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Manual of Establishing a Newborn Screening Program

Majid Alfadhel , Saif Al Saif & Abdullah Al Zaben

Manual of Establishing a Newborn Screening Program

Diagnosis & Management of Screened Disorders

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لا يجوز نشر أي جزء من هذا الكتاب، أو اختزال مادته بطريقة الاسترجاع، أو نقله على أي نحو، وبأي طريقة، سواء كانت إلكترونية أو ميكانيكية أو بالتصوير أو التسجيل أو خلاف ذلك.

إلا بموافقة الناشر على ذلك كتابة ومقدماً.

Designed by: Mr. Fuad Tafesh

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Contribution of the Authors

Dr. Majid Alfadhel performed the majority of work associated with preparing, writing and printing the book. Dr. Abdullah Al Zaben & Saif Al Saif edited the book.

Preface

The analysis of different molecules by tandem mass spectrometry (TMS) has been available since the early 1980s, but it is only in the 1990s when its use in neonatal screening programs began to be recognized. It is a cost effective test to diagnose several disorders, and it only requires a few drops of blood from the newborn. TMS allows for rapid, simultaneous analysis and detection of plenty of disorders of hemoglobin, endocrine, amino acids, organic acids, and fatty acid metabolism (1).

This novel discovery pushed several countries all over the world to implement expanded newborn screening programs (NBS) aimed at detecting disorders at their earliest or asymptomatic stages to prevent complications and save many lives. Currently, many countries have population based NBS programs covering a wide range of genetic disorders. Now data is available from several centers including North America (2), Latin America (3), Europe (4), and Australia (5).

A newborn screening program (NBS) is one of the most successful programs of preventive medicine. It is a complex program requiring multidisciplinary team collaboration including: neonatologists, nurses, biochemical geneticists (metabolic), endocrinologists, NBS lab professionals, NBS coordinators and metabolic dietitians. The rationale of newborn screening (NBS) programs is to detect abnormal metabolite in a presymptomatic baby and then provide solid management measures which likely and significantly improve the natural history of the disease.
According to a 2010 census, the population of Saudi Arabia is approximately 29 million with a growth rate of 3% (6, 7). The annual live birth rate is estimated at 569,000 neonates. However, consanguineous marriages are prevalent in the Saudi society and are estimated at between 51%-56% of the total marriages which take place. Roughly 30% of them are 1st degree cousins (8, 9, 10, 11). As a result of this high consanguinity rate, the prevalence of autosomal recessive disorders in general and inborn errors of metabolism specifically is exceedingly high causing great social and economic burden.
Although NBS program was developed more than 9 years ago, still the population coverage is suboptimal. Several factors have contributed to this including lack of biochemical geneticists, qualified laboratories, and metabolic dietitians and experts in the field that could manage such complex disorders.
The reader of this book will easily understand the essential requirements of establishing an NBS program and their role in the process of NBS. An understanding of the whole process will foster for readers a strong inclination to grasp the importance of close cooperation between all team members for the care of these affected neonates. We hope that this book could be utilized as a scientific guide to start and maintain newborn screening programs (NBS) in more hospitals.

Inborn Errors of Metabolism

This group of inherited diseases, inborn errors of metabolism (IEM), is caused by the absence or deficiency of specific enzymes, its cofactor or a transporter essential for the body's cell function. This may lead to the accumulation of toxic metabolite(s) that cause serious damage to vital organs or cause biochemical or electrolyte imbalance.

IEM disorders are rare individual diseases but are common as a group of diseases. The incidence rate is around 1 per 1000 births. These disorders usually manifest early in life with clinical or biochemical modifications that may not be recognized early.

Often times the damage is irreversible, and the best way to manage these children is to screen them before they show such signs or symptoms.

Diagnosed cases are registered and special diet, drug therapy and/or enzyme replacement are provided to maintain normal metabolism.

Examples are clearly shown in the booklet, and they contain only those IEM diseases covered by the newborn screening program.

Chapter 1

Newborn Screening Program in Saudi Arabia

With great effort from Saudi health authorities including: Prince Salman Center for Disability Research (PSCDR), King Faisal Specialist Hospital and Research Center (KFSH-RC), in addition to the Ministry of Health (MOH), the national NBS program was launched in the year 2005. Phase I includes 24 birth centers and about 120,000 newborns aiming to have overall coverage of the Kingdom in 10 years based on funding readiness (12). Currently, this program involves more than 139 hospitals distributed over all the regions of Saudi Arabia, and these hospitals belong to different health sectors including: the Ministry of Health, King Faisal Specialist Hospital and Research Center (KFSH-RC), the Ministry of National Guard, the Ministry of Defense and others. The overall incidence rate of screened disorders is approximately 1:1000.

The disorders included in the NBS program are as follows:

Inborn Errors of Organic Acids Metabolism

1. HMG-CoA Lyase Deficiency (HMG)
2. Isovaleric Acidemia (IVA)
3. Methylmalonic Acidemia (MMA)
4. Propionic Acidemia (PA)
5. Beta-ketothiolase Deficiency (BKT)
6. 3-Methylcrotonyl-CoA Carboxylase Deficiency (3MCC)
7. Glutaric Aciduria type-I (GA-I)

Inborn Errors of aminoacids metabolism

8. **Phenylketonuria (PKU)**
9. **Maple Syrup Urine Disease (MSUD)**

Urea Cycle Defects

10. Argininosuccinate Lyase Deficiency (ASL)
11. Citrullinemia (ASS)

Fatty Acid Oxidation Defects

12. Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
13. Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)

Inborn Errors of Carbohydrates

14. Galactosemia

Vitamin Responsive Disorder

15. Biotinidase Deficiency

Endocrine Disorders

16. Congenital Hypothyroidism (CH)
17. *Congenital Adrenal Hyperplasia (CAH)*

Chapter 2

Stakeholders of NBS & Their Responsibilities

- ***Biochemical Genetic (Metabolic) consultant:*** *She/he will receive positive results for the NBS program for Inborn Errors of Metabolism (IEM) and ensure that all affected infants with IEM receive appropriate confirmatory testing, counseling, and initiation of treatment as soon as possible. If the Biochemical Genetic consultant is not available, then a pediatrician who has special training in IEM could stabilize the patient and initiate the management until referral to a higher medical center.*

- ***Endocrinology consultant:*** *She/he will receive positive result for NBS program for endocrinological disorders (ED) and ensure that all affected infants with ED receive appropriate confirmatory testing, counseling, and initiation of treatment as soon as possible.*

- ***Neonatology consultant:*** *She/he will coordinate and help the specialized consultant (Biochemical Geneticist and Endocrinologist) to ensure that all affected infants receive appropriate confirmatory testing, counseling, and initiation of treatment as soon as possible.*

- ***NICU and Admission Nursery nurses:*** *They shall be responsible for collecting blood samples, ensuring the appropriate sampling of blood/urine, etc. from newborns and that all submitted samples are registered in the special form as well as the appropriate handling and transfer of blood samples to NBS labs.*

- ***NBS Lab:*** *It is responsible for analysis, interpretation of the samples and reporting the results to NBS coordinators, the Neonatologist, the Biochemical Genetic (Metabolic) consultant, and the Endocrinology consultant.*

- ***NBS nurse coordinator****: She /he observes and maintains the flow of the program and is expected to:*
    - *Ensure hospital supply of test cards.*

- *Teach nurses how to fill the cards & save them.*
- *Be responsible for checking on babies' demography for follow-up.*
- *Organize sample collection and receiving.*
- *Be responsible for following up test results.*
- *Be responsible for recalling patients for repeat tests and confirmation.*
- *Be responsible for logging all confirmed cases.*

- ***Metabolic dietitian:***
  - *Ensure availability of home formula supplies including aminoacids mixture e.g.: CYCLINEX®-1 (for infants and toddlers) or CYCLINEX®-2 (for children; adolescents, and adults)* see appendix 1.
  - *Ensure that the baby receives the required amount of natural protein: for infants - breastmilk or regular formula as a natural protein source: for older children - the source of natural protein is from regular diet.*
  - *Ensure availability and compliance of the mother with weighing skills, appropriate preparation of formula and 3-day diet records prior to clinic visits.*
  - *Monitoring of amino acid concentrations. The frequency of amino acid monitoring varies by age, metabolic stability, compliance, and regional clinical practice. For rapidly growing infants, monitoring weekly is recommended.*

- ***Clinical pharmacist:***
  - *Ensure availability of hyperammonemia scavengers in all parts of the hospital including ER, NICU and PICU.*
  - *Ensure availability of inborn errors of metabolism (IEM) medications.*

Unavailability of stakeholder at NBS site:

Essential stakeholders like Biochemical Genetic (Metabolic) consultants or metabolic dietitians cannot always guaranteed. This makes the establishment of NBS programs in targeted sites very difficult. However, until there is availability of such experts, the hospital should make a specific team that specifically cares for positive newborn screening program babies.

This team should consist of a:

- *General pediatric consultant*
- *Pediatric dietitian*
- *Clinical pharmacist*
- *Neonatology consultant*
- *Nurse coordinator*

This team should be trained by Biochemical Genetic specialists until they become familiar with initial management of these disorders, stabilize them, and refer them to referral centers. They should then follow them up with referral centers on a regular basis.

Chapter 3

Blood Sampling & Related Issues:

Before the sample is taken, parents are informed about the newborn screening program, and a flyer summarizing all relevant aspects is handed over to them. The collection of a blood specimen in the filter paper card is performed by nursing staff in the nursery. However, prior to the application of the blood to the filter paper, the nurse will document the required data on the data sheet including all medical information.

Time of blood sampling

The appropriate time for blood sampling for a term baby is between the 24th and the 72nd hour of life. For preterm babies, we need to repeat the sample when the baby is full term. If the baby gets discharged before term, we need to collect blood samples just before discharge. Then repeat the NBS when the baby reaches term.

The time of blood sampling may influence the results of the screening tests. Very early sampling will lead to a high rate of "false positive" results, and therefore we need to repeat sampling. In endocrine disorders, there is a TSH surge immediately after birth and a high cross reactivity of placental steroids while screening for CAH. In metabolic disorders, early sampling may cause "false negative" results. For example, PKU or homocystinuria, the accumulation of abnormal amino acids in the blood, does not occur until after birth when the clearance via the placenta suddenly stops. The velocity of the rise of the specific amino acid in the blood varies depending on the severity of the defect and the protein intake. On the other hand, late sampling may cause undue delay in treatment initiation in affected children. Blood for newborn screening has to be collected regardless of age before early discharge, before blood transfusion, and before treatment with corticosteroids and dopamine is initiated. If the first screening was performed before 24 hours, it has to be followed by a second screening test at the age of 48 to 72 hours.

Collection of the blood sample

Immediately after completion of the data on the card, take the blood sample before preparing the next card. The most common sampling procedure is a capillary heel prick performed by the bedside nurse in the postnatal ward, but venous blood may be used with special precautions so as not to affect the quality of screening.

Heel prick procedure

- *Put on gloves for personal safety.*
- *Do not touch the circles for blood collection on the filter paper.*
- *Place the infant's feet lower than the level of the heart in order to increase blood flow to the foot.*
- *Warm the heel for three to five minutes using a warm moist towel at a temperature no greater than 41°C.*
- *Cleanse the puncture site with a sterile alcohol pad and allow the heel to air dry.*
- *Puncture the heel with a sterile lancet in the areas shown in the picture.*
- *Wipe away the first drop of blood with a sterile gauze pad.*
- *Allow another large drop of blood to form.*
- *Apply very gentle intermittent pressure with the thumb.*
- *Avoid excess squeezing or "milking" as it contaminates the blood sample with tissue fluid.*
- *Allow the blood drop to touch one side of the filter paper circle and let the blood soak through the paper to completely fill the circle.*
- *Do not press the paper against the puncture site (it will interrupt blood flow).*
- *Do not apply successive drops of blood to the same circle.*

- *After blood collection, elevate the foot above the body and gently press the puncture site with a sterile gauze pad or cotton swab until the bleeding has stopped.*
- *Check for adequacy of the blood sample after finishing.*

Venous blood

Native venous blood may be used without the need to mention this on the card. However, common mistakes about venous blood sampling that make the sample inadequate for NBS are:

1. The blood is taken from a tube containing additives like heparin and or EDTA.
   Please let blood drop directly from a needle on the filter paper or from a syringe with no additives.
   EDTA is most dangerous because it will cause false positive results in CAH screening and false negative results in CH.
2. The blood is taken from a line where amino acids or fat solutions are infused. *This will result in false positive results and an unnecessary second sample.*
3. There is an overload of the filter paper with blood which may again cause the need for a second sample.

Identification of inadequate samples

- *The blood did not completely soak through the filter paper.*
- *The blood sample appears layered, clotted, supersaturated, scratched or abraded.*
- *Specimen exhibits serum rings; or appears diluted, discolored or contaminated.*
- *The blood is scanty and not sufficient.*
- *The data on the card is incomplete.*

Drying of the blood sample

The blood sample on the filter paper dries within 3-4 hours in the special shelf available in every postnatal ward. Direct exposure to heat and moisture has to be strictly avoided. Exposure to humid heat will destroy the enzymes biotinidase and galactose-1-P uridyltransferase (GALT). This will cause false positive results in the screening for biotinidase deficiency and classical galactosemia.

Transfer of samples to the laboratory

When the blood on the card is dry, the cards are transported to the laboratory to be processed, analyzed and interpreted by the Biochemical Geneticist.

Procedures:

1. *Inform and educate the family about your intention to collect blood for newborn screening, and get their agreement.*
2. *Wash hands with soap and warm water and dry thoroughly.*
3. *Prepare pediatric lancet, sterile gauze, gloves, band-aids and filter paper labeled with the patient's demographics. Prepare the baby's heel for the prick by gentle rubbing.*
4.

   Clean the prick site with an alcohol swab, air-dry, hold the baby's heel firmly and prick using the lancet.

5.

Remove the first blood drop with a sterile gauze and throw it away. Allow a new good size drop to form and lay it to one circle of the filter paper. Allow other drops to form and fill out the remaining circles. Clean the prick site with an alcohol swab, and apply the band-aid.

6.

Allow blood spots on the filter paper to dry for 3 to 4 hours, stack the cards as shown in the picture, and then put all the cards in the envelope and wait for the courier services for collection.

Checking Samples' Validity:

Valid Specimen

Allow a sufficient amount of blood to soak through to completely fill the preprinted circle on the filter paper. Fill all required circles with blood. Do not layer successive drops of blood or apply blood more than once in the same collection circle. Avoid touching or smearing spots.

Invalid specimens

1. Specimen quantity insufficient

Possible Causes:

• Removing filter paper before blood has completely filled the circle or before blood has soaked through to the second side.

• Applying blood to filter paper with a capillary tube.

• Touching filter paper before or after blood specimen collection with gloved or ungloved hands, hand lotion, etc.

