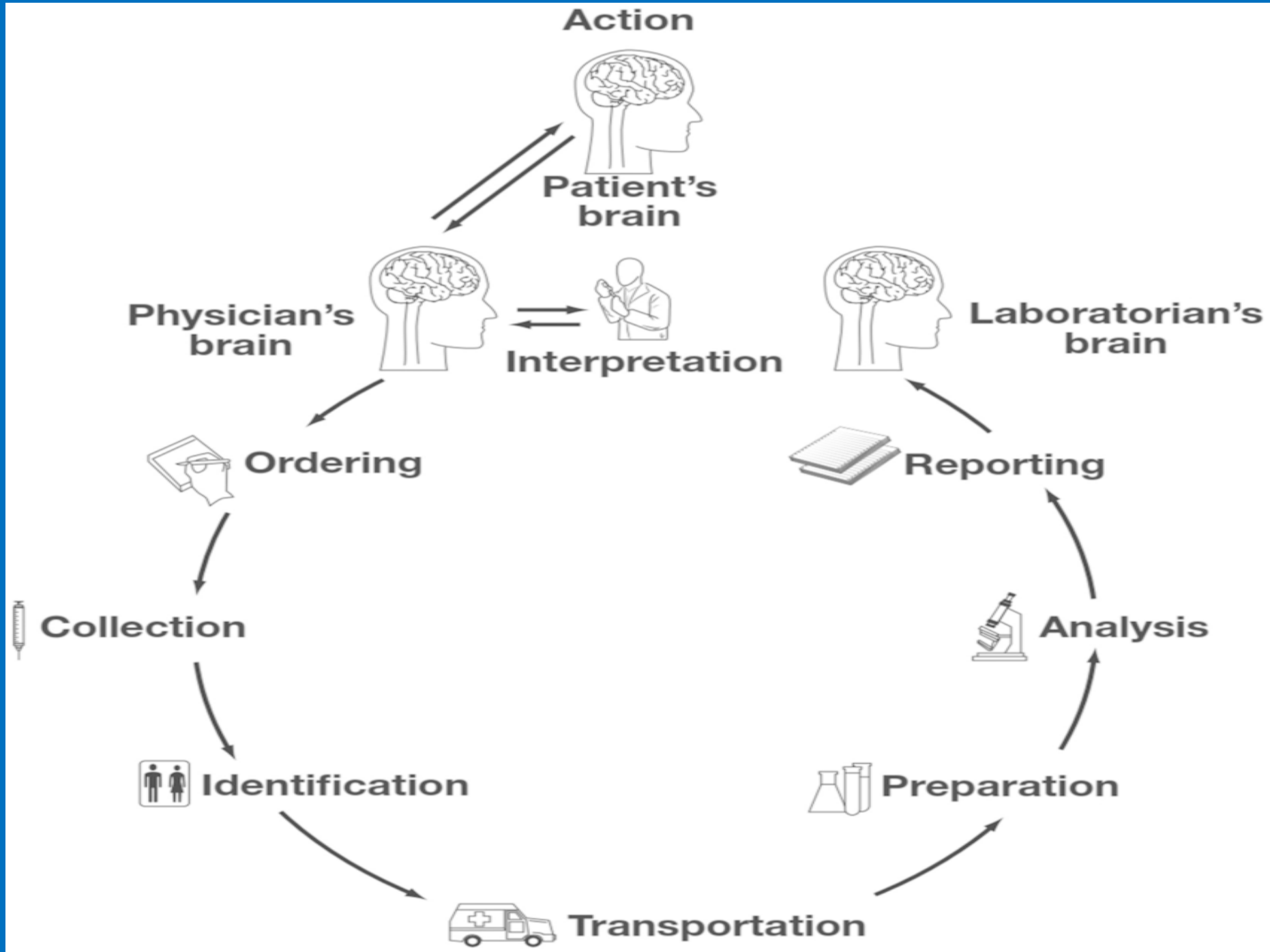
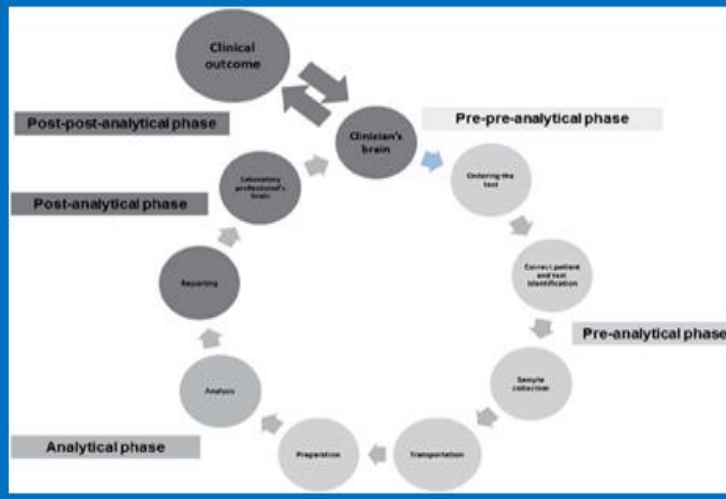

Hematology
Hemoglobinopathies
Pulmonary
Cystic Fibrosis
Genetics
Metabolism
Organic Acidurias
Fatty Acid Oxidation Disorders
Amino Acidopathies
Other Enzyme Deficiencies
Endocrine
Congenital Endocrinopathies
Hearing
Congenital Hearing Loss
Immunology
Severe Combined Immunodeficiency
Cardiology
Critical Congenital Heart Disease

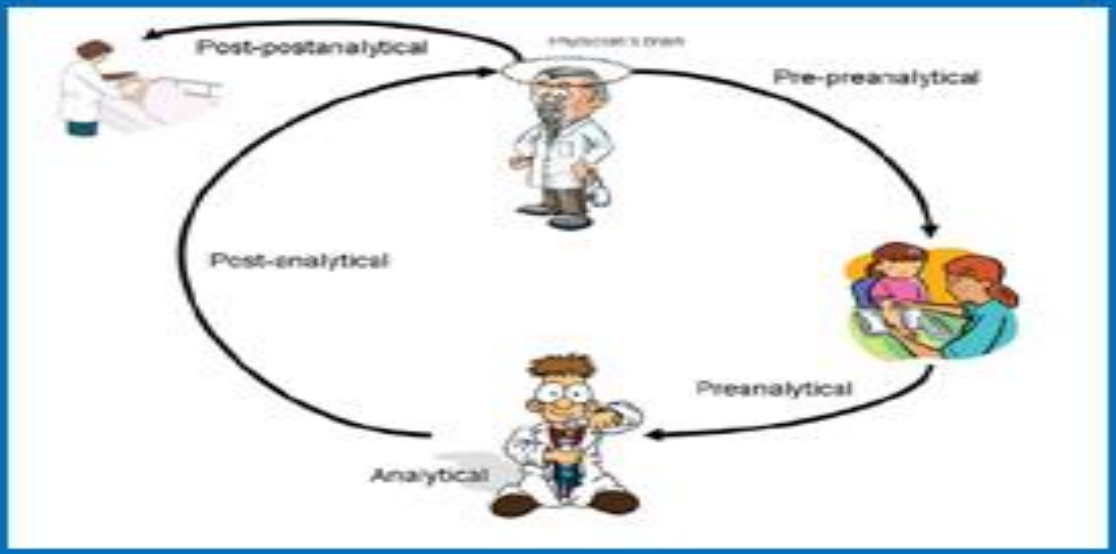
The Brain-to-Brain Loop Concept for Laboratory Testing

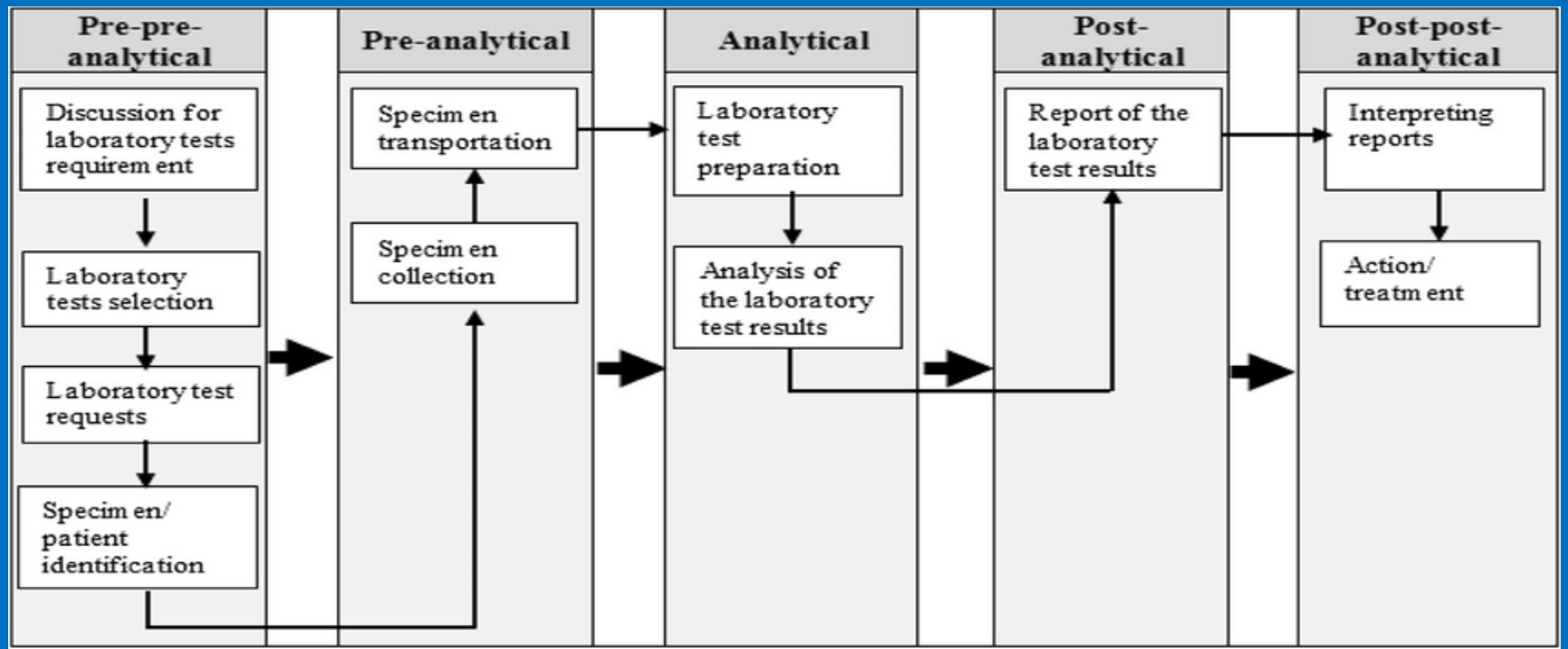
- *Forty years ago, Lundberg introduced the concept of the brain-to-brain loop for laboratory testing.*
- *In this concept, in the brain of the physician caring for the patient, the first step involves the selection of laboratory tests and the final step is the transmission of the test result to the ordering physician.*


Action
Patient's brain
Physician's brain
Interpretation
Laboratorian's brain
Ordering
Reporting
Collection
Analysis
Identification
Preparation
Transportation



- *Many intermediary steps,*
- *preanalytic, before performance of the test;*
- *Analytic and relate to the actual performance of the test;*
- *postanalytic and involve transmission of test results into the medical record.*
- *Errors have since been considered as preanalytic, analytic, and postanalytic.*


Post-postanalytical
Pre-preanalytical
Post-analytical
Preanalytical
Analytical


Pre-pre-analytical
Discussion for laboratory tests requirem ent
Laboratory tests selection
Laboratory test requests
Specim en/ patient identification
Pre-analytical
Specim en transportation
Specim en collection
Analytical
Laboratory test preparation
Analysis of the laboratory test results
Post-analytical
Report of the laboratory test results
Post-post-analytical
Interpreting reports
Action/ treatm ent

- the generation of any laboratory test result consists of 9 steps, including :
- ordering
- collection
- identification (at several stages),
- transportation
- separation (or preparation)
- analysis
- reporting
- interpretation
- action

- most errors in the loop do not fall within the analytic phase, nor do they occur most often within the preanalytic and postanalytic steps under the control and/or jurisdiction of laboratory professionals.
- Declining student interest in the field of laboratory medicine,as medical technicians/technologists and as doctoral

level laboratory directors, has been highlighted, particularly

- In the last decades, improvements in reliability and standardization of analytic techniques, reagents, and instrumentation, and advancements in information technology, along with quality control and assurance methods decreased by more than 10-fold

the analytic error rate.