• Allowing filter paper to come in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.

2. Specimen appears scratched or abraded.

Possible Cause:

• Applying blood with a capillary tube or other device.

3. *Specimen not dry before mailing.*

Possible Cause:

- *Sending specimen before drying for a minimum of three hours.*

4. *Specimen appears supersaturated.*

Possible Causes:

- *Applying excess blood to filter paper, usually with a device.*
- *Applying blood to both sides of filter paper.*

5. *Specimen appears diluted, discolored or contaminated.*

Possible Causes:

- *Squeezing or "milking" of area surrounding the puncture site.*
- *Allowing filter paper to be exposed to gloved or ungloved hands or substances such as alcohol, formula, antiseptic solutions, water, hand lotion or powder, etc., either before or after blood specimen collection.*
- *Exposing blood spots to direct heat.*

6. *Specimen exhibits serum rings.*

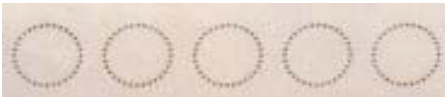
Possible Causes:

- *Squeezing or "milking" of area surrounding the puncture site.*
- *Allowing filter paper to be exposed to gloved or ungloved hands or substances such as alcohol, formula, antiseptic solutions, water, hand lotion or powder, etc., either before or after blood specimen collection.*
- *Exposing blood spots to direct heat.*

7. *Specimen appears clotted or layered.*

Possible Causes:

- *Touching the same circle on the filter paper to blood drop several times.*
- *Filling circle on both sides of filter paper.*



8. *No blood.*

Possible Cause:

- *Failure to obtain blood specimen.*

Things to Remember:

1. *Collect specimen between 24 to 72 hours of life.*
2. *For preterm infants, collect just before discharge "unless symptomatic."*
3. *Fill in all required information in a legible manner. Use Gregorian calendar for the dates.*
4. *Poor or over saturation of blood spotting on the filter paper is not acceptable.*
5. *Protect specimens from moisture and heat.*
6. *Do not pack the dried blood spot specimen in airtight leak-proof plastic bags. Use clean paper envelopes.*

Chapter 4

Interpretation of Abnormal Results

In this chapter, we will put all 17 disorders in one table that includes the disorder's clinical name, the abnormal metabolite, the abnormal values and the confirmatory test.

Table 1: Interpretation of abnormal results:

Disorder	***Abnormal results***	***Cut-off values (µmol/l)***	***Confirmatory methods***
PKU **Phenylketonuria**	*Increase phenylalanine (Phe) and phenylalanine/Tyrosine (Tyr) ratio*	*Phe: >180* *Phe/Tyr ratio >2*	*Repeat NBS, Plasma aminoacids and urine for organic acids, Pterin analysis in urine, Tetrahydrobiopterin (BH4) loading test* *DHPR-activity in DBS*
MSUD **Maple Syrup Urine Disease**	*Increase leucine/isoleucine ratio, increase valine*	*leucine/isoleucine ratio >245* *Valine >290*	*Repeat NBS, Plasma aminoacids and urine for organic acids, enzyme activity in WBC, mutation analysis*
PA **Propionic acidemia**	*Increase C3 and C3/C2*	*C3 >10* *C3/C2 >0.4*	*Repeat NBS, Plasma aminoacids and urine for organic acids and mutation analysis*
HMG-CoA Lyase Deficiency (HMG)	*Increase C5(OH) and C6DC*	*C5(OH) > 0.281* *C6DC >0.04*	*Repeat NBS, Plasma aminoacids and urine for organic acids, enzyme activity in WBC and mutation analysis*
Isovaleric Acidemia (IVA)	*Increase C5*	*>1.53*	*Repeat NBS, Plasma aminoacids and urine for organic acids, enzyme activity in WBC and mutation analysis*

3-Methylcrotonyl-CoA Carboxylase Deficiency (3MCC)	*Increase C5(OH)*	*C5(OH) > 0.281*	*Repeat NBS, urine for organic acids (for the infant and mother), enzyme activity in WBC and mutation analysis*
Beta-ketothiolase Deficiency (BKT)	*Increase C5(OH) and increase C5:1*	*C5(OH) > 0.281* *C5:1 >0.031*	*Repeat NBS, urine for organic acids, enzyme activity in fibroblasts and mutation analysis*
Disorder	***Abnormal results***	***Cut-off values (µmol/l)***	***Confirmatory methods***
Glutaric Aciduria type-I (GA-I)	*Increase C5DC, C5DC/(C8+C10), C5DC/C16*	*C5DC>0.3*	*Repeat NBS, urine for organic acids, enzyme activity in WBC and mutation analysis*
Citrullinemia (ASS)	*Increase citrulline*	*Citrulline >70*	*Repeat NBS, plasma aminoacids, urine for aminoacids, urine for orotic acid, enzyme assay in WBC and mutation analysis*
Arginosuccinase Deficiency (ASL)	*Increase citrulline*	*Citrulline >70*	*Repeat NBS, plasma aminoacids, urine for aminoacids, urine for orotic acid, enzyme assay in WBC and mutation analysis*
MCAD deficiency ***medium-chain acyl-CoA dehydrogenase***	*Increase C8 , C6, C10 and C8/C10*	*C6> 0.35* *C8>0.32* *C10> 0.3* *C8/C10 > 3.3*	*Repeat NBS, urine for organic acids, enzyme activity in WBC and mutation analysis*

VLCAD deficiency ***very long-chain acyl-CoA dehydrogenase***	*Increase C14:1, C14:1/C2, C14:1/C12*	*C14:1>0.4*	*Repeat NBS, urine for organic acids, enzyme activity in WBC and mutation analysis*
Biotinidase deficiency	*Low biotinidase level*	*<50 U/dL*	*Send for serum biotinidase and mutation analysis*
Galactosemia	*Low GALT level*	*<3.5 U/dL*	*Enzyme assay in blood and mutation analysis*
CH ***congenital hypothyroidism***	*High TSH*	*>21 µU/mL*	*Repeat TSH and free T4* *Thyroid scan and thyroid ultrasound*
CAH ***congenital adrenal hyperplasia***	*High 17(OH)P*	*>60nmol/l*	*Repeat 17 hydroxyprogesterone, serum electrolytes, ACTH stimulation test* CYP21 *gene mutation analysis*

DHPR: Dihydropteridine reductase; HMG: 3-hydroxy-3-methylglutaryl, PKU: phenylketonuria; MSUD: Maple Syrup Urine Disease; PA: propionic acidemia; MMA: methylmalonic acidemia; VLCAD: very long-chain acyl-CoA dehydrogenase; MCAD: medium-chain acyl-CoA dehydrogenase; CH: congenital hypothyroidism; CAH: congenital adrenal hyperplasia.

Chapter 5

False Positive & False Negative Results

We should know that not every positive result of NBS is true for a specific disorder; on the other hand, it is very important to understand the newborn screening program screens for these specific diseases, and there are many diseases we cannot yet screen for. Furthermore, a child exhibiting symptoms of a metabolic disorder that has repeatedly had a normal newborn screen by MS/MS may still have that disease. As with any biochemical test performed on newborns, MS/MS is a screening test, and it will miss a diagnosis if a pathognomic metabolite does not exceed the control range.

- ***False negative result:***
  False negative results of NBS could occur in case of early collection (<24 hours) or in case of blood transfusion or exchange transfusion. Therefore, if any of the aforementioned happens, then the NBS should be repeated at 72 hours in case of early sample collection or after 120 days in case of blood or exchange transfusion.

- ***False positive result:***
  False positive result of NBS could occur in several conditions. Table 2 summarize many example of these conditions. Therefore, if any of these artifacts happened, then the NBS should be repeated.

Table 2: The artifact and false positive results of NBS:

Condition	***Compound(s) involved***	***Change***
Prematurity	*C0*	*Decrease*
MCT oil	*C8,C10(C6-DC,C8-DC*	*Increase*
Liver enzyme immaturity or Liver disease	*Tyrosine , methionine and phenylalanine*	*Increase*
TPN	*Multiple amino acids*	*Increase*
Renal immaturity	*17(OH) progesterone*	*Increase*
Non-fasting sample	*Multiple amino acids*	*Increase*
Ketogenic diet	*C2, C4(OH)*	*Increase*
Sunflower/olive oil	*C14:2*	*Increase*

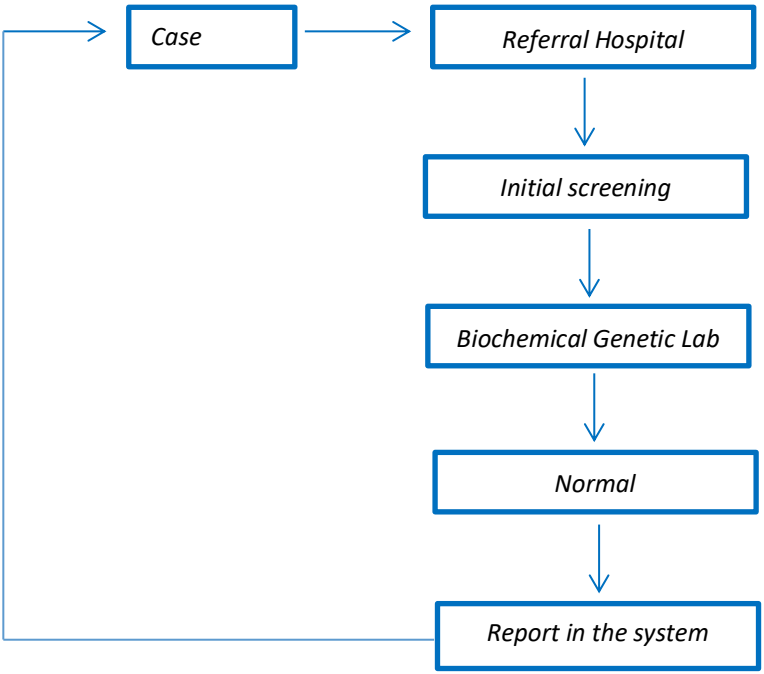
Lactic acidosis	*C2*	*Increase*
Valproic acid	*C8, C10*	*Increase*
Carnitine supplement	*C0, C2, C3 and others*	*Increase*
Benzoate	*C0*	*Increase*
Dialysis	*C0*	*Decrease*
Short gut syndrome	*C0*	*Decrease*
Early specimen collection (<12 or <24 hours)	*Unreliable testing of endocrinopathies and congenital adrenal hyperplasia (CAH) due to stress associated with birth or due to the immaturity of fetal thyroid.*	
Hyperbilirubinemia	*C3*	*Increase*
Prolonged fasting, diabetes and ketosis	*Leucine/isoleucine and valine*	*Increase*
Hyperinsulinemia, obesity	*Leucine/isoleucine and valine*	*Decrease*
Maternal vitamin B12 deficiency	*C3*	*Increase*
Antibiotics; ampicillin, cefotaxime	*C5*	*Increase*
Dextrose, IV	*C16-OH carnitine*	*Increase*
Heat, humidity or long transport time	*Galactose*	*Decrease*
Heat, humidity or long transport time	*Biotinidase*	*Decrease*

Chapter 6

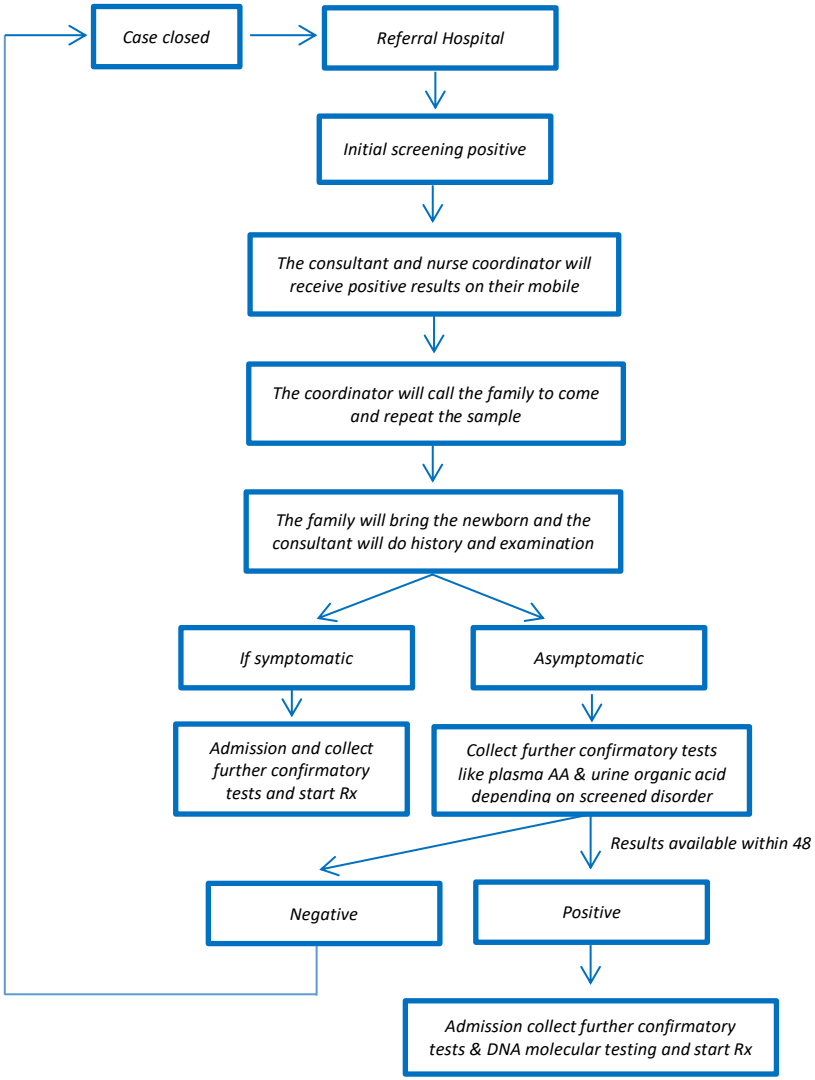
Clinical Algorithms for Newborn Screening Programs (NBS)

The results of NBS are either: normal or positive or rejected.

1 Algorithm For Collecting The Initial NBS Sample



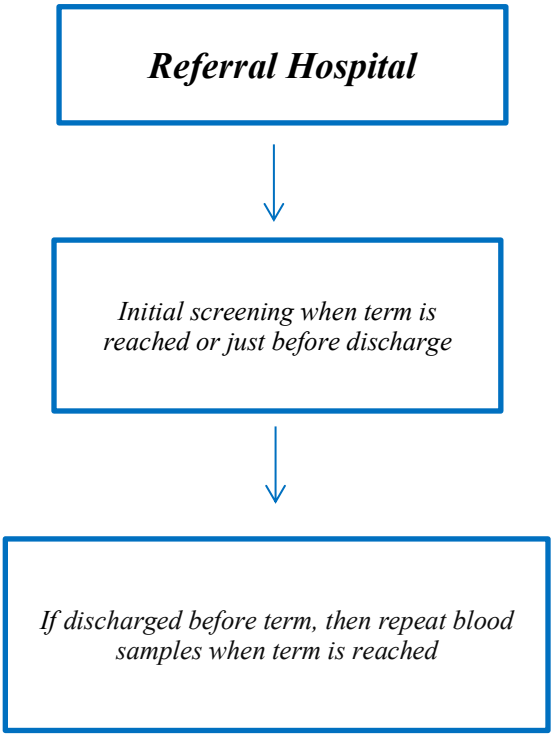
#2 Algorithm for Collecting & Follow up of Positive NBS Samples



#3 Algorithm for Collecting & Follow up of Preterm Babies

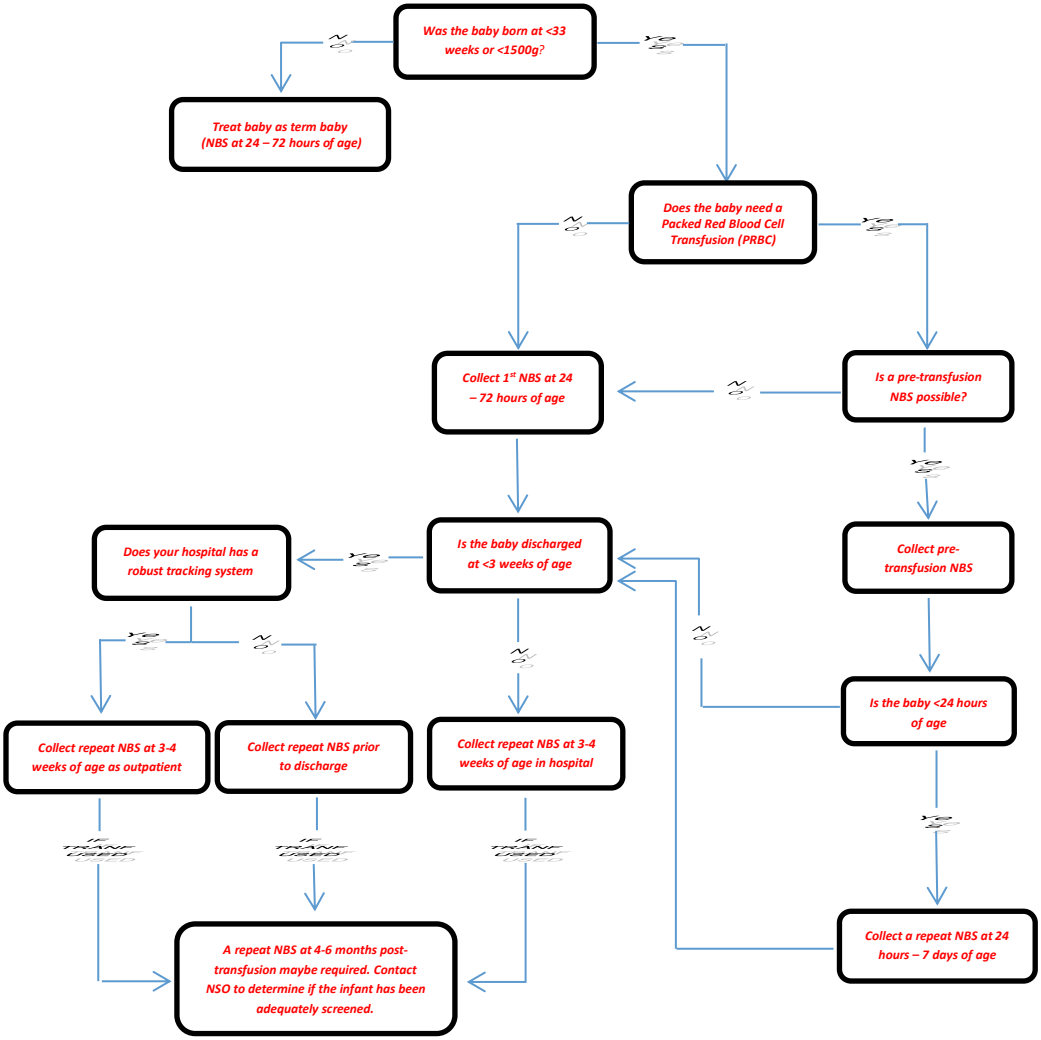
Newborn screening for preterm babies is challenging due to several factors. These newborns are more likely to have false-positive or false-negative screens, require multiple repeat screens, or have screening done under conditions that make interpretation difficult. In addition, the special care required for these infants may compete with, or distract from, obtaining NBS at the optimal times, resulting in incomplete or missed. Additionally, lack of the reference ranges in preterm may make the life difficult for the clinicians receiving the result of NBS.
The approaches for such group are heterogeneous and vary from country to country. In this section will present two common algorithm.

Algorithm A:

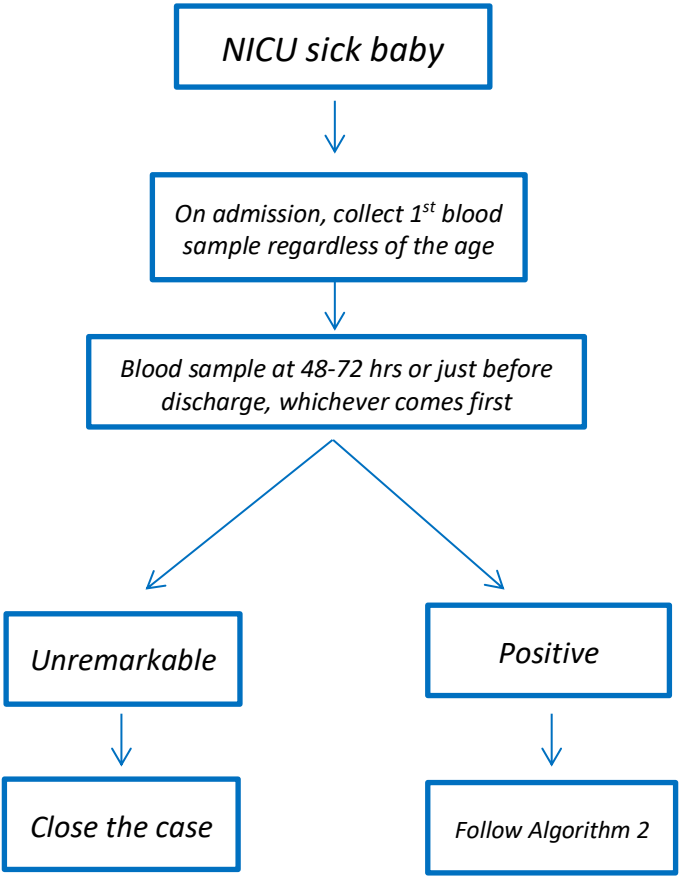

Referral Hospital
Initial screening when term is reached or just before discharge
If discharged before term, then repeat blood samples when term is reached

Algorithm B:

Premature or Very Low Birth Weight Babies +/- PRBC Transfusions



4 Algorithm for Collecting & Follow Up of Sick Babies at NICU



Chapter 7

Summary of Screened Disorders

(13, 14, 15, 16):

1. Phenylketonuria (PKU)

- ***Background:***

  PKU is an inherited inborn metabolism error in which affected individuals cannot use the aminoacid phenylalanine appropriately leading to accumulation in the blood, potentially causing serious brain damage.

- ***Etiology:***

  Mutations in the phenylalanine hydroxylase (PAH) gene produce a defective enzyme that is unable to metabolize or breakdown phenylalanine properly.

- ***Clinical Features***

  Babies with PKU are clinically indistinguishable from healthy babies for the first few weeks of life. Without treatment, however, phenylalanine accumulation will cause severe and irreversible mental retardation, eczema, and reduced hair, skin and iris pigmentation (due to reduced melanin synthesis).

  Children with untreated PKU may also have a distinctive "mousy" odor. Phenylalanine levels start rising within 24 hours of birth. Newborns with elevated levels on blood spot analysis are investigated further.

- ***Diagnosis***

  The diagnosis is confirmed by measuring the levels of plasma phenylalanine and tyrosine in the blood and urine for organic acids. Other tests may also be done to rule out other causes of high phenylalanine levels. A diagnosis of PKU can also be confirmed by genetic analysis of the PAH gene.

  Diagnostic testing is arranged by a Biochemical Genetics (metabolic) specialist.

- ***Classifications:***

  There is a standard classification, yet the most suitable one is Goldberg's et al. 1998 classification which is:

a. ***Classical PKU:*** *>1200 µmol/l(>20mg/dL).*
b. ***Moderate PKU:*** *600-1200 µmol/l(10-20mg/dL) and tolerates 350-400mg of PHe/day.*
c. ***Mild PKU:*** *600-1200 µmol/l(10-20mg/dL) and tolerates 400-600mg of PHe/day.*
d. ***Non PKU hyperphenylalaninemia:*** *<600 µmol/l(< 10 mg/dL).*
e. ***BH4 dependent PKU:*** *Reduction of Phe level >30% on day 2 and day 3 after BH4 loading tests, and this category consists of 5 disorders depending on the deficient enzyme:*
    1. *Guanosine triphosphate cyclohydrolase deficiency (GTPCH).*
    2. *6-pyrovyltetrahydrobiopterin synthase deficiency (PTPS).*
    3. *Dihydropteridine reductase deficiency (DHPR).*
    4. *Pterin-4a-carbinolamine dehydratase deficiency (PCD).*
    5. *Sepiapterin reductase (SR) deficiency.*

These kinds of disorders have different clinical features than other forms of PKU, and their clinical features are mainly related to dopamine, norepinephrine and serotonin deficiency which includes: immobility, parkinsonism, dystonia, oculogyric crisis, sleepiness, insomnia, dysphagia, temperature instability, hypotonia, ptosis, seizure and cerebellar symptoms.

- ***Management of confirmed positive cases:***

Once diagnosis is confirmed, then a BH4 loading test is performed to differentiate between classical PKU and BH4 dependent PKU.

- ***BH4 loading test:***

A BH4 loading test is performed as follows: the blood Phe should be >400 µmol/l. Additionally, there should be constant protein intake during the test, and the patient should not have any acute illness.

- ***Dosage:***

Day 1: no medication given
Day 2 and Day 3: BH4 20mg/kg PO once daily

- ***Sample collections:***

  Day 1, 2, 3: blood for Phe, Tyr and pterins at T0, T8, T16, T24.
  At the same time: urine for pterins and enzyme assay for DHPR level on dried blood spot should be collected.

- ***Interpretation:***

  Reduction of Phe level >30% on Day 2 and Day 3: then the patient has BH4 dependent or biosynthesis defects. If there is no response, then the patient has classical PKU.

- ***Management of classical PKU:***

  The patient should be referred to a metabolic dietitian, and a low protein diet with low phenylalanine intake should be started as soon as possible to prevent mental retardation and other problems. Some phenylalanine is required by the body for essential normal growth and development; thus it should not be eliminated from the diet. Frequent monitoring of plasma amino acid levels, weight gain, and development are recommended.
  Also, adult women diagnosed and treated for PKU are at risk to deliver babies with microcephaly, poor growth, and/or mental retardation if these women's phenylalanine levels are persistently elevated during pregnancy. Therefore, pregnant women diagnosed with PKU should also be monitored closely. A Biochemical Genetics specialist (Metabolic) and a Metabolic Genetics dietitian should coordinate the treatment.

2. Maple Syrup Urine Disease (MSUD)

- ***Background:***

  Maple Syrup Urine Disease (MSUD) occurs when the amino acids leucine, isoleucine, and valine cannot be broken down in the body. These are called "branched-chain" amino acids. This leads to accumulation of these amino acids in the blood and development of toxic symptoms of MSUD.

- ***Etiology:***

  In MSUD, the enzyme called "branched chain ketoacid dehydrogenase" (BCKAD) is either deficient or not working properly because of mutation in either BCKDHA, BCKDHB, or DBT genes.

- ***Clinical Features:***

  Although babies with MSUD are normal at birth, without treatment, they begin to have symptoms as soon as they are given protein, usually in the first few weeks of life. The symptoms of MSUD include poor suckling motion and lack of appetite with weight loss, vomiting, and lethargy which, if untreated, could progress to encephalopathy and eventually coma. Patients usually have maple syrup odor of cerumen and are observed to have bicycling abnormal movements. Interestingly, these patients usually do not have hyperammonemia or metabolic acidosis.

  There are also milder forms of MSUD with a later age of onset; the presentation is variable.

- ***Diagnosis:***

  The diagnosis is achieved by measuring the levels of amino acids in the blood and organic acids in the urine. The finding of high leucine, isoleucine, valine and alloisoleucine in the blood is a characteristic of MSUD. Enzyme testing and genetic testing of the MSUD genes may also be used to confirm the diagnosis.

- ***Management:***

  A low protein diet that is low in leucine, isoleucine, and valine is recommended for children with MSUD. A Biochemical Genetics specialist (Metabolic) and a Metabolic Genetics dietitian should coordinate the treatment. Supplementation with thiamine may also be considered.

3. HMG-CoA Lyase Deficiency (HMG)

- ***Background:***

  It is an organic acid disorder and ketogenesis defect due to a deficiency in one of the enzymes involved in catabolism of leucine. This enzyme is also important in producing ketone bodies, an important source of energy. This leads to the accumulation of harmful substances in the blood and a deficiency in ketone bodies.

- ***Etiology:***

  It is due to deficiency of the HMG-CoA lyase enzyme because of a mutation in the HMGCL gene.

- ***Clinical Features:***

  The patients are usually normal at birth. The patients present in the first few weeks or months of life with hypoketotic hypoglycemia and metabolic acidosis triggered by an illness or prolonged fasting. Other symptoms include lethargy, failure to thrive, vomiting, hypotonia, liver dysfunction and hyperammonemia. They may also have hypotonia and seem irritable. In the long term, repeated episodes may cause brain damage, learning problems, or mental retardation. The presentation of HMG lyase deficiency is variable, and there may be individuals with the disorder who are asymptomatic.

- ***Diagnosis:***

  It is achieved by measuring the acylcarnitine profile which shows high C5 (OH) and C6DC and urine organic acids which show increased quantities of 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids. The diagnosis is confirmed by DNA molecular testing of the HMGCL gene or enzyme assay in WBC or cultured fibroblasts.

- ***Management:***

  Low protein and/or low fat diet may be recommended to children with HMG lyase deficiency. Supplementation with carnitine may

also be considered. Treatment can prevent metabolic crises and their sequelae. A Biochemical Genetics specialist and a Metabolic Genetics dietitian should coordinate the treatment.