- The brain-to-brain loop in laboratory testing represents working paradigm to better establish the physician-laboratory and the physician-patient relationship.
- It is essential to maintain laboratory information within the right clinical context, avoiding the risk of inappropriate test requests and result interpretation.

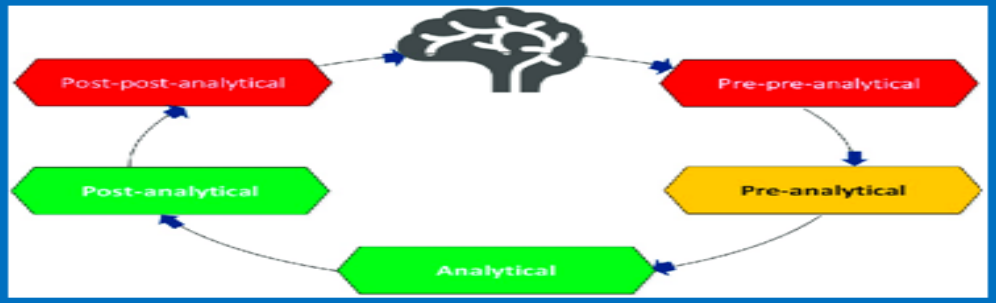
Analytical Issues/Quality Requirements

- 5. The director of the testing laboratory should be a board-certified doctoral scientist or physician with specialized training and/or experience in biochemical genetics. **Must understand the technology (MS/MS), and have sufficient knowledge of biochemical genetics**
-
- 6. Known concentrations of non-isotopic amino acid reference calibrators should be prepared in an appropriate aqueous matrix.
- For ion-exchange chromatography, two different compounds eluting in important parts of the chromatogram should be used as internal standards. For tandem mass spectrometry, stable-isotope amino acid internal standards should be used when possible

at ambient temperature in a dry environment at room temperature or 4°C

before analysis

- At 4°C the decrease in the concentrations of C6 and C8 is about 4% per year. In specimens stored at -20°C the decrease in the concentration of acetylcarnitine is less than 10% per year and those of C6, C8 and C10 are less than 3%.



- 8. Specimens should be deproteinized prior to analysis.
- 9. Chemical derivatization of amino acids is required for detection (e.g., ion-exchange chromatography).
- 10. Chemical derivitization of amino acids is recommended to enhance assay sensitivity and specificity (e.g., MS/MS).
- 11. Amino acids should be analyzed quantitatively by

a reliable technique, such as automated cation exchange liquid chromatography.

- 12. Amino acids should be analyzed quantitatively by a reliable technique, such as electrospray ionization tandem mass spectrometry.

13. Identification of amino acids by ion-exchange chromatography should primarily be based on chromatographic retention time, and retention time relative to an internal standard. Quantitation should be based on the recovery of the internal standard in each specimen compared to the recovery of the internal standard in the calibrators.

- 14. Qualitative screening methods, such as thin-layer chromatography (TLC), should not be used for amino acid analysis.
- 15. At least two control mixtures should be analyzed daily to monitor the ongoing performance of the analytic process.
- 16. Age-matched reference intervals (normal ranges)for reported amino acids should be established or verified by the test in laboratory for the population being investigated.

Post-Analytical Issues/Quality Requirements

- 17. Interpretation of test results should be based on relative amino acid levels and …, pattern recognition, and correlation of positive and negative findings.
- 18. Test reports should include appropriate patient and specimen information, test results, and clinical interpretation.
- 19. Substances that have the potential to interfere with the analysis should be identified and taken into account during interpretation.

Non IEM

- There are a range of non-specific causes which include:
- Major illness e.g. organ failure (disorders associated with liver dysfunction) (ie,PHE AND TYROSINE)
- Transient illness
- Premature – liver maturity
- Diet / feed
- Parenteral nutrition /TPN
- Analytical error – unlikely with triplicate testing
- Contamination of card

- Cut off: Gray zone /pathologic zone
- Importance low or high metabolite
- Common metabolite in several diseases specific cut off for each disease
- History clinical status Diet/Formula/medication/Transfusion
- Metabolite base/enzymology(Enzyme assay)
- If initial result is an alert, or abnormal results are obtained on two different NBS specimens, further testing is recommended to establish diagnosis

Definition and Use of Primary and Secondary Markers

- Primary markers (analytes) – used to establish presumptive positives.
- Secondary markers – used in conjunction with primary analyte results to assign risk
- Isolated elevations of secondary markers are considered unimportant

Secondary metabolites and criteria for mild elevation of primary marker

- For PKU: PHE/TYR ratio > 3
- For MSUD: LEU+ILE and VAL
- For PA and MMA: C3/C2 ratio > 0.4
- For MCAD: C8/C10 ratio > 3
- For VLCAD: C14:1/C12:1 ratio > 3
- For LCHAD: C16-OH plus at least one of the following: C18:1-OH, C16, C18:1
- For CPT-II/CAT: C16 and C18:1
- No suitable secondary markers for C3-DC, C4, C5, C5-OH, C5-DC

Action taken by co-ordinator

- Low risk: contact physician of record, check clinical status of pt., request second blood spot specimen,
- recommend follow-up testing if symptomatic
- Moderate risk (includes positive test on repeat specimen from above and/or presence of secondary markers): request follow-up testing; recommend referral to regional metabolic center if child symptomatic
- High risk: recommend immediate referral to metabolic center, follow-up testing and initiate appropriate therapy regardless of clinical status

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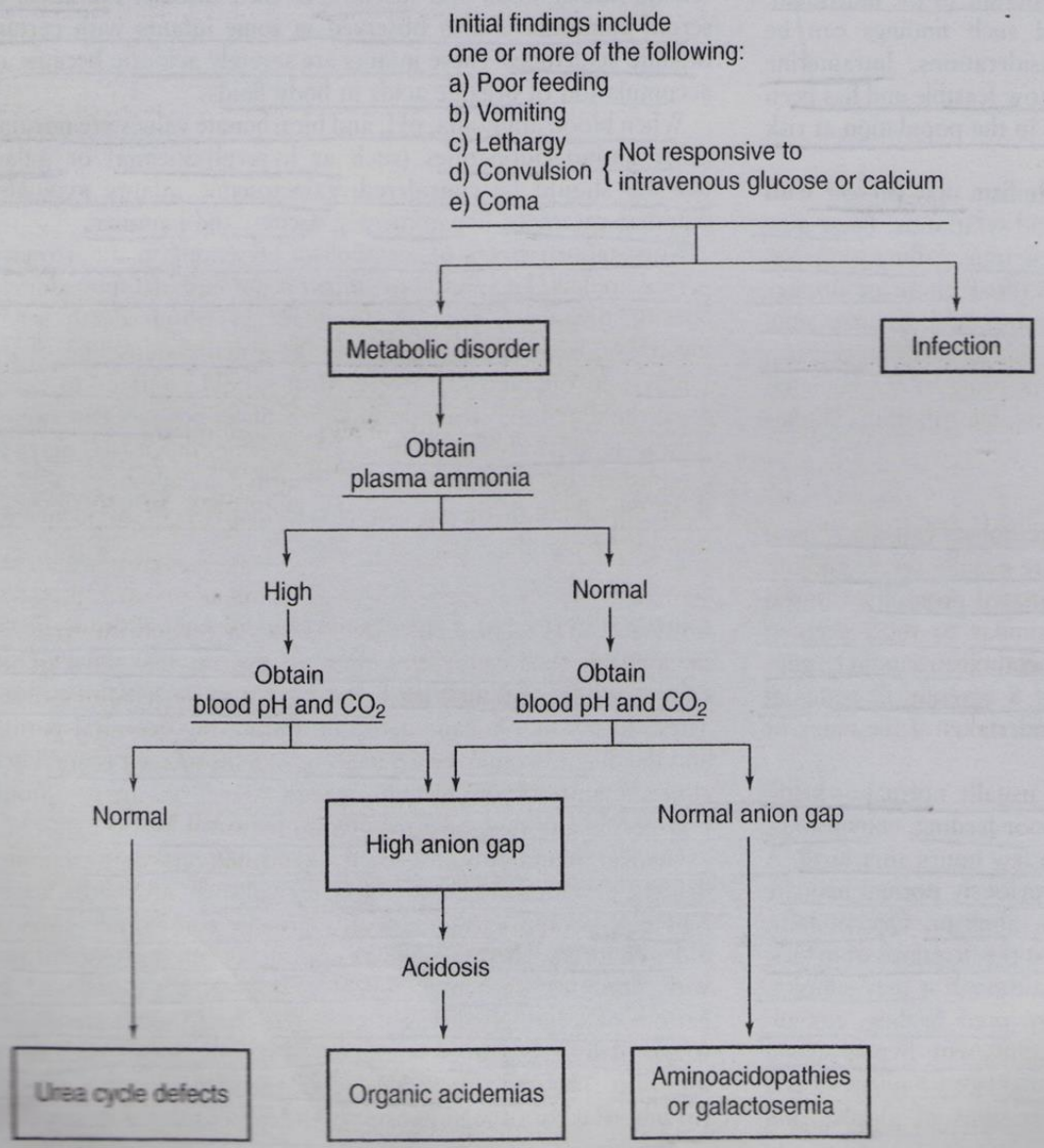

Figure 84-1. Clinical approach to a newborn infant with a suspected metabolic disorder. This schema is a guide to the elucidation of some of the metabolic disorders in newborn infants. Although some exceptions to this schema exist, it is appropriate for most cases.

- Which diseases are diagnosed by LC/Mass
- a)Diagnostic for:
- FAOD
- Aminoacidopathies
- UCD
- b)Suggestive for
- Organic acidemia
- Mithocondrial disorders
- In suspicion to organic acidemia, differention and confirmation by urine GCMS is necessary.