4. Isovaleric Acidemia (IVA)

- ***Background:***

  It is an organic acid disorder due to a deficiency in one of the enzymes involved in the catabolism of leucine. This leads to the accumulation of harmful substances in the blood which is isovaleryl coA, and that leads to dangerous symptoms of IVA.

- ***Etiology:***

  It is due to deficiency in the isovaleryl coA dehydrogenase enzyme because of a mutation in the IVD gene.

- ***Clinical Features:***

  The patient is usually normal at birth. The clinical presentation is highly variable. Severe neonatal onset, which is the most common form, is characterized by poor feeding, vomiting, and somnolence in the first days of life in a previously healthy infant, followed by lethargy, seizures, coma, and death if not treated. It is frequently accompanied by metabolic acidosis with high anion gap, ketonuria, hypoglycemia & hyperammonemia. The characteristic odor of sweaty feet may be present. The vomiting may be severe enough to suggest pyloric stenosis. For the chronic intermittent form, a milder form of the disease presents later in life in which the first clinical manifestations (vomiting, lethargy, acidosis or coma) may not appear until the infant is a few months or few years old. In both forms, acute episodes of metabolic decompensations may occur during the catabolic state such as an infection. Other complications include: pancreatitis and cardiomyopathy. Some patients are asymptomatic. Investigations show severe metabolic acidosis with ketonuria, hypoglycemia and hyperammonemia.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows high C5 and urine organic acids which show increased quantities of 3-OH isovaleric acid, isovaleryl glycine. The diagnosis is confirmed by DNA molecular testing of IVD gene or enzyme assay in WBC or cultured fibroblasts.

- ***Management:***

  A low protein diet that is low in leucine is recommended for children with IVA. Carnitine and glycine supplementation. Treatment can prevent metabolic crises and their sequelae. A Biochemical Genetics (Metabolic) specialist and a Metabolic Genetics dietitian should coordinate the treatment.

5. Propionic Acidemia (PA)

- ***Background:***

  It is an organic acid disorder due to a deficiency in one of the enzymes involved in catabolism of propionyl CoA. Sources of propionyl CoA are from catabolism of isoleucine, valine, methionine, threonine (50%), odd-chain fatty acids (25%), cholesterol side chains, thymine, and uracil and gut bacterial activity (25%). This leads to the accumulation of harmful substances in the blood which leads to toxic symptoms of PA.

- ***Etiology:***

  It is due to deficiency of the propionyl coA carboxylase enzyme because of a mutation in either PCCA or PCCB genes.

- ***Clinical Features:***

  The patient is usually normal at birth. The clinical presentation is highly variable. Severe neonatal onset, which is the most common form, is characterized by poor feeding, vomiting, and somnolence in the first days of life in a previously healthy infant, followed by lethargy, seizures, coma, and death if not treated. It is frequently accompanied by metabolic acidosis with high anion gap,

ketonuria, hypoglycemia, hyperammonemia and pancytopenia. For the chronic intermittent form, a milder form of the disease is present later in life in which the first clinical manifestations (vomiting, lethargy, acidosis or coma) do not appear until the infant is a few months or few years old. In both forms, acute episodes of metabolic decompositions may occur during a catabolic state such as an infection. Other complications include: cardiomyopathy, arrhythmia, growth impairment, intellectual disability, seizures, basal ganglia lesions and pancreatitis. Other rarely reported complications include optic atrophy, hearing loss, premature ovarian insufficiency (POI), and chronic renal failure. Investigations show severe metabolic acidosis with ketonuria, hypoglycemia, hyperammonemia, neutropenia (with or without thrombocytopenia), or anemia.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows a high C3 and C3/C2 ratio. Plasma aminoacids show increased glycine. Urine organic acids show increased quantities of propionic acid, 3-OH propionic acid, methyl citric acid, propionyl glycine and tiglylglycine. The diagnosis is confirmed by DNA molecular testing of either PCCA or PCCB genes or enzyme assay in WBC or cultured fibroblasts.

- ***Management:***

  A protein restricted diet that is low in isoleucine, valine, methionine, threonine and odd chain fatty acids is recommended to children with PA. Carnitine, metronidazole (flagyl) and biotin supplementation is suggested. There is no evidence to support the use of sodium benzoate or alkali therapy (sodium bicarbonate or polycitra) in chronic management of PA; therefore, its use is controversial. Treatment can prevent metabolic crises and their sequelae. A Biochemical Genetics (Metabolic) specialist and a Metabolic Genetics dietitian should coordinate the treatment.

6. Methylmalonic acidemia (MMA)

- ***Background:***

  Methylmalonic Acidemia is an organic acid disorder due to a deficiency in one of the enzymes involved in catabolism of methylmalonyl CoA. This leads to the accumulation of harmful substances in the blood which leads to toxic symptoms of MMA.

- ***Etiology:***

  The causes of methylmalonic acidemia are as follows:

  1. *Non IEM:*
     a. *Vitamin B12 deficiency*
     b. *Bacterial gut metabolism*
     c. *Maternal vitamin B12 deficiency*
     d. *Pernicious anaemia*
     e. *Gastroenteritis in very young infants*
     f. *Short bowel syndrome*
     g. *Malnutrition*
  2. *IEM:*
     a. *Methylmalonyl CoA mutase deficiency caused by mutation in the MUT gene.*
     b. *Transcoblamin II deficiency caused by mutation in the* TCN2 *gene.*
     c. *Cobalamin A, B, C, D, F caused by mutation in either MMAA, MMAB, MMADHC, and LMBRD1.*
     d. *Methylmalonyl CoA epimerase deficiency caused by mutation in the MCEE gene.*

- ***Clinical Features:***

  There are 4 main phenotypes: Infantile/non responsive B12 type, which is the most common form, Intermediate/B12 responsive type, late onset/childhood, and benign/adult form. The clinical features are similar to propionic acidemia; however, the renal complications like interstitial nephritis, renal tubular acidosis and hyperuricemia are more prevalent in MMA. Investigations in

mutase type show severe metabolic acidosis with ketonuria, hypoglycemia, and hyperammonemia.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows a high C3 and C3/C2 ratio. Urine organic acids show increased quantities of methylmalonic acid, methyl citric acid and tiglylglycine. Plasma aminoacids show increased glycine. Total homocysteine and vitamin B12 in the baby and mother should be measured to differentiate between different causes of MMA. The diagnosis is confirmed by DNA molecular testing of involved genes or enzyme assay in WBC or cultured fibroblasts.

- ***Management:***

  A protein restricted diet that is low in isoleucine, valine, methionine, threonine and odd chain fatty acids is recommended to children with MMA. Carnitine, m metronidazole (flagyl) and vitamin B12 supplementation is suggested. There is no evidence to support the use of sodium benzoate or alkali therapy (sodium bicarbonate or polycitra) in chronic management of MMA; therefore, its use is controversial. Treatment can prevent metabolic crises and their sequelae. A Biochemical Genetics (Metabolic) specialist and a Metabolic Genetics dietitian should coordinate the treatment.

7. Beta-ketothiolase Deficiency (BKT)

- ***Background:***

  Beta-ketothiolase deficiency is one of ketolysis defects due to a deficiency in one of the enzymes involved in catabolism of isoleucine. This leads to the accumulation of harmful substances in the blood which leads to toxic symptoms of BKT.

- ***Etiology:***

  It is due to deficiency of the β-ketothiolase enzyme because of a mutation in the ACAT1 gene.

- ***Clinical Features:***

  Most patients present this condition in the first 2 years of life with recurrent episodes of ketoacidosis. Episodes of decompensation generally start with tachypnea and vomiting followed by dehydration and a decreasing level of consciousness. Some patients also have seizures. Investigations show severe metabolic acidosis with ketonuria. Blood glucose, lactate and ammonia levels are normal in most cases. Several patients remain asymptomatic until adulthood and rarely present in the neonatal period.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows high C5 (OH) and C5:1 and urine organic acids that show increased quantities of 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid and tiglylglycine in urine. The diagnosis is confirmed by DNA molecular testing of ACAT1 gene or enzyme assay in cultured fibroblasts.

- ***Management:***

  Avoid fasting and a sick-day regime with glucose/maltodextrin. A low protein and/or low fat diet may be recommended to children with beta-ketothiolase deficiency. Supplementation with carnitine may also be considered. Treatment can prevent metabolic crises and their sequelae. A Biochemical Genetic specialist and a Metabolic Genetic dietitian should coordinate the treatment.

8. 3-Methylcrotonyl-CoA Carboxylase Deficiency (3MCC)

- **Background:**

It is an organic acid disorder due to deficiency in one of the enzymes involved in the catabolism of leucine.

- ***Etiology:***

  It is due to deficiency in the 3-methylcrotonyl CoA carboxylase enzyme because of a mutation in MCCC1 or MCCC2 genes.

- ***Clinical Features:***

  The presentation of 3-MCC deficiency is variable, and there may be individuals with this disorder who are asymptomatic or do not develop symptoms until later in life. Occasionally, asymptomatic women who have 3MCC deficiency will have infants who appear to have 3MCC deficiency on the newborn screen but are found later not to be affected. This finding is a transient state in the newborn as it is a reflection of the mother's metabolic status. Some patients present at a few months of life with vomiting, lethargy and metabolic crises triggered by URTI or other catabolic states. Other patients may have developmental delay or hypotonia.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows high C5 (OH) and also urine organic acids that show increased quantities of 3-hydroxy-isovaleric acid and 3-methylcrotonyl glycine. The diagnosis is confirmed by DNA molecular testing of MCCC1 or MCCC2 genes or enzyme assay in WBC or cultured fibroblasts. Furthermore, a blood acylcarnitine profile and urine organic acids analysis should be done for the mother to exclude maternal 3-MCC deficiency.

- ***Management:***

  Several patients do not require treatment, and there is growing evidence that suggests that this disorder is a benign condition. However, some investigators recommend the following measures for treatment of this condition including: prevent fasting, especially when the child is ill. In an acute symptomatic episode, intravenous D10% and fluids and carnitine are given. In the long term, supplementation with carnitine may also be considered. A Biochemical Genetic specialist and a Metabolic Genetic dietitian should coordinate the treatment.

9. Glutaric Aciduria type-I (GA-I)

- ***Background:***

  It is an organic acid disorder due to deficiency in one of the enzymes involved in the catabolism of lysine, hydroxylysine, and tryptophan. This enzyme is called glutaryl CoA dehydrogenase. This leads to accumulation of glutaric acid, 3-hydroxy glutaric acid and other harmful substances in the blood and urine.

- ***Etiology:***

  It is due to glutaryl CoA dehydrogenase enzyme deficiency because of mutation in the GCDH gene.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, some may have macrocephaly. They may also have hypotonia and seem jittery and irritable. Without treatment, babies may have episodes of metabolic crisis with encephalopathy, which can progress to coma and eventually death. The first episode usually occurs at infancy and can be triggered by URTI or other catabolic states. Other symptoms that include hypotonia, progressive muscle spasms, poor balance, poor coordination, dystonia, chorioathetoid movement may be observed. Also, there may be seizures and metabolic acidosis. GAI could be misdiagnosed as child abuse because of acute or chronic subdural and/or retinal hemorrhages. MRI brain scans usually show temporal hypoplasia as a characteristic feature. As the child grows older, the risk of crises and symptoms decrease. In fact, the presentation of GAI is variable, and there may be individuals with the disorder who are asymptomatic.

- ***Diagnosis:***

  It is achieved by measuring an acylcarnitine profile which shows highly increased C5DC, C5DC/(C8+C10), C5DC/C16 ratios and urine organic acids that show increased Glutaric acid and 3-OH-glutaric acid levels.

- ***Management:***

  A low protein diet, special metabolic formula, carnitine as well as riboflavine are often recommended to children with GAI. They should also avoid fasting. Treatment can prevent metabolic crises and their sequelae. In an acute symptomatic episode, IV D10% and fluids can be given, along with other medications that can help the body rid itself of harmful substances and decrease the level of acid in the blood. A Biochemical Genetic specialist and a Metabolic Genetic dietitian should coordinate the treatment.

10. Argininosuccinase Deficiency (ASL)

- ***Background:***

  It is one of the urea cycle defects due to deficiency of one of the enzymes that is involved in the cycle. Other names include: argininosuccinic aciduria, and argininosuccinate lyase deficiency. As a result of this deficiency of this enzyme, hyperammonemia occurs, which leads to irreversible brain damage.

- ***Etiology:***

  It is due to deficiency of the argininosuccinate lyase enzyme because of mutation in the ASL gene.

- ***Clinical Features:***

  The most common and most severe form is characterized by hyperammonemia within the first few days after birth. Newborns typically appear healthy for the first 24 hours, but over the next few days, they develop vomiting, lethargy, hypothermia and refuse to accept feeding. Tachypnea and respiratory alkalosis are early findings. If untreated, it will lead to worsening lethargy, seizures, coma, and eventually death. Other clinical findings include: hepatomegaly and trichorrhexis nodosa (coarse and friable hair). There is a late onset form which ranges from episodic hyperammonemia (triggered by acute infection or stress or by non-compliance with dietary restrictions and/or medication) to

cognitive impairment, behavioral abnormalities, and/or learning disabilities in the absence of any documented episodes of hyperammonemia.

- ***Diagnosis:***

  The diagnosis is confirmed by measuring amino acid levels in blood and urine. Argininosuccinic acid and orotic acid levels are elevated in the urine. Citrulline levels will be elevated in the blood, while arginine levels will be low. DNA molecular testing of the ASL gene as well as enzymatic studies in RBC or fibroblasts are helpful in confirming the diagnosis.

- ***Management:***

  A low protein diet and a special medical formula (Cyclinex®) are often recommended in children with this disorder. A medications called sodium phenylbutyrate, sodium benzoate and arginine may be considered in patients with recurrent episodes of hyperammonemia. Children should not be kept without food for extended periods of time. This special diet can prevent hyperammonemic episodes and their sequelae. In an acute symptomatic episode, IV D10% and fluids can be given, along with arginine and Ammonul® to decrease the level of ammonia in the blood. A Biochemical Genetics specialist and a Metabolic Genetics dietitian should coordinate the treatment.

11. Citrullinemia:

- ***Background:***

  It is one of the urea cycle defects due to deficiency of one of the enzymes that is involved in the urea cycle. Other names include: citrullinemia type 1 and argininosuccinate synthetase (ASS) deficiency. As a result of the deficiency of this enzyme, hyperammonemia occurs, which leads to irreversible brain damage.

- ***Etiology:***

  It is due to deficiency of the argininosuccinate synthetase enzyme because of mutation in the ASS1 gene.