- **Diagnosable Components:**
  - **LC Mass: 48 Components**
  - **GCMS: 135 Components (To 178)**

1)TSH

2)17-OH Progesterone

3)Biotinidase

4)Galactose

5)Phenylalanine

6)Immunoreactive trypsin

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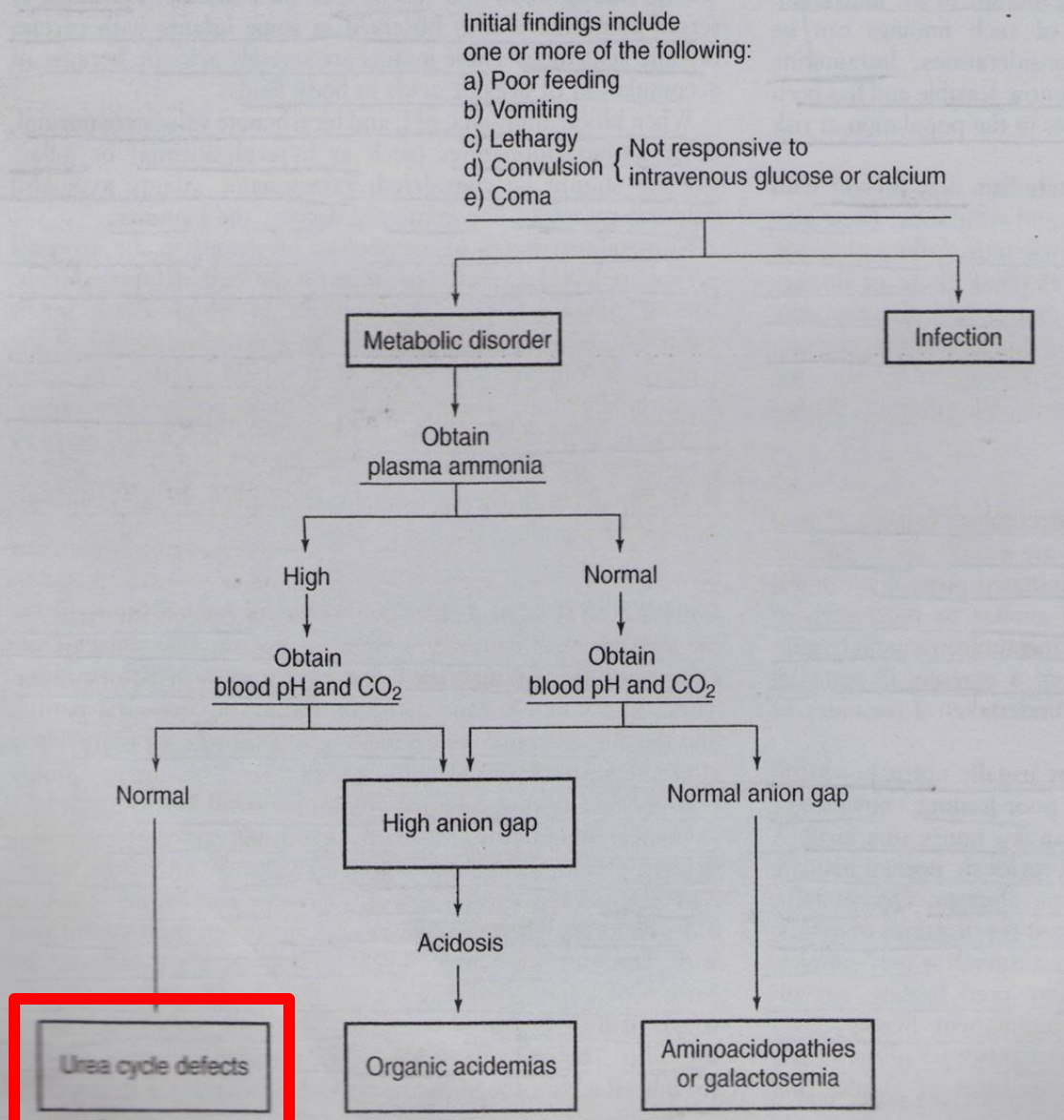

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1) **UCD:** elevation of ammonia without metabolic acidosis (sometimes respiratory alkalosis)

Normal Level of ammonia:

- Fulterm < 100 μmol/L (< 1.7 μg/ml)
- Preterm < 150 μmol/L (< 2.6 μg/ml)
- Children < 35 μmol/L (< 0.6 μg/ml)

Pathologic Level of ammonia:

Neonate: > 150 μmol/L (> 2.6 μg/ml)

Ch.-Ad: > 100 μmol/L (> 1.7 μg/ml)

MS/MS: Increased level of

- Citruline
- Glutamic acid
- Aspartic acid
- Alanine

Urine GCMS: Increased level of:

- orotic acid
- uracil

Orotic acid

- Indic.: Suspected heterozygous OTC deficiency, urea cycle defects carbamyl phosphate disorder, disorders of pyrimidine

metabolism, mitochondrial disorders, allopurinol test

- Method: HPLC, MS-MS, capillary electrophoresis
- unexplained elevations also in other disorders, e.g. Rett syndrome, Lesch-Nyhan syndrome,
- “benign orotic aciduria

Elevation of other amino acids

- **Citrulline:** DD: citrullinaemia:↑ Cit; argininosuccinic aciduria:↑ Cit,↑ Asa,

Renal disease

- Confirmation: AA plasma and urine
- **Arginine:** Argininaemia; low sensitivity, Arg frequently normal in newborns
- Confirmation: AA plasma and urine
- **Glycine:**Non-ketotic hyperglycinaemia
- Confirmation: AA plasma (if symptomatic: plasma + CSF)

Follow-up testing for elevated citrulline

- *Possible diagnosis: citrullinemia (ASD);*
- *argininosuccinic aciduria(ASLD)*Plasma amino acids - elevated Cit, also Asa in ASLyase
- Urine amino acids (grossly elevated arginino-succinic acid (Asa) is diagnostic of ASL def)
- Urine organic acids - orotic acid may be elevated
- Confirmation:
- Argininosuccinate synthetase (ASS) activity in liver or cultured fibroblasts
- Argininosuccinate lyase (ASL) deficiency is confidently diagnosed from Asa levels

Differential diagnosis

Plasma citrulline	*Other features*	*Diagnosis*
Low (usually)	↑↑ Orotic acid	Ornithine transcarbamylase deficiency
	Specific acylcarnitines and organic acids	Organic aciduria, e.g. propionic or methylmalonic aciduria
	↓–n Orotic acid	Carbamylphosphate synthase deficiency N-acetylglutamate synthase deficiency Ornithine aminotransferase deficiency (newborns)
>30 µM	↑ Orotic acid	Lysinuric protein intolerance
>50 µM	↓–n Orotic acid, ↑ lactate	Pyruvate carboxylase deficiency (neonatal)
100–300 µM	↑ Argininosuccinate	Argininosuccinic acidaemia
>1,000 µM	↑ Orotic acid	Citrullinaemia

Follow-up testing for elevated arginine

- *Possible diagnosis: arginase deficiency*
- Plasma amino acids - marked elevation of Arg
- Urine amino acids - elevated Arg, Lys, Cys ,Orn
- Urine organic acids - orotate
- Confirmation: Arginase activity (RBC)

Follow-up testing for elevated ornithine

- *Possible diagnosis: HHH syndrome; gyrate atrophy*
- Plasma amino acids - markedly elevated Orn
- • Urine amino acids - elevated Orn, homoCit
- • Urine organic acids - orotic acid
- • Confirmation:
- elevated ammonia in addition to Orn and increased
- excretion of homocitrulline (homoCit) are diagnostic of
- HHH syndrome - a mitochondrial membrane
- transporter defect (ORNT1)
- • ornithine aminotransferase activity in lymphocytes
- (gyrate atrophy)

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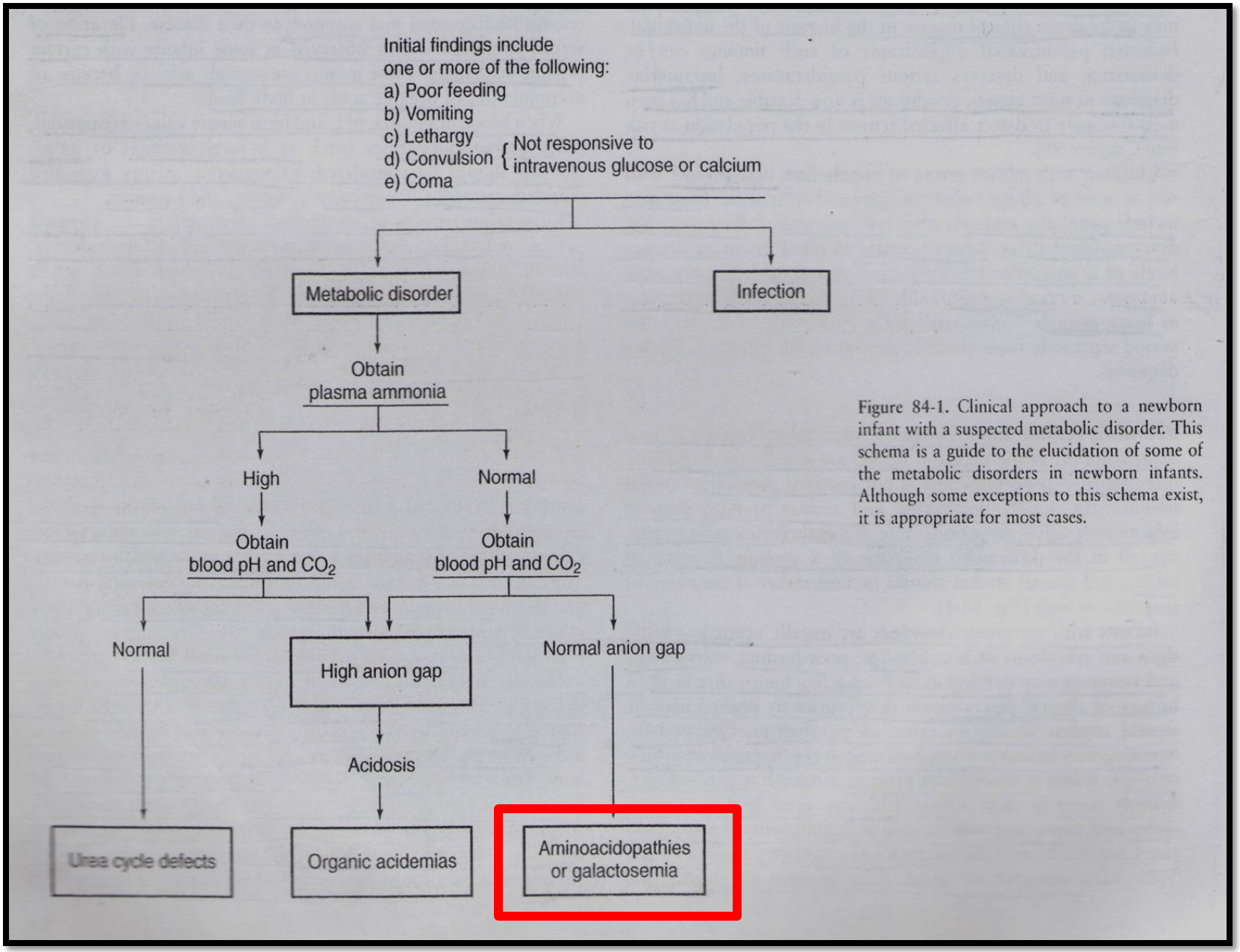

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- The most common abnormality in NBS for amino acids is elevated tyrosine; most cases are NOT tyrosinemia I, II or III (these are very rare)
- The most common urea cycle defect, OTC deficiency, is not currently detectable by MS/MS (possibility of low citrulline?)
- It is not clear that Tyr-I, NKH, HHH, Hyperprolinemia or Arginase deficiency are detectable in the neonate (< 5d of age)

Aminoacidopathies

2/A) PKU:

High blood level of phenylalanine (usually above than 10mg/dl) is diagnostic in:

- **HPLC method**
- **MS/MS method** **(mass spectrometry/ mass spectrometry)**

GCMS: Increased level of metabolites:

- Phenyl acetate
- Phenyl lactate
- Phenyl pyrovate

Phenylketonuria (PKU;)

- Metab.: ↑ Phe, ↓ Tyr, ↑ Phe/Tyr ratio
- Confirm.: AA plasma; exclude cofactor deficiency pterines in urine, DHPR activity in DBS; consider BH4 test
- DD: Prematurity, liver disease/hepatic failure, parenteral nutrition; ↑ Phe + ↑ Tyr: tyrosinaemia type 2 or 3, transient hypertyrosinaemia (premature neonates
- Neonatal Presentation: None

Pre-analytical aspects PHE

- **Potential for false negatives PHE**

Missing sample spot in the plate well

Transfusions at least 72h

Delays in transit

Physiological reasons

Potential for false positives

Contamination of the sample

Non-sample source contamination

Physiological reasons

2/B) Tyrosinemia: Blood level elevation of tyrosine:

- HPLC method ,MS/MS method **(mass spectrometry/mass spectrometry)**
  - **GCMS:** Elevation of:
  - Succcinylacetone
  - N-acetyltyrosine
  - 4 -HPPA
  - 4 -HPLA
  - 4 -HPAA

Follow-up testing for elevated tyrosine

Possible diagnosis: tyrosinemia type I, II or III

- Plasma amino acids - elevated Tyr
- Urine organic acids (elevated tyrosine metabs;
- succinylacetone is diagnostic of type I)
- **TYR II or III - Elevated TYR with normal SUAC**
- Clinical history (hepatorenal phenotype - type I; oculocutaneous phenotype - type II)
- DD:types 2 and3, transient hypertyrosinaemia (mainly premature neonates)
- *Note: transient tyrosinemia of the newborn is by far the most common cause of elevated Tyr*

Follow-up testing for elevated glycine

- *Possible diagnosis: NKH (nonketotic hyperglycinemia)*
- CSF amino acids - elevated glycine
- Plasma amino acids - elevated glycine
- Urine organic acids - rules out other metabolic causes for elevated glycine
- Confirmation:
- Ratio of CSF: plasma glycine > 0.08
- Reduced activity of the glycine cleavage system (liver)

Follow-up testing for elevated proline

- *Possible diagnosis: hyperprolinemia type I or type II*
- Plasma amino acids - elevated proline
- Urine organic acids (to rule out lactic acidosis and check for P5C)
- Confirmation:
- Type II - P5C dehydrogenase deficiency - by marked elevation of D1-pyrroline 5-carboxylate (P5C) in urine and plasma
- Type I - proline oxidase deficiency - by exclusion of type II

Follow-up testing for elevated methionine

- *Possible diagnosis: homocystinuria or hypermethioninemia*
- Plasma amino acids - elevated methionine and/or total plasma homocysteine
- Confirmation:

Cystathionine ß-synthase activity in lymphocytes or fibroblasts (if Hcys and Met elevated)

- Methionine adenosyl transferase activity (if Met only elevated) in liver

Potential for false negatives MET

Transfusions

Delays in transit / sample deterioration

Physiological reasons

Potential for false positives :

Liver disease (for example due to tyrosinaemia type I or galactosaemia), parenteral nutrition, and methionine adenosyl transferase (MAT) deficiency can give rise to an elevated methionin concentration in the newborn period.

Homocystinuria

- Metab.: ↑ Hcy; more common: ↑ Met (2nd tier Hcy from DBS, where available)
- **Abnormal Screen Result: Elevated MET**
- Elevated MET/PHE
- DD: Liver failure (↑ Met and Tyr); MAT I/III (↑ Met only)
- Confirm.: AA plasma, Hcy
- Neonatal Presentation: None

- Homocystinuric patients can be sub-divided into two important biochemical phenotypes:
- Pyridoxine responsive (screen undetectable)
- Pyridoxine unresponsive (screen detectable)

Raised total homocysteine concentrations are also seen in some rarer inborn errors of metabolism (MTHFR deficiency and defects of vitamin B12 metabolism) and in maternal B12 deficiency but these would not be detected by screening as they are associated with low, rather than high, methionine concentrations.

Total homocysteine (tHcy)

- Blood should be centrifuged within 45 min to obtain EDTA or heparin plasma or serum. For exact

measurement it is important to treat plasma or serum with a reducing agent that converts all Hcy species into the reduced form, HcyH, which is measured either directly or after derivatisation.

- *Normal values* (*fasting*): children < 10 yrs: 3.5–9 µmol/l; > 10 yrs: 4.5–11 µmol/l; women premenopausal 6–15 µmol/l; post-menopausal 6–19 µmol/l; men 8–18 µmol/l.

Maple syrup urine disease

- Metab.: ↑ XLE (= Leu + Ile + Allo-Ile + OH-Pro), ↑ Val, ↑ XLE/Ala
- **Abnormal Screen Result:**

Elevated LEU+ILE

- Elevated VAL
- Elevated LEU+ILE/PHE
- Elevated VAL/PHE
- DD: Total parenteral nutrition, hydroxyprolinaemia, probably non-disease
- Confirm.: AA plasma (Allo-Ile)

Pre-analytical aspects MSUD

- **Potential for false negatives**

Delays in transit / sample deterioration

Physiological reasons

transfusions

Potential for false positives :

MS/MS analysis does not differentiate leucine from isoleucine or hydroxyproline. While elevation of leucine and isoleucine both result from MSUD, increased hydroxyproline may indicate the rare benign condition hydroxyprolinaemia.

increased leucine concentration in galactosaemia or other severe liver disease

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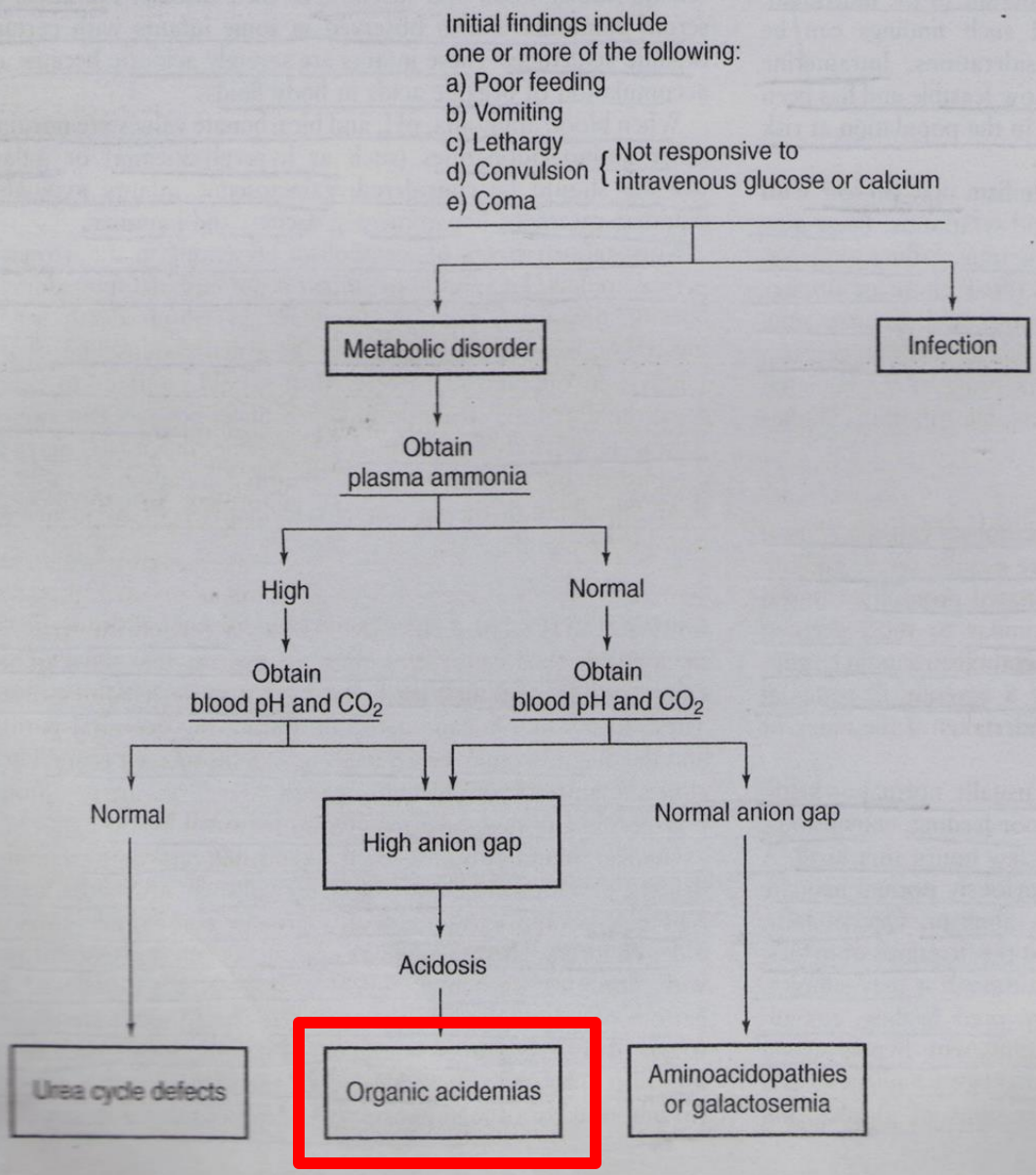

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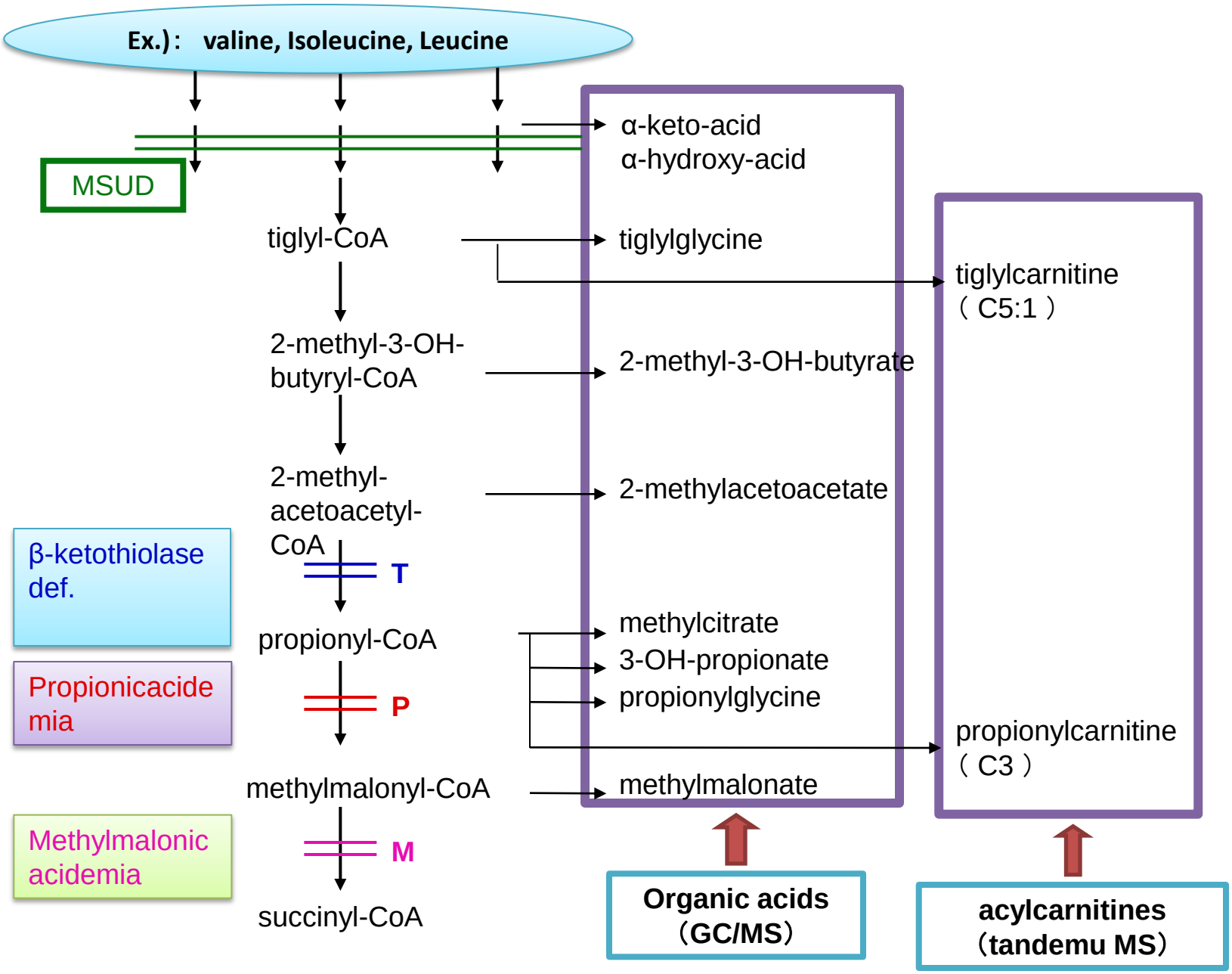
Organic acids (OA)