- ***Clinical Features:***

  The most common and most severe form is characterized by hyperammonemia within the first few days after birth. Newborns typically appear healthy for the first 24 hours, but over the next few days, they develop vomiting, lethargy, hypothermia and refuse to accept feeding. Tachypnea and respiratory alkalosis are early findings. If untreated, it leads to worsening lethargy, seizures, coma, and eventually death. Other clinical findings include: hepatomegaly and elevated liver enzymes. There is a late-onset form which ranges from episodic hyperammonemia (triggered by acute infection or stress or by non-compliance with dietary restrictions and/or medication) to cognitive impairment, behavioral abnormalities, and/or learning disabilities in the absence of any documented episodes of hyperammonemia.

- ***Diagnosis:***

  The diagnosis is confirmed by measuring amino acid levels in blood and urine. Orotic acid levels will be elevated in the urine. Citrulline levels will be elevated in the blood, while arginine levels will be low. DNA molecular testing of the ASS1 gene as well as enzymatic studies in fibroblasts may also be helpful in confirming the diagnosis.

- ***Management:***

  A low protein diet and a special medical formula (Cyclinex®) are often recommended in children with this disorder. Dietary supplementation with arginine is also recommended. A medication called sodium phenylbutyrate and sodium benzoate may be considered in patients with recurrent episodes of hyperammonemia. Children should not be kept without food for extended periods of time. This special diet can prevent hyperammonemic episodes and their sequelae. In an acute symptomatic episode, IV D10% and fluids can be given, along with

arginine and Ammonul® to decrease the level of ammonia in the blood. A Biochemical Genetic specialist and a Metabolic Genetic dietitian should coordinate the treatment.

12. Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)

- **Background:**

  MCAD is a fatty acid oxidation defect due to deficiency in one of the enzymes (Medium-chain acyl-CoA dehydrogenase) involved in the breakdown of medium chain fatty acids (between 4 and 12 carbons long). Individuals who are missing this enzyme have an accumulation of "medium-chain" fatty acids which cause the symptoms of the disease.

- ***Etiology:***

  Mutations in the ACADM gene are a potential cause of the Medium-chain acyl-CoA dehydrogenase (MCAD) enzyme deficiency.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, they present symptoms in their first few months of life such as poor feeding, vomiting, lethargy and hypoketotic hypoglycemia. These symptoms are frequently accompanied by seizures, Reye-like syndrome with hepatomegaly, elevated liver enzymes and hyperammonemia which could progress to coma and eventually death, if not treated. Some infants suffer from sudden infant death syndrome.

- ***Diagnosis:***

  The diagnosis of MCAD deficiency is established by measuring the acylcarnitine profile (Tandem MS) in the blood which shows an increased C8, C6, C10 and C8/C10 ratio. Urine organic acids show dicarboxylic aciduria and increased quantities of hexanoylglycine, phenylpropionylglycine, octanoyl glycine and suberylglycine.

Diagnosis is confirmed by enzyme testing in WBC or fibroblasts or DNA molecular testing of the ACADM gene.

- ***Management:***

  Frequent feedings ensure that the child with MCAD deficiency does not undergo any prolonged period of fasting. This is very effective in preventing metabolic crises and their sequelae. In an acute symptomatic episode, IV glucose should be given as soon as possible. Supplementation with carnitine and/or uncooked cornstarch as a source of glucose may also be considered. A Biochemical Geneticist should coordinate the treatment.

13. Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)

- **Background:**

  VLCAD is one of the fatty acid oxidation defects due to deficiency of one of the enzymes involved in the breaking down of long chain fatty acids (between 12 and 18 carbons long). Individuals missing this enzyme have an accumulation of "very long-chain" fatty acids that cause the symptoms of the disease.

- ***Etiology:***

  Mutations in the ACADVL gene are a potential cause of Very long-chain acyl-CoA dehydrogenase (VLCAD) enzyme deficiency.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, they present symptoms in their first few months such as poor feeding, vomiting, lethargy, hypoketotic hypoglycemia and cardiomyopathy. These symptoms are frequently accompanied by seizures and muscle weakness which could progress to a coma and eventually death, if not treated. There are also forms of VLCAD deficiency that present in later years reaching adulthood characterized by exercise intolerance, muscle pain, rhabdomyolysis and myoglobinuria. Investigations show increased

creatine kinase (CK) levels and liver transaminases. Patients may have acidosis and/or increased lactic acid and ammonia in the blood.

- ***Diagnosis:***

  The diagnosis of VLCAD deficiency is established by measuring the acylcarnitine profile (Tandem MS) in the blood sample which shows an increased C14:1, C14:1/C12 ratio and C14:2. Urine organic acids show dicarboxylic aciduria. Diagnosis is confirmed by enzyme testing in WBC or fibroblasts or DNA molecular testing of the ACADVL gene.

- ***Management:***

  Frequent feedings ensure that a child with VLCAD deficiency does not undergo any prolonged period of fasting. This is very effective in preventing metabolic crises and their sequelae. In an acute symptomatic episode, IV glucose should be given as soon as possible. Supplementation with carnitine may also be considered. Restrict long-chain-fatty acids (> 14 carbons) to 15-25% of energy, carbohydrates 60% and proteins 15-25%. Use metabolic formula (ProViMin® or Monogen®). A Biochemical Geneticist (Metabolic) and Metabolic Dietitian should coordinate the treatment.

14. Galactosemia

- ***Background:***

  It is an inborn error in carbohydrates due to a deficiency in one of the enzymes that is involved in the breakdown of simple sugar galactose.

  Galactose is primarily a part of a larger sugar called lactose, which is found in all breastmilk, dairy products and many baby formulas. The signs and symptoms of galactosemia result from accumulation of toxic galactose metabolites and the inability to use galactose to produce energy.

- ***Etiology:***

  Mutations in the GALT gene are a potential cause of the galactose-1- phosphate uridyl transferase enzyme deficiency.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, at their first few weeks of life after drinking milk, they start presenting symptoms such as vomiting, diarrhea, weight loss, failure to gain weight, poor feeding, jaundice, lethargy, hypoglycemia, liver damage, cataracts, bleeding, and E. coli sepsis. Even with early treatment, however, children with galactosemia are at increased risk of developmental delays, speech problems (verbal dyspraxia), abnormalities of motor function, and osteoporosis. In females, premature ovarian failure is known as a long term possible outcome.

- ***Duarte Variant Galactosemia:***

  NBS allows for detection of more cases with Duarte variant galactosemia, sometimes called just Duarte galactosemia or "DG", which also results from mutations in the GALT gene. However, instead of carrying severe mutations in both copies of their GALT gene, patients carry one copy of the gene with a severe (G) mutation and a second copy with Duarte (D) mutation. A child with Duarte variant galactosemia, therefore, generally has one parent who is a carrier for a severe (G) GALT mutation and one parent who is a carrier for the Duarte variant (D). Patients with Duarte variant galactosemia usually show about 25% of the normal level of GALT activity in red blood cells. Newborns with Duarte variant galactosemia may not show any symptoms, such as jaundice. Experts, therefore, disagree about whether infants with Duarte variant galactosemia should be put on a lactose/galactose restricted diet, and no one knows whether older children or adults with Duarte variant galactosemia are at increased risk for long-term complications. Reported studies give mixed or inconclusive results, so more research is needed to answer the concern sufficiently. Also, girls and women with

Duarte variant galactosemia are not believed to be at risk for premature ovarian insufficiency.

- ***Diagnosis:***

  The diagnosis of Galactosemia is established by measuring the amount of galactose, galactose-1-phosphate, and enzymatic activity of galactose-1-phosphate uridyltransferase (GALT) in the blood sample. The diagnosis is confirmed by DNA molecular testing of the GALT gene.

- ***Management:***

  A galactose-restricted diet is effective in preventing many of the complications of galactosemia, including liver and kidney problems. It may also reduce the risk for developmental delays. A Biochemical genetic specialist (Metabolic) and a Metabolic Genetic dietitian should coordinate the treatment.

15. Biotinidase Deficiency

- ***Background***

  It is one of the vitamin responsive disorders due to a deficiency in one of the enzymes that is involved in recycling the biotin which is vitamin (B7). This enzyme is called Biotinidase. Biotin, in turn, is important as an enzyme cofactor. Free biotin is needed to activate Carboxylase enzymes by binding at a specific site. Carboxylases are essential in the production of fats, carbohydrates and for the breakdown of proteins. This process is blocked if an individual has Biotinidase deficiency.

- ***Etiology***

  Mutations in BTD genes are a potential cause of the Biotinidase enzyme deficiency.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, they present symptoms in their first few months of life with poor

feeding, vomiting, lethargy and intractable seizures. It is frequently accompanied by hearing loss, conjunctivitis and visual problems, skin rash, and alopecia. Later, ataxia and developmental delay became apparent if not treated.

- ***Diagnosis:***

  The diagnosis of Biotinidase deficiency is established by measuring the enzymatic activity in the blood sample. Individuals with profound biotinidase deficiency have less than 10% of mean normal serum biotinidase enzyme activity. Individuals with partial biotinidase deficiency have 10%-30% of mean normal serum biotinidase enzyme activity. DNA molecular testing of BTD gene confirms the diagnosis.

- ***Management:***

  Supplementation with biotin (10-20mg) can prevent symptoms of Biotinidase deficiency. A Biochemical Genetics specialist should coordinate the treatment.

16. Congenital Hypothyroidism (CH)

- ***Background:***

  It is one of the endocrine disorders due to a deficiency of thyroid hormones. Individuals' thyroid hormones are important for the normal function of all the body's organs and for the brain's development.

- ***Etiology:***

  The most common causes of congenital hypothyroidism are partial or total failure of the thyroid gland to develop and defects in the thyroid hormone synthesis. Congenital hypothyroidism is a heterogeneous disorder and likely to have many different causes, both genetic and non-genetic.

- ***Clinical Features:***

  The affected babies are usually normal at birth; however, some may have jaundice, constipation, lethargy, hypotonia, and feeding problems. If not diagnosed and treated quickly, they are at risk for mental retardation and failure to thrive.

- ***Diagnosis:***

  The diagnosis is confirmed with a repeat measurement of TSH and free thyroxine using a blood sample. Further tests can include thyroid scans.

- ***Management:***

  Thyroxine supplementation for life. An Endocrinology specialist should coordinate the treatment.

17. Congenital Adrenal Hyperplasia (CAH)

- ***Background:***

  It is one of the endocrine disorders in which the adrenal gland has an impaired ability to make cortisol and aldosterone due to a mutation in one of the enzymes required for their synthesis. Cortisol is important in coping with physical stress, and aldosterone is important for controlling salt balance. The underproduction of these hormones may mean that an infant cannot regulate salt and fluids, leading to a crisis of tremendous salt loss and possibly death. In addition, because of the block in certain hormonal pathways, excess male hormones are produced.

- ***Etiology:***

  The most common cause of CAH is a deficiency of the 21-hydroxylase (21-OH) enzyme, a crucial enzyme in cortisol and aldosterone synthesis. Levels of hormone precursors such as 17-hydroxyprogesterone and androgens are elevated.

- ***Clinical Features:***

  CAH is different from many of the other conditions in the newborn screen in that girls may be symptomatic at birth. With the overproduction of male hormones during pregnancy, some chromosomally female babies have masculinization of the external genitalia to varying degrees. Some female babies and all male babies appear normal at birth but can be at risk for a salt loss crisis. These babies may present with failure to thrive, vomiting, dehydration, hypotension, hyponatremia, hyperkalemia, and may go into shock and die. This crisis occurs within the first few weeks of life. In the long term, individuals with CAH may continue to have an imbalance in certain hormones, leading to symptoms such as precocious puberty, short stature, or reduced fertility.

- ***Diagnosis:***

  The diagnosis of CAH is established by measuring the 17-OHP and other adrenal hormones in the blood. DNA molecular testing of the CYP21A2 gene may help in confirming the diagnosis.

- ***Management:***

  Replacement of deficient hormones is an effective means of preventing a salt-wasting crisis and preventing long-term complications as indicated above. Parents of female babies who have had virilization may opt for surgery to improve the appearance of the external genitalia. An Endocrinology Specialist should coordinate the treatment.

Chapter 8

Management of screened disorders

Management of Screened Disorders:

While the management of endocrinopathies screened by NBS is quite clear and rarely challenging, the management of inborn errors of metabolism screened by NBS is clearly complex and challenging.
Such observation could be explained by the following:

1. *Availability of endocrinologists over the Kingdom and awareness of general pediatricians about the management of Congenital Hypothyroidism and Congenital Adrenal Hyperplasia.*
2. *Rarity of Biochemical Genetics (Metabolic) specialists and Metabolic Dietitians over the Kingdom.*
3. *Lack of educational resources about the management of these disorders.*
4. *Lack of focus on IEM in pediatric residency training programs.*

Therefore, in the management section we will focus on IEM screened by NBS rather than CH and CAH as the management of the latter is well known.

The management of IEM could be subdivided into 4 parts:

1. *Management of acute emergency crises.*
2. *Transition to long term management.*
3. *Long term management (home and outpatient visits).*
4. *Nutritional Management.*

Emergency Protocols for Patients with Inborn Errors of Metabolism (IEM)

The most common and early presentation of IEM screened by NBS program is acute hyperammonemia. The baby is usually normal at birth and then presents on the 3rd day of life suffering from poor feeding, vomiting, lethargy, hyperventilation resulting often in respiratory alkalosis, and irritability that rapidly progresses to seizures, deep coma, and even death if not urgently treated. Acute hyperammonemia is usually the presentation of urea cycle disorders

[Argininosuccinase deficiency (ASL) and Citrullinemia (ASS)] or organic acidemia [Methylmalonic Acidemia (MMA) and Propionic Acidemia (PPA)]. In case of urea cycle disorders respiratory alkalosis usually present while in organic acidemia, metabolic acidosis is common associated clinical finding. Other presentation at the same age is the presentation of Maple Syrup Urine Disease (MSUD), which is leucine encephalopathy in the form of poor feeding, lethargy, impaired level of consciousness and bicycling abnormal movements.

In this section, we will focus on emergency management of acute hyperammonemia and leucine encephalopathy.