- Organic acids are analysed in urine, only in exceptional circumstances in other body fluids. The method of choice is gas chromatography-mass spectrometry (GC-MS); quantitation of specific
- OA is possible with stable isotope dilution assays.
- OA in plasma, CSF or vitreous fluid if no urine sample can be obtained, e.g. post mortem


Ex.)： valine, Isoleucine, Leucine
MSUD
α-keto-acid
α-hydroxy-acid
tiglyl-CoA
tiglylglycine
tiglylcarnitine
（C5:1）
2-methyl-3-OH-butyryl-CoA
2-methyl-3-OH-butyrate
2-methyl-acetoacetyl-CoA
2-methylacetoacetate
β-ketothiolase def.
T
propionyl-CoA
methylcitrate
3-OH-propionate
propionylglycine
Propionicacidemia
P
propionylcarnitine
（C3）
methylmalonyl-CoA
methylmalonate
Methylmalonic acidemia
M
succinyl-CoA
Organic acids
（GC/MS）
acylcarnitines
（tandemu MS）

- Acylcarnitine profile is helpful:
  - ↑ **C_3** (propionyl carnitine)→ *P.A
    *MMA
    *MCD
  - ↑ **C_5** (Isovaleryl carnitine): IVA
  - ↑ **C_5OH** (3-hydroxy isovaleryl carnitine)→
    - BKT
    - MCD
    - MCC
    - HMGL
    - IVA
    - 2M-3HBA
  - ↑ **C_4DC** (Methyl malonyl carnitine): MMA
  - ↑ **C_5DC** (Glutaryl carnitine): GA_1

Acylcarnitines in Organic Acidemias: Primary Markers

- **Acylcarnitine species Disorder to be considered**
- **C3 PA, MMA, MCD**
- **C4 IBCD, (SCAD, MAD)**
- **C5 IVA, 2MBCD ,(MAD)**
- **C5:1 (with C5-OH) SKAT, 3-MCC**
- **C5-OH 3-MCC, HMGL, SKAT, MCD, 3-methylglutaconyl hydratase def**
- **C3-DC MA**
- **C5-DC GA-I,**
- **C6-DC (with C5-OH) HMG**

- **Urine organic acid analysis is diagnostic for differentiation:**

P.A→
- MC
- PG
- 3HPA

- **MMA→**
  - MC
  - PG
  - MMA
  - 3HPA

- **MCD→**
  - MC
  - 3HPA
  - methyl crotonylglycine

- **Biotinidase deficiency→**
  - ↓ Biotinidase enzyme
  - GCMS: ↑ MCG- 3HPA- MC

- **IVA→** ↑ IVG
- **BKT:** 2M 3HBA, TG
- **GA_1:** GA, 3HGA
- **HMGL:** 3-hydroxy 3-methylglutaric acid,

  3-methylglutaconic acid

- **MSUD→**
  - HPLC, MS/MS : ↑ leucine, valine, isolucine
  - U.GCMS: ↑ ketoisoralerate, α keto 3-methylvalerate,α ketoisocaproate

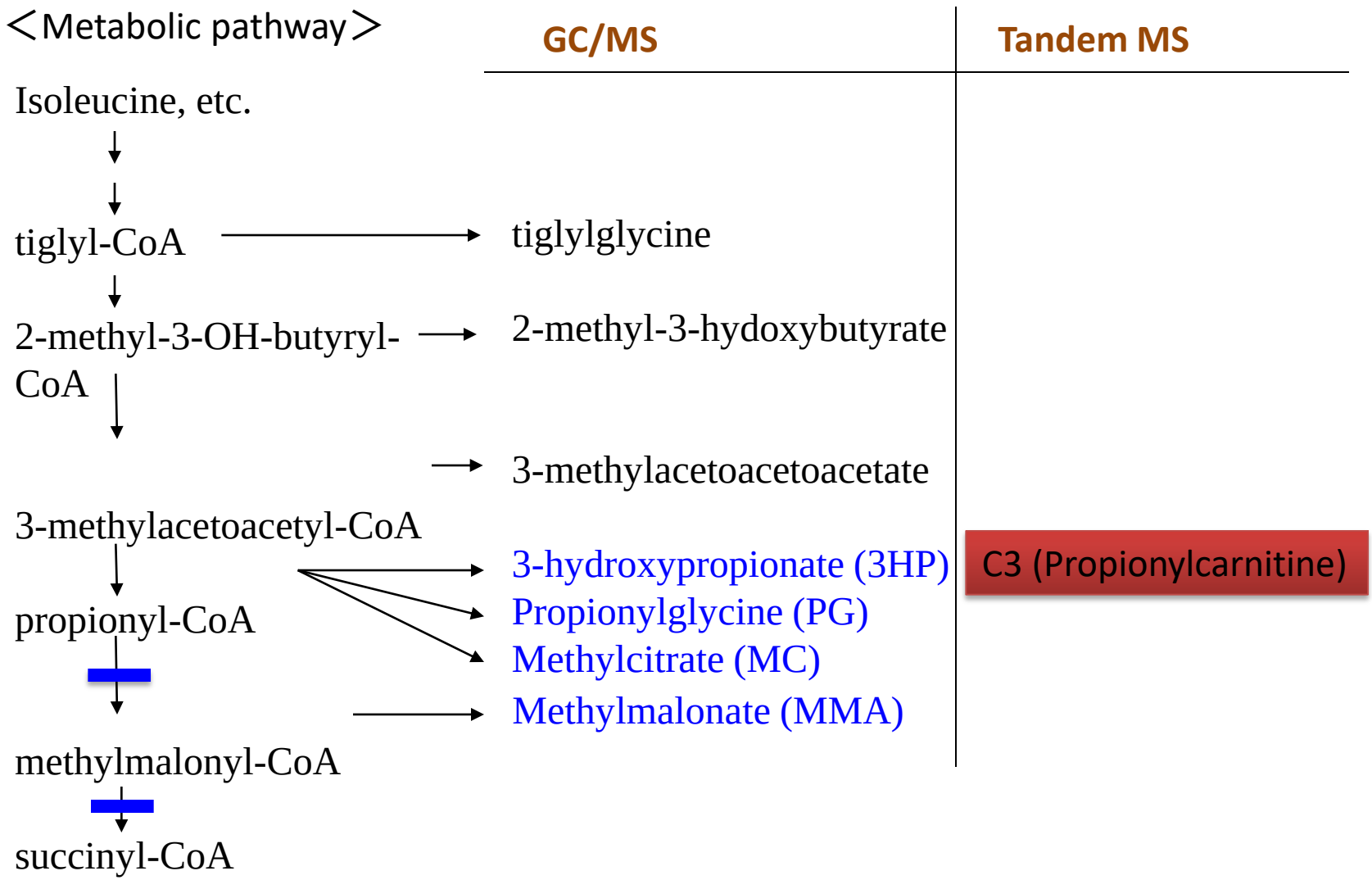
- **Elevated C3 (C3/C0, C3/C2, C4DC)**
- **Abnormal Screen Result: Elevated C3 (propionyl carnitine)**
- Elevated C3/C2 Elevated C3/C16
- **when the C3 is greater than 10 µM and the C3/C2 and/or C3/C16 is elevated or when the C3 is greater than 15 µM, regardless of the ratio levels**
- DD: Propionic aciduria ,methylmalonic aciduria; cobalamin disorders, FIGLU(Glutamate formiminotransferase deficiency),Succinyl CoA synthase deficiency
- many false positive cases
- Confirm.: Acylcarnitines (plasma), OA (urine

Methylmalonic Acidemia with Homocystinuria (CBL C, D, F)

- Abnormal Screen Result: Elevated C3 (propionyl carnitine)
- Decreased MET (Methionine)
- Elevated C3/C2

Ex1）elevation of C3 in tandem MS

Methylmalonicacidemia



Elevated C5 (C5/C2)

(isovaleryl carnitine) Isovaleric acidemia is a disorder of leucine (LEU)

DD: Isovaleric aciduria ,2-methylbutyric aciduria ,possibly non-disease,

- Confirm: Acylcarnitines plasma, OA urine
- In OA:Lactic, 3OH-BUTYRIC, **ISOVALERYLGLYCINE**, HIPPURIC, CITRIC, ISOVALERYLGLUTAMATE

Pre-analytical aspects C5

- **Potential for false negatives :**

Transfusions

Delays in transit / sample deterioration

Physiological reasons

Potential for false positives :

Pivaloylcarnitine is isobaric with isovaleryl carnitine and can result in false positive results

pivalic derivatives present in nipple creams and AB

Glutaric aciduria type 2 is often associated with an increase in C5, C8 and C5-DC

2-methylbutyryl carnitine is elevated in short/branched chain acyl-CoA dehydrogenase deficiency (SBCAD) ,2-methyl butyryl co A dehydrogenase deficiency and is isobaric with isovalerylcarnitine and causes a positive screening result

OA:**2-METHYLBUTYRYLGLYCINE,**2-ETHYL-3OH-PROPIONIC, ALPHA-KG, HIPPURIC, CITRIC

Follow-up testing for elevated C5

- *Possible diagnosis: isovaleryl-coA dehydrogenase deficiency,2-methylbutyryl-coA dehydrogenase deficiency (2-MBCD),multiple acyl-coA dehydrogenase(MAD deficiency)*
- **Plasma acylcarnitine analysis - elevated C5 (+ others in MAD deficiency)**

Follow-up testing for elevated C5-DC

- *Possible diagnosis: Glutaryl-coA dehydrogenase deficiency (GA-I)(* Glutaric aciduria type 1)
- Metab.: ↑ C5DC (= glutaryl-CoA
- **Elevated C5DC (glutaryl carnitine) + C6OH (3-OH hexanoyl carnitine)**

Urine OA analysis - glutaric acidemia“classical”:3OH-GLUTARIC,GLUTARIC

- **Urine organic acids analysis - glutaric acidemia“low excretor” - glutaric acid not observed! :3OH-GLUTARIC**

Pre-analytical aspects C5-DC

- **Potential for false negatives :**

Transfusions

Delays in transit / sample deterioration

Physiological reasons

Potential for false positives :

C6OH acylcarnitine is isobaric with C5-DC acylcarnitine

- elevated C6OH acylcarnitine is seen in association with ketosis

Glutaric aciduria type 2 is often associated with an increase in C5, C8 and C5-DC acylcarnitines,

Elevated C5OH+C4DC

- **Elevated C5OH &C4DC(methyl malonyl carnitine)**
- DD:1. Multiple carboxylase deficiency ,C3 Elevate
- 2.HMG-CoA lyase deficiency ,also ↑ C6DC
- 3.3-Methylcrotonylglycinuria(3MCC) (possibly non-disease) ***Maternal 3-MCC:*** In some newborns, the elevated C4DC+C5OH is reflective of maternal 3-MCC levels.
- 4.3-Methylglutaconic aciduria I (probably non-disease in childhood)also C6:1
- 5.3-Oxothiolase deficiency, also ↑ C5:1
- Confirm.: Acylcarnitines plasma, OA urine

Follow-up testing for elevated C5-OH (3-OH isovaleryl carnitine)

- **Plasma acylcarnitine analysis –**
- **elevated C5-OH; also with C5:1 in 3-MCC and SKAT,**
- **or with C6DC in HMG;**
- **or with C3** (propionyl carnitine) **in MCD (holocarboxylase synthetase def).**

Urine organic acids analysis

- moderate or marked elevation of 3OH-isovalerate, with 3-methylcrotonylglycine(3-MCC);

or with 3-methylglutaconic and 3Methyl-3OH-glutaric acids , 3-METHYLGLUTARIC(HMG);

or with 3-methylglutaconic acid (glutaconic aciduria type I);

or with metabolites of propionic acidemia in MCD.

- In ß-ketothioase deficiency (SKAT), there is marked elevation of 2-methyl-3-OH-butyric and 2-methylacetoacetic acids, with tiglylglycine.

Biotinidase deficiency

- Method: Determination of biotinidase activity (% normal); residual activity < 10% = severe deficiency,
- 10–20(–30)% = partial deficiency
- Exposure of test card to humid heat may cause denaturation of enzymes and consecutively a false positive result
- Confirm.: Biotinidase analysis in serum/plasma

Beta Ketothiolase Deficiency

- **Elevated C4DC (methyl malonyl carnitine) + C5OH (3-OH isovaleryl carnitine)**
- C5:1 Tiglyl- BKT, MCC, MHBD,MCD
- C4-DC Methylmalonyl-/succinyl- MMA a , SUCLA2

2-Methyl 3-OH Butyric Aciduria (2M3HBA)

- **Elevated C4DC (methyl malonyl carnitine) + C5OH (3-OH isovaleryl carnitine)**
- Elevated C5:1 (tiglyl carnitine)
- Neonatal Presentation: Usually none

Elevated C4(butyryl carnitine)

- DD: SCAD deficiency ,IBD deficiency

(Isobutyryl Glycinuria)Ethylmaonic Enchephalopathy also C5

- Probably non-diseases (→ C4-acylcarnitine is excluded from NBS programmes in several

countries):

- outpatient assessment, continue breast feeding
- Confirm.: Acylcarnitines (plasma), OA (urine
- Neonatal Presentation: Nonete
- OA In EE:Ethylmalonic acid,isovaleryl glycine

Follow-up testing for elevated C-4

- *Possible diagnosis: isobutyryl-coA dehydrogenase deficiency(IBCD), (SCAD deficiency, MAD deficiency)*
- **Urine organic acids analysis - elevated isobutyrylglycine in IBCD**
- LACTIC, ALPHA-KG, ACONITIC, CITRIC, 4OH-HIPPURIC

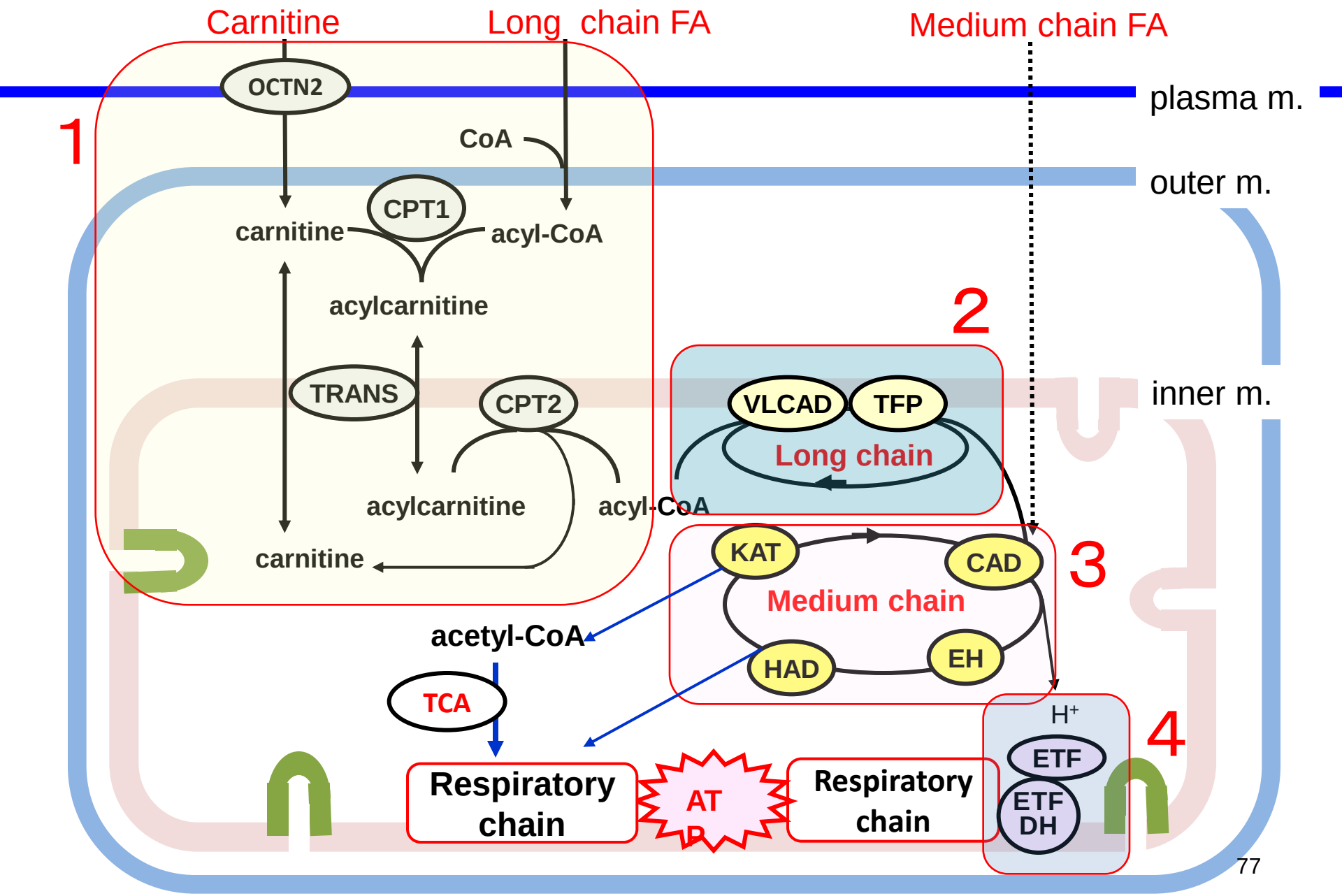
- Urine organic acids analysis - marked elevation of ethylmalonic and 2-methylsuccinic acids,butyrylglycine (“classical” SCAD);

modest elevation of ethylmalonic (“mild variant” SCAD); one or more of the following modestly elevated: ethylmalonic acid, adipic acid, glutaric acid, butyrylglycine, isobutyrylglycine,

isovalerylglycine, hexanoylglyine,

suberylglycine (MAD)

Mitochondrial β-oxidation



Acylcarnitines in FAO defects: Summary

Acylcarnitine species	Disorder to be considered
C0	Transporter defect
C4	SCAD, MAD
C5 (with C4)	MAD
C6 (with C8; C10:1)	MCAD
C8	MCAD
C10 (with C8, C10:1)	MCAD
C10:1 (with C8)	MCAD
C14:1	VLCAD

Acylcarnitines in FAO defects: Summary

Acylcarnitine species	Disorder to be considered
C14:1-OH (with C16-OH)	LCHAD/TFP
C16 (usually with C18:1)	CPT-II, CAT
C18:1 (with C16)	CPT-II, CAT
C16-OH	LCHAD, TFP
C18:1-OH (with C16-OH)	LCHAD, TFP
C16 Low (with C18:1)	CPT-I
C18:1 Low (with C16)	CPT-I

- Assessment of acylcarnitine profile with MS/MS method is diagnostic:
- In urine GCMS, findings is nonspecific→

  - **Dicarboxylic aciduria:** ↑ suberate, sebacate, adipate
  - **Non ketosis:** ↓ A.A- ß HB

- **PCD:**
  - ↓ Co (Free carnitine)
  - ↓ Long chains (↓ C16 - C18)

- C16 (Hexadecanoyl carnitine)
- C 18 (octadecanoyl carnitine)

- **CPT_1:**
  - ↑ Co
  - ↓ Long chains (↓ C16- C18)

- **SCAD:**
  - ↑ C_4 (Butyryl carnitine)
  - ↑ EM- M.S
- **MCAD:**
  - ↑ C_8 (octanoyl carnitine)
  - ↑ H.G, S.G
- **SCHAD:** ↑ C_4OH (3-hydroxy butyryl carnitine)
- **VLCAD:** ↑ $C_{14:1}$ (tetradecenoyl carnitine)

- TFP
- LCHAD

→ ↑ C_{16OH}- C_{18OH}

C_{16OH}: (3-hydroxyhexadecanoyl carnition)
C_{18OH}: (3-hydroxyoctadecanoyl carnitine)

- **CPT_2**
- **CACT**

→ ↑ C_{16}- $C_{18:1}$

C_{16}: Hexadecanoyl carnitine
$C_{18:1}$: octadecenoyl carnitine

Elevated C0

- Diagn.: CPT1 deficiency: ↑ C0/(C16 + C18)
- Confirm.: Acylcarnitines (plasma), carnitine status
- Secondary to rhabdomyolysis

Very low C0

- DD: Carnitine transporter deficiency ,organic acidurias, prematurity; if FTR normal: test mother for carnitine deficiency
- Confirm.: OA urine, carnitine status, fractional tubular re-absorption (FTR) of carnitine
- **Plasma acylcarnitine analysis - low C0 (usually <10**
- **µM); low acylcarnitine signals generally**
- **Urine organic acids analysis - non-specific findings;**
- **absence of dicarboxylic acids.**

Carnitine Uptake/Transport Deficiency (CUD)

- **Low C0 (free carnitine)**
- **C3 (propionyl carnitine) + C16 (palmitoyl carnitine) < 2**
- ***Maternal CUD*** - In some newborns, the low free carnitine is reflective of maternal CUD.
- **C0+C2+C3+C16+C18:1/Cit informative marker**
- **Low C0 :**medications including valproate, other…
- Secondary carnitine deficiencies,
- insufficient dietary intake,
- Renal tubulopathy,

C 2
(Acetylcarnitine)

- Elevate in:Carnitine supplementation or ketosis (deficiency if low)
- HMG CoA synthase deficiency(3-hydroxy-3 methyl glutary CoA synthase deficiency)
- Conf:plasma AC,in organic acid crotonylglysine/4 hydroxy- 6 methyl-2-pyrone
- Low C2 in CUD/Ethylmalonic Enchephalopathy
- ,CPT2,MCAD

Carnitine Palmitoyl Transferase Type I Deficiency (CPT IA)

- Primary High Markers
- Elevated C0
- **Elevated C0 (Free Carnitine)/C16 (palmitoyl carnitine) + C18 (octadecanoyl carnitine) ratio**
- Primary Low Markers
- **Low C16 (palmitoyl carnitine)**
- **Low C18 (octadecanoyl carnitine)**
- Low C18:1
- Low C18:2
- OA: **unremarkable. No specific diagnostic metabolites.**