1. Emergency Management of Acute Hyperammonemia (17, 18):

- *Basic life support (CAB).*
- *Stop all sources of protein both enteral and parenteral nutrition for a maximum of 24 to 48 hours.*
- *Check glucose level (Gluco-Checks).*
- *Insert an IV line (central and peripheral) and take blood for Ammonia (NH3), blood gases, chem1(Na, K, Cl, urea, creatinine), & CBC, blood C/S (peripheral and central if patient has a central line). Liver transaminases, Ca, alkaline phosphatase as STAT order.*
- *Ammonia should be taken with precaution (without tourniquet, transported in ice-water to the laboratory, separated within 30 minutes of collection and analyzed immediately).*
- *Start 1.5 to double maintenance I.V.F as D10% 0.45NS + KCL 30meq/l until the serum K result is available and then adjust accordingly (see below).*
- *Call the pharmacy to prepare the medications and Intra-lipid (see dosages in Table 4).*
- *Call the Biochemical Geneticist (metabolic) on call.*

- *Consider starting insulin if hyperglycemia develops (glucose >10mmol/l at dose of 0.05-0.1 unit/kg/hour and titrate up until blood glucose is controlled. (Keep GlucoChecks 6.5-10 mmol/L). Total glucose requirements (mg/kg/minutes) are dependent on the age (0-1year: 8-10, 1-3years:7-8, 4-6years:6-7, 7-12years:5-6, adolescent: 4-5, adults: 3-4).*
- *If ammonia >100µmol/l in infants, children and adults; and >150µmol/l in neonates (start loading dose of combined sodium benzoate and sodium phenylacetate (AMMONUL®) and Arginine (see table 4).*
- *Start IV Intra-lipid 20% 2-3gram/kg/day to give additional calories (if fatty acid oxidation defects are excluded).*
- *If the patient is on combined sodium benzoate and phenylacetate (AMMONUL®) or arginine, give KCl 40 meq/l because it causes hyperchloremic hypokalemic metabolic acidosis. KCl can be given through peripheral line up to 60 meq/l; rate must not exceed 0.125meq/kg/hour.*
- *Start dialysis if ammonia > 500µmol/l in neonates and children and there is no response to the medical treatment within 4 hrs. Consult ICU and Nephrology team if you anticipate starting dialysis in the next few hours.*
- *Reloading has to be done carefully, in particular during the first 24 hrs., as cumulative doses of >750 mg/kg/24 h of combined sodium benzoate and phenylacetate (AMMONUL®) have been shown to be associated with development of toxicity (vomiting, lethargy). Reloading should be done only in neonates with severe disorders or those who are undergoing dialysis and should be spaced at least 6 hours.*
- *In an undiagnosed acute case, also start N-carbamylglutamate (Carbaglu®). It only exists as an enteral form, so it is generally given by nasogastric (NG) tube. Give 100 mg/kg once followed by 50 mg/kg q 6 hrs. Once it is clear that the patient does not have N-acetylglutamate synthase (NAGS) or Carbamoyl phosphate synthetase I (CPS1) deficiency or organic acidemias, it should be stopped.*

- *In an undiagnosed acute case, start L-carnitine IV 100 mg/kg/day divided q 6hr, hydroxycobalamin 1mg IM/IV, and biotin 10mg IV/PO.*
- *Give glucose polymers or protein free formulas (for example: Prophree or Polycose or Maxijul) through PO/NG as tolerated to give additional calories.*
- *DO NOT decrease dextrose rate or amount, and DO NOT STOP calorie delivery in the acute stage for any reason (e.g. medications, fluid bolus, or hyperglycemia) as this can precipitate hypoglycemia and catabolism which will further worsen the patient's condition.*
- *Call the Metabolic Dietitian on call.*
- *If patient has a known diagnosis, DO NOT STOP other oral chronic medications (in case of vomiting, convert to IV forms if available).*
- *Antibiotics may be started if there is any evidence of sepsis. Ammonia, electrolyte and blood gases analysis need to be done at regular intervals during this acceleration of the management stage. The frequency is dictated by the patient's condition and the speed at which results can be obtained.*
- *Protein should be reintroduced within 24-48 hours of initiation of therapy even if the patient is on dialysis.*

Table 3: Laboratory testing that should be done in any patient suspected of having hyperammonemia:

Ammonia
Glucose
Venous/arterial blood gas
Electrolytes (including calcium)
Anion gap
Blood urea nitrogen (BUN)
Creatinine
Liver transaminases
Bilirubin

Albumin
Alkaline phosphatase
Lactate
Lipase (if symptoms indicate)
Blood Culture
Complete blood count
Acylcarnitine profile (Tandem MS), total and free carnitine
Plasma amino acids
Save a blood sample for DNA banking and DNA testing (if genotype is not known)
Urine ketones (healthy infants should not have any)
Urine organic acids (obtained within 2 hrs of presentation by any method of collection)
Urine for orotic acid

Table 4: Medication used in acute management of hyperammonemia and their dosages:

Medications	Mechanism of action	Dosage	Route	Adverse reaction
Sodium benzoate*	Conjugation with glycine to form hippuric acid	Weight≤20kg: 250mg/kg as loading dose over 90 minutes followed by 250-500 mg/kg/day Weight>20kg: 5.5gram/m^2as loading dose over 90 minutes followed by 5.5gram/m^2/day[a]	IV	Cardiovascular: Hypotension Dermatologic: Injection site reaction Electrolytes imbalance: Hyperglycemia hypokalemia, hypernatremia Gastrointestinal: Vomiting, diarrhea CNS: Altered mental status, seizure, cerebral edema Other: Fever
Sodium phenylacetate*	Conjugation with glutamine to form phenylacetylglutamine	Same as above	IV	Same as above

AMMONUL®[1] [*]	*Contains both sodium benzoate and sodium phenylacetate*	*Same as above*	*IV (ammonul could be given through peripheral line on limited basis)*	*Same as above*
L-Arginine	*As an intermediate metabolite in the urea cycle it can improve the flow through the urea cycle and thereby improve ammonia removal*	*250-400 mg/kg/day as loading dose over 90 minutes followed by 250 mg/kg/day*	*IV*	*Hyperchloremic metabolic alkalosis, hypokalemia, elevated BUN and creatinine levels, flushing, nausea, vomiting, abdominal cramps, bloating, numbness, headache*
Carbaglu® (carglumic acid)[2]	*Replace N-acetylglutamate (NAG) as an activator of mitochondrial carbamoyl phosphate synthetase (CPS1), the first enzyme of the urea cycle*	*100mg/kg bolus per NG tube* *then 25–62.5mg/kg every 6h*	*NG*	*Abdominal pain, diarrhea, vomiting, anemia, otitis media, tonsillitis, nasopharyngitis, fever, headache*

BUN, Blood urea nitrogen

[1]: It is supplied as a vial of 50 ml or 5000mg constitute of concentrated, aqueous 10% sodium benzoate and 10% sodium phenylacetate solution. Thus, each mL provides 100 mg of sodium benzoate and 100 mg of sodium phenylacetate in water. According to the prescribing information, AMMONUL must be diluted with sterile Dextrose Injection, 10% (D10W) at ≥ 25 mL/Kg before administration.

[*]: It should be given through a central line; however, it could be given peripherally on limited bases.

[a]: If on hemodialysis/hemodiafiltration, maintenance doses should be increased to 350mg/kg/day (or proportional increase for body surface-based dose calculation).

[2]: It is supplied as a 200mg tablet, 5 or 60 tablets in a polypropylene bottle with a polyethylene cap and desiccant unit.

2. Management of Leucine Encephalopathy Due to MSUD (19)

- *Basic life support (CAB).*
- *Stop all sources of protein central and parenteral nutrition.*
- *Check GlucoChecks.*
- *Insert an IV line and take bloods for the following: blood gas, Chem 1(Na, K, Cl, urea, creatinine), ammonia, and urine analysis to check ketones in addition to plasma amino acids.*
- *Call Biochemical Genetics lab (Metabolic) for urgent processing of plasma aminoacids.*
- *Start immediately one and a half to double maintenance I.V.F as 10% Dextrose + KCL 20 Meq/l. Re-adjust according to lab results (Keep GlucoChecks 6.5-10mmol/L). If hyperglycemia develops, consider starting insulin at doses of 0.01-0.05 unit/kg/hour and titrate up until blood glucose is controlled.*
- *Start IV intralipid 20% at 2-3 g/kg/day to provide additional calories.*
- *Call the pharmacy to expedite the delivery of medications.*
- *Call the Biochemical Geneticist (metabolic) on call.*
- *Give glucose polymers or protein free formulas (for example: Prophree or Polycose or Maxijul) through PO/NG as tolerated to give additional calories.*
- *Identify and treat the infection or other causes of the metabolic stress.*
- *When there is nausea or vomiting, give Granisetron10 to 40 microgram / kg, infused over 3 to 5 minutes.*
- *Call the Metabolic Dietician on call to help with dietary management.*
- *Begin enteral therapy if tolerated with a metabolic formula (Ketonex®-), 2.5-3.5 g/kg/day.*
- *Maintain phenylalanine, tyrosine, histidine, methionine, tryptophan, and threonine within normal.*

- *Begin 1% solutions of Isoleucine and Valine when levels approach upper treatment range. Start supplements at 20-30 mg/kg/day (1.5 to 3.0 ml/kg). Dosage ranges between 20-120 mg/kg/day and adjust based on plasma amino acids results.*
- *It is important to realize that isoleucine and valine levels may drop rapidly and that very low levels (isoleucine < 100 umol/L and valine < 200 umol/L) will keep the leucine level from dropping by limiting protein synthesis. Low levels will also allow more leucine to enter the brain by providing less transport competition and thus will produce or enhance brain edema and neurological complications.*
- *Monitor plasma amino acids and urine for ketones and ammonia daily until the patient has improved clinically and leucine has come back to the upper treatment range.*
- *Consider dialysis if there is persistently high plasma leucine despite above measures or leucine encephalopathy.*

3. Transition to Long Term Management (Wards protocol):

- *Continue implementing the above measures including high caloric intake, aminoacids mixture (metabolic formula with close monitoring of ammonia level, blood gas and electrolytes) until normalization of their levels.*
- *Ensure appropriate caloric intake and medication dosages by calculating calories and medication dosages/ kg daily and document that information in the chart.*
- *Provide remaining prescribed energy with Polycose® or Pro-Phree ®.*
- *Discontinue IV lipid when target calorie intake can be achieved through other sources because long administration of lipids may cause fatty liver.*
- *A patient needs daily evaluation from the Metabolic Dietitian with gradual introduction of natural protein 50%*

of normal intake at home, and then upgrade to 100% if the patient has improved clinically and there is normalization of ammonia level, blood gas and electrolytes.

- *Titrate IVF according to PO intake until it is discontinued.*
- *Discharge the patient if the following parameters have been achieved:*

    1. *Normal clinical status as home before crisis.*
    2. *Normal plasma ammonia level achieved (see appendix 2).*
    3. *Normal electrolytes and blood gas.*
    4. *For newly diagnosed cases: parents are educated about the disease natural history, formula, medications and sick day protocol.*
    5. *For newly diagnosed cases: emergency card is provided to the parents (see example of emergency card at appendix).*
    6. *Family is comfortable with preparation of formula and sick day protocol.*
    7. *Calculate the dosage of medications and ensure there are appropriate dosages / kg.*
- *Arrange an appointment with the General Metabolic Genetics Clinic 2 week after discharge with plasma aminoacids and electrolytes, and prealbumin prior to appointment.*

4. Long Term Management of Urea Cycle Disorders (Home and Outpatient Visits)(20-28)

a. Nutritional Management:

The aim of dietary management includes:

- *Normal weight gain, linear growth, and head growth.*
- *Normal psychomotor development, as assessed by serial examinations and valid developmental screening tools (e.g., Denver Developmental Screening Test II).*
- *Protein is restricted in the diet and is further supplemented with aminoacids mixture to provide additional amino acids without the offending amino acids.*
- *Avoidance deficiencies of the essential amino acids, fatty acids, and micronutrients.*

Metabolic Dietitians should ensure the following:

- *Home formula supplies include aminoacids mixture eg: CYCLINEX®-1 (for infant and toddler), CYCLINEX®-2 (for children; adolescents, and adults) see appendix 3.*
- *For infants: breastmilk or regular formula as a natural protein source. For older children: the source of natural protein is from regular diet.*
- *Compliance of the mother with weighing skills, appropriate preparation of formula and 3-day diet records prior to clinic visit.*
- *Monitoring of amino acid concentrations. The frequency of amino acid monitoring varies by age, metabolic stability, compliance, and regional clinical practice. For rapidly growing infants, monitoring weekly is recommended (see monitoring section).*

Table 5: Treatment ranges for target amino acids in urea cycle disorders:

Amino Acid (µmol/l)	***(2-4 hr postprandial)***			
	Newborn	***1-3m***	***3m-6y***	***6-18y***
GLYCINE (GLY)	*106-254*	*105-222*	*125-318*	*158-302*
ALANINE (ALA)	*132-455*	*134-416*	*148-475*	*193-545*
GLUTAMINE (GLU)	*243-822*		*475-746*	*360-740*
LYSINE (LYS)	*71-272*	*37-168*	*85-218*	*108-233*
ARGININE (ARG)	*17-119*	*21-74*	*32-142*	*44-130*
ISOLEUCINE (ILE)	*27-80*	*32-87*	*13-81*	*38-95*
LEUCINE (LEU)	*61-183*	*43-165*	*40-158*	*79-174*
VALINE (VAL)	*78-264*	*96-291*	*85-334*	*156-288*
CITRULLINE (CIT)	*3-36*	*6-36*	*8-47*	*19-52*

Note: The optimal level depends on the normal range for age established in the local laboratory.