Carnitine Palmitoyl Transferase Type II Deficiency (CPT II)

- Primary Markers
- Elevated C16 (palmitoyl carnitine)
- Elevated C18
- Informative Markers
- Elevated C12
- Elevated C16OH
- Elevated C18:1 (oleyl carnitine)
- OA: **either normal, or showing dicarboxylic aciduria and 3-hydroxydicarboxylic aciduria with reduced ketones when fasting. No specific diagnostic metabolites.**

Carnitine/Acylcarnitine Translocase Deficiency (CACT)

- Primary Markers
- Elevated C16 (palmitoyl carnitine)
- **Elevated C18 (octadecanoyl carnitine)**
- Informative Markers
- Elevated C12
- Elevated C16OH
- Elevated C18:1 (oleyl carnitine)

Elevated long-chain acylcarnitines

- DD: ↑ C16, C18; low C0: carnitine translocase or CPT2 deficiency
- ↑ C14:1, C14, C14:1/C4, C14:1/C12:1, etc.: VLCAD deficiency
- ↑ C16OH, C18:1OH LCHAD/MTP deficiency
- C12-OH 3-Hydroxy dodecanoyl- LCHAD/TFP deficiency/C14-OH
- Confirm: Acylcarnitines (plasma), OA (urine), carnitine status

Very Long Chain Acyl Co-A Dehydrogenase Deficiency (VLCAD)

- Primary Markers
- Elevated C14:1 (tetradecenoyl carnitine)
- **Elevated C14:1/C2 ratio**
- **High Secondary Markers**
- **Elevated C12 (dodecanoyl carnitine)**
- **Elevated C12:1 (dodecenoyl carnitine)**
- Elevated C14 (tetradecanoyl carnitine)
- **Elevated C14:2 (tetradecadienoyl carnitine)**
- **Elevated C16 (palmitoyl carnitine)**
- **Urine organic acids analysis - either normal, or showing**
- **dicarboxylic aciduria with reduced ketones when fasting**

Long Chain 3-OH Acyl Co-A Dehydrogenase Deficiency (LCHAD) and Trifunctional Protein Deficiency (TFP)

- Abnormal Screen Result:
- Primary Marker
- Elevated C16-OH (3-OH palmitoyl carnitine)
- Secondary Markers
- Elevated C14:1 (tetradecenoyl carnitine)
- Elevated C14 (tetradecanoyl carnitine)
- Elevated C18 (octadecanoyl carnitine)
- Elevated C18:1-OH (3-OH oleyl carnitine)
- C14-OH/C12-0H
- OA: either normal, or showing dicarboxylic aciduria and 3-hydroxydicarboxylic aciduria with reduced ketones when fasting. No specific diagnostic
- metabolites for LCHAD; 3-OH-monocarboxylic acids might accumulate in TFP deficiency

Malonic aciduria /Medium/Short Chain 3-OH acyl CoA Dehydrogenase Deficiency (M/SCHAD

- Metab.: ↑ C3DC, C4OH
- **Elevated C3DC (malonyl carnitine) + C4OH (3-OH butyryl carnitine)**
- **Elevated C3DC (malonyl carnitine) + C4OH (3-OH butyryl carnitine)/C10 (decanoyl carnitine) ratio**
- **C10-OH**
- **OA:Malonic acid**
- C4OH In SCHAD OA:3 Hydroxy Glutaric acid

Pre-analytical aspects C8
Factors affecting the screening results

- C8 concentrations decrease slightly with increasing birth weight and in general, males have slightly higher C8 concentrations than females;
- **Potential for false negatives :**
- Transfusions could result in a false negative result, At least 72 hours is recommended

Dextrose administration in a sick neonate with MCADD prior to blood collection may reduce octanoylcarnitine levels.

It is known that C8 falls in older infants (after approximately 1 month of age)

False positive C8

- ***Premature/sick infants*** - Some special formulas and breast milk fortifiers fed to premature/sick infants contain medium chain triglycerides (MCT) as the primary fat source. These feedings may cause false elevations of some acyl carnitines analyzed in MCAD screening, particularly C8, C10:1 and C8/C10.
- Hypoxia/stress induced lipolysis/riboflavin deficiency or deficient mother/valproate therapy/mithocondrial myopathy/ Physiological stress / Early sampling , contamination
- MAD DEFICIENCY:C4,C5,C6,C8,C10,C12,C14,C14:1

Elevated medium-chain acylcarnitines

- Diagn.: MCAD deficiency: ↑ C8, C8/C2, C8/C12
- **Abnormal Screen Result: Primary Markers**
- **Elevated C8 (octanoyl carnitine)**
- Elevated C10 (decanoyl carnitine)
- Elevated C10:1 (decenoyl carnitine)
- Secondary Markers
- **Elevated C6 (hexanoyl carnitine)**
- Elevated C8/C10
- Confirm.: Acylcarnitines (plasma), OA
- (urine- **elevated hexanoylglycine and suberylglycine, often with 5-OH-hexanoic acid, also with dicarboxylic acids when fasting. Variants can be normal**

Medium Chain Ketoacyl CoA Thiolase Deficiency (MCAT)

- Abnormal Screen Result: Elevated C8 (octanoyl carnitine
- C8-OH
- C6
- Dienoyl Co-A Reductase Deficiency (DE RED)
- Elevated C10:2 (decadienoyl carnitine)
- C10:2/C10

Glutaric Aciduria Type II (GA II) (C4– C18)

- multiple acyl Co-A dehydrogenase deficiency (MADD)
- Primary Markers
- Elevated C4 (butyryl carnitine)
- Elevated C5 (isovaleryl carnitine)
- Secondary Markers
- Elevated C6 (hexanoyl carnitine)
- Elevated C8 (octanoyl carnitine)
- Elevated C10 (decanoyl carnitine)
- Elevated C10:1
- Elevated C12
- Elevated C12:1
- Elevated C14
- **Elevated C14:1 (tetradecenoyl carnitine)**
- Elevated C16OH
- Elevated C5DC

3/C) Mitochondrial disorders:

- There is not any specific finding in acyl carnitine profile
- High blood level of:
  - Lactate
  - L/P ratio
- Metabolic acidosis

- U.GCMS: increased level of:
  - Lactate
  - 3-hydroxybutyrate
  - Acetoacetate
  - Fumarate
  - Succinate
  - Malate
  - 2- ketoglutarate

Galactose (Gal) and galactose metabolites

- Findings: – Galactose (plasma, dried blood spots); pathological if > 10 mg/dl (0.55 mM)
- – Galactose-1-phosphate (erythrocytes); pathological if > 0.5 mg/dl (19 µM)
- – Galactitol (urine); pathological if > 10 mmol/mol creatinine
- – Enzyme studies (erythrocytes): GALT, galactokinase, epimerase
- – Mutation studies (EDTA whole blood)

Pre-analytical aspects

- Galactosemia
- *Measurement of blood spot galactose-1-phosphate-uridyl-transferase (GAL-1-PUT)*
- *Thin-layer chromatography of sugars (galactose) using dried blood spots*
- *Measurement of blood spot galactose-1-phosphate (GALP)*

Galactosaemia

- Method: Gal-1-P uridyltransferase (GALT) activity; quantitation of galactose (Gal) and Gal-1-P (either in parallel or as second tier tests; in GALT and UDP-Gal epimerase [GALE]deficiencies almost all galactose [> 90%] is Gal-1-P).
- DD: ↓ GALT activity: classical galactosaemia (GALT deficiency)
- ↑ Gal: inborn errors of Gal metabolism : GALT/GALE/GALK deficiencies);
- liver failure (various causes); open ductus venosus arantii

- GALT activity may be false normal after erythrocyte (exchange) transfusion. Exposure of test card to humid heat may cause denaturation of enzymes and consecutively a

false positive result for GALT activity

- **Abnormal Screen Result: Elevated total galactose with low GALT: at risk for classical galactosemia.**
- **Normal total galactose with very low GALT: at risk for Duarte galactosemia, or at risk for classical galactosemia, if infant on non-lactose feeding at time of screening.**
- **Elevated total galactose with normal GALT: at risk for GALK or GALE deficiency.**
- Repeat screening for galactosemia should be done 120 days after the last transfusion.

- **If GALT is normal in the initial specimen, repeat galactosemia screening as soon as possible. NO NEED TO STOP BREAST FEEDING OR CHANGE FORMULA TYPE at this time.**
- Neonatal Presentation: GALT - hypoglycemia, jaundice, sepsis, failure to thrive
- Duarte variant galactosemia - None
- GALK - None
- GALE - Usually none

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, phosphoethanolamine	↓ 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

Table 49.6 Nutritional status and amino acid values

Factor/condition	Source	Amino acid(s) affected	Value
Diet, canned formula or milk	U	Homocitrulline	↑ H
Diet, gelatin	U	Gly	↑ H
Diet, high protein (infants)	B	Met, Tyr	↑ H
Diet, shellfish	U	Taurine	↑ H
Diet, white meat from fowl	U	Anserine, 1-methylhistidine, carnosine	↑ H
Folate deficiency	B	Homocyst(e)ine	↑ H
Kwashiorkor	B	Pro, Ser, Gly, Phe	↑ H
Kwashiorkor	B	Leu, Ile, Val, Trp, Met, Thr, Arg	↓ L
Obesity	B	Branched-chain amino acids, Phe, Tyr	↑ H
Obesity	B	Gly	↓ L
Starvation, 1–2 days (with or without vomiting)	B	Branched-chain amino acids, Gly	↑ H
Starvation, 1–2 days (with or without vomiting)	B	Alanine	↓ L
Vitamin B12 deficiency	B	Homocyst(e)ine	↑ H
Vitamin B6 deficiency	U	Cystathionine	↑ H

B blood, *U* urine, *H* high, *L* low

Table 49.7 Effects of illness/disease on amino acid values

Factor/condition	Source	Amino acid(s) affected	Value
Burn >20 % of surface area (0–7 days after injury)	B	Phe	↑H
Burn >20 % of surface area (0–7 days after injury)	U	Ala, Gly. Thr, Ser, Glu, Gln, Orn, Pro	↓L
Diabetes	B	Leu, Ile, Val	↑H
Hepatic disease	B	Tyr, Phe, Met, Orn, GABA	↑H
Hepatic disease	B	Branched-chain amino acids	↓L
Hepatoblastoma	U	Cystathionine	↑H
Hyperinsulinism	B	Leu, Ile, Val	↓L
Hypoparathyroidism, primary	U	All amino acids	↑H
Infection	B	All amino acids	↓L
Infection	B	Phe/Tyr ratio	↑H
Infection	U	All amino acids	↑H
Ketosis	B	Leu, Ile, Val	↑H
Ketotic hypoglycemia	B	Ala	↓L
Leukemia, acute	U	Advanced disease: all amino acids	↑H
Leukemia, acute	U	On therapy: Gly, Asp, Thr, Ser	↑H
Neuroblastoma	U	Cystathionine	↑H
Renal failure	U	Phe, Val	↓L
Renal failure	U	His	↑H
Renal failure	B	Phe, Cit, Cys, Gln, homocyst(e)ine	↑H
Renal failure	B	Leu, Val, Ile, Glu, Ser	↓L
Respiratory distress on oxygen	B	Cystine	↓L
Rickets	U	Gly	↑H