Biochemical target for optimal urea cycle control (30):

- *Plasma ammonia <40 µmol/L.*
- *Plasma glutamine <1000 µmol/L.*
- *Normal plasma levels of alanine, glycine, lysine, and arginine.*
- *No subnormal concentrations of essential amino acids (eg, leucine, isoleucine, valine).*
- *Normal urinary orotate excretion (<3 µmol/mmol creatinine).*
- *Normal plasma protein concentrations (eg, albumin).*

Table 6: Recommended daily nutrient intake in urea cycle disorders (33):

Age	Total Protein (g/kg)	Energy (kcal/kg)	Fluid (mL/kg)
Infants			
0 to <3 mo	2.20–1.25	150–125	160–130
3 to <6 mo	2.00–1.15	140–120	160–130
9 to <12 m	1.60–0.90	120–110	130–120
Girls and boys	(g/day)	(kcal/day)	(mL/day)
1 to <4 yr	8–12	945–1890	945–1890
4 to <7 yr	12–15	1365–2415	1365–2445
7 to <11 yr	14–17	1730–3465	1730–3465
Women	(g/day)	(kcal/day)	(mL/day)
11 to <15 yr	20–23	1575–3150	1575–3150
15 to <19 yr	20–23	1260–3150	1260–3150
≥19 yr	22–25	1785–2625	1875–2625
Men	(g/day)	(kcal/day)	(mL/day)
11 to <15 yr	20–23	2100–3885	2100–3885
15 to <19 yr	21–24	2200–4095	2200–4095
≥19 yr	23–32	2625–3465	2625–3465

Table 7: Safe daily protein intake in urea cycle disorders (20, 28):

Age	1 m	2m	3m	6m	12m	18m	2y	3y	4-6	7-10	11-12
Protein intake (gm/kg/day)	1.77	1.50	1.36	1.31	1.14	1.03	0.97	0.90	0.87	0.92	0.90

- *Protein requirements for child with urea cycle disorders varies depending on the age and growth rate and severity of the disease.*
- *For patients with severe disease, some of natural protein (a mixture of essential and non-essential aminoacid) may be replaced with an essential aminoacid mixture (20-30%).*
- *Requirements for many traced elements and vitamins in children with urea cycle disorders have not been established.*
- *Several of the supplements appear to be very low in vitamin B12 in their diet; some centers measure urinary methylmalonic acid to monitor the adequacy of their supplement.*

Table 8: Chronic medications for life:

Disorder	Sodium benzoate	Sodium phenylbutyrate (PBA)	L-Arginine (hydrochloride or free base)	L-Citrulline	Carbamylglutamate (Carbaglu®)
NAGS deficiency	Not indicated	Not indicated	Not indicated	Not indicated	10–100 mg/kg/day
CPS1deficiency	≤ 250mg/kg/day maximum :12g/day	<20 kg: ≤250mg/kg/day >20 kg: 5g/m2/day maximum:12g/day	<20kg: 100-200mg/kg/day >20kg: 2.5-6g/m2/day Maximum: 6g/day	100-200mg/kg/day Maximum: 6g/day	Not indicated
OTC deficiency	Same	Same	Same	Same	Not indicated

ASS and ASL deficiency	*Same*	*Same*	*<20kg: 100-300mg/kg/day >20kg: 2.5-6g/m2/day Maximum: 8g/day*	*Not indicated*	*Not indicated*
Arginase deficiency	*Same*	*Same*	*Not indicated*	*Not indicated*	*Not indicated*

Note:

- *Sodium PBA was considered the second choice for long-term treatment. It should be given together with sodium benzoate in patients in which benzoate alone is not enough.*
- *Serum/plasma levels of benzoate/PBA and plasma levels of arginine (aim at fasting levels of 70–120 µmol/L) should be monitored to adjust dosages in case of high or repeated doses.*
- *L-Arginine: higher doses are needed in some patients according to expert advice: 400-700mg/kg/day in children of <20kg, and, >20 kg: 8.8-15.4 g/m2/day.*
- *Sodium phenylbutyrate: higher doses are needed in some patients according to expert advice: <20: 450-600mg/kg/day, >20kg: 9-13 g/m2/day.*
- *Citrulline may be preferable. When administered, there is no need for concomitant use of L-arginine.*

b. Monitoring

I. Clinical monitoring (20, 28, 33):

- ✓ *Weight, height, and head circumference*
- ✓ *Developmental assessment*
- ✓ *Neurologic assessment*
- ✓ *Liver examination*

II. Biochemical lab monitoring :

- ✓ *Plasma amino acid and ammonia weekly since diagnosis is until 6 months of age: then every other week until 1 year and then monthly.*

- ✓ *Ammonia targets less than 40µmol/l (in other references: <80µmol/l).*
- ✓ *Plasma glutamine targets less than 1000 µmol/l; however, high glutamine concentration does not invariably indicate encephalopathy.*
- ✓ *Normal concentration of essential amino acid (e.g.: leucine, isolucine, valine).*
- ✓ *Normal urinary orotate excretion is less than 3 µmol/l creatinine.*
- ✓ *CBC, Retic, Ca, Phosphorus, AST, ALT, GGT, alkaline phosphatase, vitamin B12, zinc, selenium, urine for orotic acid, lipid profile, blood sodium benzoate and/or sodium PBA/phenylacetate assays to be done every 3 months until 1 year of age and then every 6 months.*
- ✓ *Albumin and prealbumin monthly until 1 year and then every 3 months.*
- ✓ *Plasma ferritin concentration evaluated at 6, 9, 12 months and then every 6 months.*
- ✓ *Magnetic resonance imaging (MRI) early on in each coma or stroke-like episode and at 2-year intervals.*

III. Table 9: Other monitoring parameters :

Age (years)	0.5	1	2	3	4	5	6	9	12	15
EEG	*	*		*		*				
Bone Age	*		*		*		*	*	*	*
Bone densitometry		*		*		*		*		

IV. Clinic follow up:

- ✓ **Metabolic and Dietitian clinic:** *every 2 months until 1 year of age and then every 3 months until 6 years of age: then every 6 months until 17 years of age: then every year.*

- ✓ ***Neurology clinic:*** *at Dx then every 6 months until 1 year: then every 2 years until 9 years of age and then at 15 years of age.*
- ✓ ***Psychology clinic:*** *at 3 years of age: then every 2 years until 9 years of age and then subsequently at 13 and 15 years.*
- ✓ ***Physiotherapy :*** *at Dx then at 4 months, 8 months, 18 months, 3 years, and 5 years.*
- ✓ ***Occupational therapy:*** *at 18 months and 3 years.*
- ✓ ***Social service:*** *at Dx then at 1 year and 3 years.*

Note: *These are general guidelines for monitoring that could be adjusted on individual bases.*

c. Sick day management:

- *In females with deterioration during or prior menstruation, instituting a "sick day" dietary regimen for the premenstrual or menstrual period would seem reasonable.*
- *Home sick-day regimen is also indicated in case of URTI, trauma, surgery etc.*
- *Reduce protein intake to 50% for 24 to 48 hrs.*
- *Increase total calories by 10 to 25%.*
- *1 -1.5 times increased fluid intake.*
- *Maximize Na phenylbutyrate if possible.*
- *Maximize arginine hydrochloride if possible.*
- *Aggressively treat the underlying illness.*
- *If the patient does not improve within 24-48 hrs, bring the patient to ER.*

d. Management with immunizations:

- *Give sick day formula 50% for 24 hours.*
- *Manage fever >38 °C with ibuprofen. Acetaminophen should be avoided because this drug could be hepatotoxic, especially in large doses.*
- *If the condition does not improve within 24 hours, bring to ER.*

e. Management with surgical procedure:

- *Ensure that the patient is at his usual state of health prior to procedure.*
- *Ensure stability of metabolic parameters including leucine, isoleucine and valine prior to procedure.*
- *Plasma aminoacids, chem1, blood gas prior to procedure.*
- *High caloric intake with IVF D10% and lipids which provide 110 to 120% of normal energy needs starting 12-24 hours prior to procedure.*
- *After surgery, follow the guidelines mentioned in the Transition to long term management (Wards protocol) section of this manual.*

f. Liver transplantation:

- *Liver transplantation should ideally be carried out before irreversible neurological damage and/or repeated crises, generally between 3 and 12 months of age and/or 5 kg body weight.*
- *It is for those suffering recurrent metabolic decompensation and hospitalizations despite medical therapy as well as those with poor quality of life.*
- *Standard orthotopic liver transplantation (OLT) is preferred to auxiliary liver transplantation because it has fewer complications.*
- *Transplantation of liver lobes from living relatives can reduce waiting times and gives results comparable to those obtained with cadaveric organs; albeit it entails a small risk for donors. Heterozygosity for the disease in the living related donor is not a problem, except in OTCD females, although asymptomatic OTC heterozygotes have been successful donors.*

Notes 1:

- *Hemodialysis is the preferred method.*
- *Continuous arteriovenous hemodialysis (CAVHD) or continuous venovenous hemodialysis (CVVHD) with*

flow rates greater than 40 to 60 mL/min is optimal. Some centers use extracorporeal membrane oxygenation (ECMO) with hemodialysis. Although this combination of techniques provides very high flow rates (170-200 mL/min) and rapidly decreases ammonia levels, morbidity is greater because of the need for surgical vascular access.

- *Nitrogen scavenging therapy needs to be continued during hemodialysis.*
- *Continue nitrogen scavenging therapy for 12-24 hours after the patient has been stabilized and is able to accept enteral feeds and medications.*

Notes 2:

Table 10: Normal ammonia level by age:

Age	Upper limit (umol/l)
0-7 day	**94**
8-30 days	**80**
1m-15yrs	**48**
>16	**26**

Note: The optimal level depends on the normal range for age established in the local laboratory.

Notes 3:

AMMONUL® (information from Ucyclyd Pharma, Inc)

- *AMMONUL® contains 30.5 mg of sodium per mL of undiluted product. Thus, AMMONUL® should be used with great care, if at all, in patients with congestive heart failure or severe renal insufficiency, and in clinical states in which there is sodium retention with edema. If an adverse reaction does occur, discontinue administration of AMMONUL®, evaluate the patient, and institute appropriate therapeutic countermeasures.*
- *Administration through a central line, however, could be given peripherally if central line is not available.*

Administration through a peripheral line may cause burns.

- *Can be given at the same line with potassium.*

5. Long Term Management of Maple Syrup Urine Disease (Home and Outpatient Visits)(19, 22, 29, 30-33)

a. Nutritional management:

The aim of dietary management includes:

- *Normal weight gain, linear growth, and head growth*
- *Normal psychomotor development, as assessed by serial examinations and valid developmental screening tools (e.g., Denver Developmental Screening Test II)*
- *Age-appropriate tolerance of leucine, isoleucine, and valine, with stable plasma BCAA concentrations and BCAA concentration ratios*
- *Avoidance of essential amino acids, fatty acids, and micronutrient deficiencies*

A Metabolic Dietitian should ensure the following:

- *Home formula supplies include BCAA-free powder (Ketonex-1 (for infant and toddler), Ketonex-2 (for children; adolescents, and adults).*
- *For infants: breastmilk or regular formula as a natural protein source. For older children: the source of natural protein is from regular diet.*
- *Supplementation with 10-mg/ml (1%) solutions of isoleucine, valine, and leucine and fortified formula with*

glutamine and alanine. The dose is adjusted according to plasma amino acids result.

- *Monitoring of amino acid concentrations. The frequency of amino acid monitoring varies by age, metabolic stability, compliance, and regional clinical practice. For rapidly growing infants, monitoring weekly is recommended.*

Suggested clinical parameters for asymptomatic infant or young child include the following:

- *Normal age and weight adjusted energy intake.*
- *Protein as essential and non-essential amino acids: 2-3 g/kg/day (see table 12).*
- *Appropriate leucine tolerance. The dietary requirement for BCAAs varies as a function of age, growth rate, calorie intake, illness, and residual in vivo BCKAD enzyme activity. In persons with classic MSUD (0%-2% enzyme activity), leucine tolerance in mg/kg/day is 65-85 for neonates, 20-40 for children, and 10-15 for adults.*
- *Isoleucine and valine supplements as needed to maintain a plasma valine to leucine concentration ratio (mol:mol) of 2 or greater, and an isoleucine to leucine ratio of 0.5 to 1. Isoleucine supplements can periodically be suspended based on plasma amino acid monitoring, but continuous valine supplementation is prudent because its low affinity for the blood-brain barrier*
- *LAT1 transporter makes it especially vulnerable to competitive inhibition by leucine.*

Table 11: Treatment ranges for target amino acids in MSUD (39)

Amino Acid	***(2-4 hr postprandial)***
ALA	***150-500 umol/L***
GLUT	***400-800 umol/L***
ALLO	***0***
ILE	***150-300 umol/L***
LEU	***100- 250 umol/L***
VAL	***200-400 umol/L***

Table 12: Recommended daily nutrient intake (39)

Age	***Nutrient***					
	ILE (mg/kg)	*LEU (mg/kg)*	*VAL (mg/kg)*	*Protein(g/kg*	*Energy (Kcal/kg)*	***Fluid (ml/kg)***
Infants						
0 to <3 mo	*36-60*	*60-100*	*42-70*	*3-3.5*	*120 (95-145)*	*125-150*
3 to <6 mo	*30-50*	*50-85*	*35-60*	*3-3.5*	*115 (95-145)*	*130-160*
6 to <9 mo	*25-40*	*40-70*	*28-50*	*2.5-3*	*110 (80-135)*	*125-145*
9 to <12 mo	*18-33*	*30-55*	*21-38*	*2.5-3*	*105 (80-135)*	*120-135*
Girls & Boys	*ILE (mg/day)*	*LEU (mg/day)*	*VAL (mg/day)*	*Protein (g/day)*	*Energy (Kcal/day)*	*Fluid (ml/day)*
1 to < 4 yr	*165-325*	*275-535*	*190-400*	*≥30*	*1,300 (900 - 1800)*	*900- 1,800*
4 to < 7 yr	*215-445*	*360-695*	*250-490*	*≥35*	*1,700 (1300 - 2300)*	*1,300- 2,300*
7 to < 11 yr	*245-470*	*410-785*	*285-550*	*≥40*	*2,400 (1650 - 3300)*	*1,650- 3,300*

Table 13: Normal target aminoacids references by age

Amino Acid (µmol/L)	*(2-4 hr postprandial)*			
	Newborn	*1-3 m*	*3m-6y*	*6-18y*
LEU	*61-183*	*43-165*	*40-158*	*79-174*
ILE	*27-80*	*32-87*	*13-81*	*38-95*
VAL	*78-264*	*96-291*	*85-334*	*156-288*
GLU	*243-822*		*475-746*	*360-740*
ALA	*132-455*	*134-416*	*148-475*	*193-545*

b. Supportive measures:

- *Neuropsychiatric morbidity is first addressed with strict and consistent metabolic control. Adolescents and adults with MSUD and attention deficit hyperactivity disorder (ADHD), depression, or anxiety respond favorably to standard psychostimulant & antidepressant medications.*
- *Thiamine treatment. The existence of "thiamine-responsive" BCKAD mutants is controversial. Nevertheless, for any person with MSUD in whom the functional consequences of the mutation(s) are unknown, a four-week trial of enteral thiamine (50-100 mg/day, divided twice a day) is reasonable. However, it should be noted that significant changes in dietary therapy (e.g., BCAA or calorie intake) during the treatment period confounds interpretation of a specific thiamine effect.*

c. Look for complications of MSUD which includes:

- ***Iatrogenic essential amino acid deficiency.*** *Anemia, acrodermatitis, hair loss, growth failure, arrested head growth, anorexia, and lassitude valine.*
- ***Iatrogenic nutritional deficiencies.*** *Commercially available MSUD synthetic formulas provide marginal*

intakes of certain minerals and micronutrients, and utilize vegetable oils that contain little or no omega-3 fatty acid (linolenic, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Zinc, selenium, and omega-3 fatty acid deficiency were common among patients with classic MSUD [Strauss & Morton 2003]. Other studies have documented deficiencies of folic acid and selenium in persons treated with MSUD formula.

- ***Osteoporosis.*** *In 90% of adolescents with classic MSUD (n=10), bone mineral density of the radius and femoral neck, but not lumbar spine, were low compared to unaffected age-matched siblings. Bone fractures commonly cause transient leucinosis.*
- ***Recurrent oroesophageal candidiasis****. Candida infections are common in hospitalized persons with MSUD and may result from T-cell inhibitory effects of elevated plasma leucine iatrogenic immunodeficiency as a result of inadequate BCAA intake.*

d. Monitoring:

Table 14: Suggested monitoring schedule includes:

Age	PAA	Electrolytes, urine dipsticks for ketones	CBC, total protein, albumin, prealbumin, Se, Zn, ferritine, Ca, Mg, 25-hydroxy vitamin D, growth parameters	Clinic visits
0-3 months	weekly	weekly	2 months	2 months
3-18 months	2 weeks	2 weeks	3 months	3 months
18 month – 10 years	monthly	monthly	4 month	4 months
10-18 years	monthly	monthly	6 months	6 months

Maintain records of food intake for 3 days immediately before each plasma amino acid.