B blood, *U* urine, *H* high, *L* low

Table 49.8 Effect of medications on amino acid values

Table 49.8 Effect of medications on amino acid values

Factor/condition	Source	Amino acid(s) affected	Value
Acetaminophen	U	Acetaminophen-cysteine disulfide may interfere with determination of Phe	↑ H
N-Acetylcysteine	U	Acetylcysteine-cysteine disulfide	↑ H
Ampicillin/amoxicillin	U	Interferes with determination of Met, Phe, argininosuccinate	↑ H
Arginine infusion	B	Arg	↑ H
Arginine infusion	U	Arg, Lys, Orn, Cys	↑ H
Bile acid sequestrants (colestipol, niacin)	B	Homocyst(e)ine	↑ H
Cephalexin	U	Ninhydrin reactive metabolite	
Cyclosporin A	B	Total homocysteine	↑ H
2-Deoxycoformycin	B	Homocyst(e)ine	↓ L
Lysine aspirin	U	Lys	↑ H
Methotrexate therapy	B	Homocyst(e)ine	↑ H
Methotrexate therapy	B	Phe/Tyr ratio	↑ H
Nitrous oxide anesthesia	B	Homocyst(e)ine	↑ H
Oral contraceptives	B	Pro, Gly, Ala, Val, Leu, Tyr	↓ L
Penicillamine	U	Penicillamine disulfide, penicillamine-cysteine disulfide	↑ H
D-Phenylalanine	U	Phe	↑ H
Tamoxifen	B	Homocyst(e)ine	↓ L
Tetracycline, renal toxicity	U	All amino acids	↑ H
Valproate	B,U	Gly	↑ H
Vigabatrin/vinyl-GABA	U	β-Alanine, β-aminoisobutyrate, GABA	↑ H
Vigabatrin/vinyl-GABA	CSF	GABA, β-alanine	↑ H
Vigabatrin/vinyl-GABA	B,U	2-Aminoadipic acid	↑ H

B blood, *U* urine, *H* high, *L* low

Table 50.5 Non-IEM organic acids in urine as well as dietary/drug/bacterial artefacts

Compound	Condition
Aromatic acids (4-hydroxyphenyl)	Gut bacterial action
Mandelic acid	Albumin infusion
D-Lactic acid	Short bowel syndrome
D-2-Hydroxyisocaproic acid	Short bowel syndrome
D-Phenyllactic acid	Short bowel syndrome
3-Hydroxyisovaleric acid	Valproate medication
Glutaric acid	Gut bacterial action
3-Hydroxypropionic acid	Gut bacterial action
Methylmalonic acid	Vitamin B_{12} deficiency
Ethylmalonic acid	Vitamin B_2 deficiency
C_{10}>C_8>C_6 dicarboxylic acids	MCT diet
7-Hydroxyoctanoic acid	MCT diet
3-Hydroxydicarboxylic acids	Coeliac disease
Succinic acid	2-Ketoglutarate decomposition.
Glycolic acid	Ethylene glycol poisoning
Pyroglutamic acid	Glutamine decomposition Flucloxacillin toxicity
Di-(2-ethylhexyl)phthalate	Nutramigen feeding Pregestimil feeding
Furane-2,5-dicarboxylic acid	Heated sugars
Furoylglycine	Heated sugars
4-Hydroxycyclohexanecarboxylic acid	Food processing
Homovanillic acid	Neuroblastoma
Vanilmandelic acid	Neuroblastoma, phaeochromocytoma
N-Acetyltyrosine	Parenteral feeding
5-Hydroxyindoleacetic acid	Carcinoid syndrome
Valproate metabolites	Depakine therapy
2-Hydroxyhippuric acid	Salicylate ingestion
Ethosuximide metabolites	Antiepileptic therapy
Keppra metabolites	Antiepileptic therapy
Phenytoin metabolites	Antiepileptic therapy

Abnormalities associated with the inherited organic aci- accumulation in the various organic acidemias. It can be

Table V: Maternal conditions affecting the newborn screening results

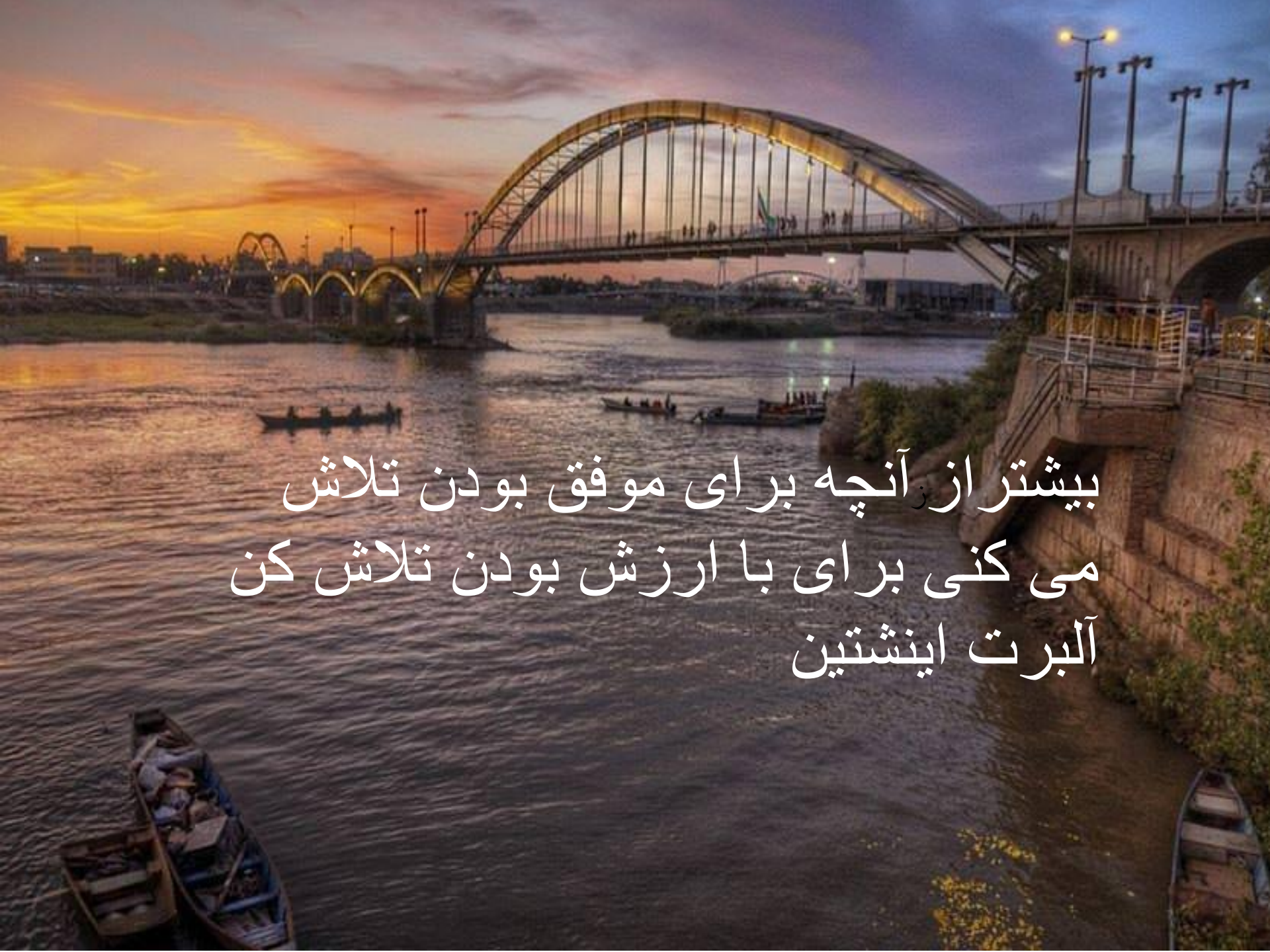
Maternal conditions	NBS analyte affected	Results in	Additional information/ duration of interference
Hyperthyroidism treated with Propylthiouracil (PTU)	Low thyroxine (T4), high TSH	Transient hypothyroidism	Until drug clears, typically 7–14 days
^{161}I (radioactive iodine) treatment during pregnancy: Before 8 weeks' gestation.	none	Euthyroid (but may cause birth defects)	Will cause maternal hypothyroidism (potential effect on fetal brain development if not treated in first trimester)
^{161}I (radioactive iodine) treatment during pregnancy: After 8 weeks' gestation (when fetal thyroid matures and traps iodine)	Low T4, high TSH	Permanent hypothyroidism	Lifelong
Steroids: prednisone, betamethasonel/ dexamethasone	Low or normal 17-0HP	Suppresses fetal adrenal function and causes false-negative results for CAH	Unknown - depends on class of steroid and dose; estimate 1–2 weeks
CAH	Elevated 17-0HP	False-positive result	Unknown-estimate 3-7 days
PKU or moderate hyperphenylalaninemias uncontrolled by diet or medications	Elevated phenylalanine; although ratio of phenylalanine - to - tyrosine (Phe/Tyr) should be normal; false-positive result	Transient hyperphenylalaninemia	12–24 hours unless infant has PKU
3-MCC deficiency	Elevated C50H	False-positive result	Unknown
Fatty liver of pregnancy or HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)	May have elevated even chain acylcarnitines	False-positive result	Unknown
Carnitine deficiency	May have low carnitine levels	False-positive result	Unknown
Vitamin B12 deficiency	Elevated propionylcarnitine (C3)	False-positive result	A number of days depending on nutrition provided

Table VI: Treatments used in special care baby unit and effects on newborn screening results

Treatment	Effect on newborn screening results	Duration of effect
Parenteral Nutrition (PN)	Elevation of multiple amino acids	4–24 hours after PN discontinued
Carnitine supplementation	Elevations of acylcarnitines; can mask carnitine transport disorders	For duration of supplementation and weeks later
Red cell transfusion and Extra Corporeal Life Support (ECLS) (pre- and postnatal transfusions)	Can mask the absence of enzymes and proteins intrinsic to the red blood cell (RBC), thereby negating results for hemoglobinopathies and galactosemia (when testing is for galactose 1 phosphate uridyl transferase (GALT) enzyme activity)	120 days after last transfusion ECLS invalidates all NBS results for analyte-specific periods of time
Dopamine	False-negative testing for CH, because levels of TSH are suppressed	Until drug therapy is stopped
Steroids	Suppressed TSH and T4; possible false-negative result for CH. May suppress 17-OHP resulting in false-negative testing for CAH	Unknown - depends on class of steroid and dose; estimate 1–2 weeks
Iodine exposure with povidone/ iodine preps	Transient hypothyroidism; lowT4, elevated TSH	Once exposure to topical iodine discontinued, resolution may take 2–6 weeks (depending on dose absorbed and other factors)
Pivalic acid antibiotic therapy	May cause elevated isovaleryl 2-methylbutyryl carnitine	Unknown

Table VII: Conditions of the infant affecting newborn screening tests

Condition of the infant	Effect on newborn screening	Duration of effect
Immature hypothalamic-pituitary thyroid axis	Low T4, normal TSH, infants with congenital hypothyroidism (CH) can be missed	Up to 6 weeks of age
Hypothyroxinemia of preterm birth	Transient hypothyroidism, lowT4; normal TSH followed by elevated TSH	Up to 6 weeks of age
Liver enzyme immaturity	Transient elevations of tyrosine, methionine, and galactose, occasionally other amino acids	A few weeks
Iodine deficiency	Transient hypothyroidism low T4, elevated TSH	Until supplemented
Acute illness	Transient hypothyroidism; low T4, elevated TSH, elevated immunoreactive trypsinogen (IRT)	Until recovered
Hypoxia	Elevated IRT	Until recovered
Liver disease	Elevated tyrosine, methionine, galactose Depression of biotinidase enzyme	Until recovered
Renal immaturity	Elevated 17-OHP, amino acids	Until recovered
Preterm	Lower biotinidase levels inversely related to gestational age	40 weeks gestational age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