Goals of laboratory monitoring:

- *Plasma leucine concentration: 150-300 µmol/L with an age-appropriate intake.*
- *Plasma isoleucine concentration approximately equal to plasma leucine concentration.*
- *Plasma valine concentration at least twofold plasma leucine concentration.*
- *Indices of calcium, magnesium, zinc, folate, selenium, and omega-3 essential fatty acid sufficiency.*
- *Normal growth parameters.*

e. Management of feeding problems:

- *General recommendations:*
  - *Monitor growth and intake of essential nutrients*
  - *Consider overnight tube feeding or limited nocturnal fasting times.*
- *Children with mild to moderate feeding problems*
  - *Use a combination of pureed foods and commercial enteral feeds to meet the nutritional prescription in addition to the medical protein and other energy modules.*
  - *Increase the frequency of meals and reduce quantity per feed.*
- *Children with severe feeding problems*
  - *G-tube feeding consisting of an enteral product to the natural protein in addition to the medical protein and other energy modules.*
  - *In cases of severe vomiting: use pharmacotherapy, consider fundoplication or J-tube feeding.*

f. Sick day management:

- *At the first sign of illness, start sick day formula.*
- *Sick-day diet to provide 120-130 kcal/kg/day for neonates or 110 to 120% of normal energy needs in older individuals.*
- *Minor illness like URTI can normally be managed with a sick day formula providing 50% of normal protein intake and high in calories and meeting or exceeding CHO requirements.*
- *Give prophree or polycose as tolerated.*
- *Give ibuprofen (10 mg/kg/dose q 6 h) when fever >38 C.*
- *Give ondansetron (0.15 mg/kg/dose q 8 h) to manage vomiting.*

g. Management with immunizations:

- *Give sick day formula 50% for 24 hours.*
- *Manage fever >38 C with ibuprofen.*
- *Give double dose of carnitine (200 mg/kg/day).*
- *If condition is not improved within 24 hours, bring to ER.*

h. Management with surgical procedure:

- *Ensure that the patient is at his usual state of health prior to procedure.*
- *Ensure stability of metabolic parameters including leucine, isoleucine and valine prior to procedure.*
- *Plasma aminoacids, chem1, urine for ketones prior to procedure.*
- *High caloric intake with IVF D10 and intralipids 20% which provide 110 to 120% of normal energy needs starting 12-24 hours prior to procedure.*
- *After surgery, follow the guidelines mentioned in the Transition to Long Term Management (Wards protocol) section of this manual.*

i. Orthotopic liver transplantation:

This is an effective therapy for MSUD patients. 37 individuals with classic MSUD (age 1.9-20.5 years) underwent elective orthotopic liver transplantation between 2004 and 2009. Plasma leucine, isoleucine, and valine concentrations were normal within six hours after transplantation in all individuals and remained so on an unrestricted diet. Metabolic cure was reflected by a sustained increase in weight-adjusted leucine tolerance from 10-40 mg/kg/day to more than 140 mg/kg/day, normalization of plasma concentration relationships among branched-chain and other essential and non-essential amino acids, and metabolic and clinical stability during protein loading and intercurrent illnesses. patients may get instable during catabolic situations.

Appendix 1:

Control of brain edema:

A decrease in blood osmolarity of more than 8 mosm/L per day can precipitate fatal brain herniation in an ill infant or child with MSUD. Close monitoring (preferably in an intensive care unit) is warranted.

Neurologic assessments to be performed on a frequent basis to monitor for brain swelling include the following:

- *Measure head circumference and fontanel size in neonates.*
- *Watch for signs of increased intracranial pressure including:*
    - *Papilledema*
    - *Disorientation, combativeness*
    - *Depressed level of consciousness*
    - *Refractory vomiting*
    - *Extremity hyperreflexia*
    - *Bradycardic hypertension*

- *Watch for signs of impending brain herniation including:*
    - *Hyperactive gag*
    - *Pupillary asymmetry*
    - *Ophthalmoplegia*
    - *Decorticate posturing*
- *Methods to minimize the possibility of brain swelling:*
    - *Elevate the individual's head*
    - *Assess total body sodium, potassium, and water balance at 12-hour intervals.*
    - *Minimize osmotic variation of the extracellular fluid in hospitalized patients by assessing weight trend, urine output, and serum and urine electrolytes every 12 hours and adjusting electrolyte and water intake accordingly.*
    - *Give Furosemide (0.5-1.0 mg/kg/dose) as needed every six to 12 hours to oppose the urinary concentrating action of vasopressin and maintain urine osmolarity at a ceiling value of 300-400 mosm/L. This allows for brisk output of isotonic urine to compensate for the large infused volume associated with hypercaloric feeding.*

Methods to manage brain swelling:

- *For weight gain, hyponatremia, or deepening encephalopathy, administer:*
    - *Furosemide: 1 mg/kg, followed by*
    - *Mannitol: 0.5-1.0 g/kg over 60 minutes, followed by hypertonic (3%-5%) saline: 2.5 mEq/kg over 60 minutes.*

Neuroimaging:

During episodes of acute encephalopathy, individuals with MSUD are typically too unstable for magnetic

resonance imaging. Cranial CT scan is used to look for major indices of cerebral edema, such as decreased volume of cerebral ventricles and basal fluid spaces, or reduced gray-white discrimination.

Appendix 2:

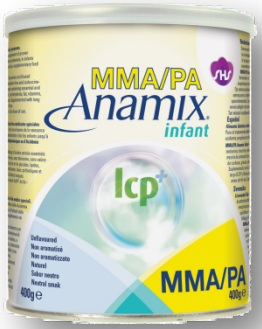
Dialysis:

Methods of invasive leucine removal, peritoneal dialysis and venovenous hemofiltration are less effective and more dangerous than short courses of continuous hemodialysis. When hemodialysis is used to treat MSUD, it must be coupled with effective nutritional management to constrain the catabolic response and prevent recurrent clinical intoxication. A combined approach to therapy, using hemodialysis with simultaneous anabolic nutritional therapy, was shown to be highly effective in one neonate with classic MSUD. Dialysis without simultaneous management of the underlying disturbance of protein turnover is analogous to treating diabetic ketoacidosis with invasive removal of glucose and ketones rather than insulin infusion. In both conditions, effective treatment depends not only on lowering concentrations of pathologic metabolites but also on controlling the underlying metabolic derangement.

6. Long Term Management of Methylmalonic Acidemia (Home and Outpatient Visits) (22, 34-43)

a. Nutritional management:

The aim of dietary management includes:



- *Normal weight gain, linear growth, and head growth.*
- *Normal psychomotor development, as assessed by serial examinations and valid developmental screening tools (e.g., Denver Developmental Screening Test II).*
- *Protein is restricted in the diet to provide limited amounts of isoleucine, methionine, threonine, valine and odd chain fatty acids and further supplemented with aminoacids mixture to provide additional amino acids without the offending amino acids (see table 15 and 16).*
- *Avoidance deficiencies of essential amino acids, fatty acids, and micronutrients.*

Metabolic Dietitians should ensure the following:

- *Home formula supplies include aminoacids mixture e.g.: Propimex-1® (for infant and toddler), Propimex-2® (for children; adolescents, and adults): see appendix 1.*
- *For infants: breastmilk or regular formula as a natural protein source. For older children: the source of natural protein is from regular diet.*
- *Compliance of the mother with weighing skills, appropriate preparation of formula and 3-day diet records prior to clinic visit.*

- *Monitoring of amino acid concentrations. The frequency of amino acid monitoring varies by age, metabolic stability, compliance, and regional clinical practice. For rapidly growing infants, monitoring weekly is recommended (see monitoring section).*

Table 15: Treatment ranges for target amino acids in MMA:

Amino Acid (µmol/L)	*(2-4 hr postprandial)*			
	Newborn	*1-3m*	*3m-6y*	*6-18y*
Glycine (GLY)	*106-254*	*105-222*	*125-318*	*158-302*
Isoleucine (ILE)	*27-80*	*32-87*	*13-81*	*38-95*
Methionine (MET)	*6-36*	*4-39*	*5-34*	*16-37*
Threonine (THR)	*65-147*	*64-225*	*40-139*	*74-202*
Valine (VAL)	*78-264*	*96-291*	*85-334*	*156-288*

Note: The optimal levels should be near the lower limit of normal ranges. These levels are based on BC Children Hospital reference ranges.

Table 16: Proposed nutrient intake when prescribing nutrition support inpatients with MMA (44)

Nutrients	*0-5m*	*5m-1y*	*1<4y*	*4<7y*	*7<11y*	*11<19y*
ILE	*110-60/kg*	*90-40/kg*	*485–735/day*	*630–960/day*	*715–1090/day*	*956–1470/day*
MET	*50-20/kg*	*40–15/kg*	*275–390/day*	*360–510/day*	*410–580/day*	*550–780/day*
THR	*125-50/kg*	*75–20/kg*	*415–600/day*	*540–780/day*	*610–885/day*	*830–1200/day*
VAL	*105/60/kg*	*80–40/kg*	*550–830/day*	*720–1080/day*	*815–1225/day*	*1105–1655/day*
Protein	*2.5/kg*	*2.5/kg*	*≥30/day*	*≥35/day*	*40/day*	*50/day*
Energy	*100-125% of RDA for age*					

Table 17: Chronic medications for life:

Medication	*Dose*	*Route*	*Comments*
Carnitine	*200-300mg/kg/day divided 2-3 times /day*	*PO*	
Hydroxocobalamin	*1 mg weekly IM or 10 mg daily orally*	*PO*	*Discontinue if not responsive phenotype*
Metronidazole	*10-20 mg/kg/day, one week on drug with three weeks off*	*PO*	
Laxative agents	*Daily use of laxatives at age/weight-appropriate doses*	*PO*	

b. Supportive measures:

- ***Sodium benzoate:*** *There is no evidence that would support a role for benzoate in chronic treatment.*
- ***Growth hormone:*** *Only considered if there is a low level of growth hormone/IGF1 and normal nutritional parameters.*
- ***Gastrostomy tube insertion:*** *Recommend consideration be given to the placement of GT tube, particularly in infants and young children with PA at the time of diagnosis.*
- ***Placement of a port-a-cath*** *(totally implantable central venous access device): May be considered when unreliable peripheral venous access is significantly compromising patient care but should be balanced by the increased risk of destabilizing infection.*
- ***Carbaglu®:*** *There is no current published evidence for its use in chronic management although ongoing studies suggest a utility for associated chronic hyperammonemia.*
- ***Antiepileptic drugs:*** *As needed.*
- ***Therapy for arrhythmia:*** *As needed.*

- ***Anti-emetics**: Ondansetron may be used on individual bases.*
- ***Alkali therapy:** There is no published evidence for the use of alkali therapy like polycitra or sodium bicarbonate on chronic bases.*

c. Look for complications of MMA which includes:

1. ***Iatrogenic essential amino acid deficiency**:*
   a. *ILE deficiency: Weight loss or no weight gain; redness of buccal mucosa; fissures at corners of mouth; tremors of extremities; decreased plasma cholesterol and ILE; increased concentrations of plasma lysine, phenylalanine (PHE), serine (SER), tyrosine (TYR), and valine (VAL); skin desquamation, and corneal de-epithelialization.*
   b. *MET deficiency: Decreased plasma MET and cholesterol and increased plasma PHE, proline, SER, THR, and TYR concentrations.*
   c. *THR deficiency: Arrested weight gain; glossitis and redness of buccal mucosa; and decreased plasma THR and globulin concentrations.*
   d. *VAL deficiency: Poor appetite, drowsiness; excessive irritability and crying in infants; weight loss or decrease in weight gain; and decreased plasma albumin concentration.*

2. ***Intellectual and developmental delay:** To maximize patients intellectual-developmental outcome, patients need the following:*
   a. *Early initiation of physical, occupational and speech therapy services to continue throughout childhood.*
   b. *Optimize nutrition.*
   c. *Avoid acute metabolic decompensation.*
   d. *Treat episodes of metabolic decompensation swiftly and aggressively.*

3. ***Tubulointerstitial nephritis with progressive impairment of renal function:***

4. ***Neurologic findings:*** *Some individuals develop a "metabolic stroke" or infarction of the basal ganglia, characteristically the globus pallidus, during acute metabolic decompensation, which can produce an incapacitating movement disorder.*

5. ***Pancreatitis***

6. ***Growth failure***

7. ***Functional immune impairment:*** *This results in an increased susceptibility to severe infections, particularly by fungal and gram-negative organisms.*
   a. *CBC with differential at diagnosis annually and as needed to follow abnormalities.*
   b. *If neutropenia is present, institute infection control precautions (isolation, gown and glove, etc.) as indicated by hospital policy.*
   c. *Expectant management with judicious use of colony stimulating factors only in cases where neutropenia is not resolving or there is evidence of bacterial infection with neutropenia.*

8. ***Optic nerve atrophy:***
   a. *Annual examination by an ophthalmologist to include visual acuity as well as visual examination of the anterior chamber and dilated evaluation of the fundus.*
   b. *Treatment of decreased visual acuity as indicated.*

9. ***Dermatologic manifestations:*** *Resembling acrodermatitis enteropathica are frequently associated with deficiency of essential amino acids, particularly isoleucine, which is excessively restricted in the diet of persons with MMA.*

10. ***Osteoporosis and osteopenia***

11. ***Cardiomyopathy.***

d. Monitoring:

Table 18: Suggested monitoring schedule includes:

Age	*Plasma aminoacids, ammonia, urine dipsticks for ketones, acylcarnitine profile, serum and urine methylmalonic acids*	*Clinic visit and dietitian visit*
0-3 months	***Weekly***	***Monthly***
3-18 months	***Every 2 weeks***	***Every 3 months***
18 months-10years	***Monthly***	***Every 3 months***
10-18years	***Every 3 months***	***Every 6 months***

Table 19: Suggested other lab and radiological monitoring schedule includes:

Test/action	*Time*
CBC, albumin, prealbumin, Se, Zn, ferritine, Ca, Mg, 25-hydroxy vitamin D, Growth parameters, chem1, bone profile, liver ezymes, thiamine, lactic acid and cholesterol	*Prior to each clinic visit*
ECG, Echocardiogram, 24-hour Holter monitoring	*At diagnosis and then yearly*
EEG	*At diagnosis and then yearly*

Ophthalmology exam	*At diagnosis and then yearly*
Referral to physical, occupational and speech therapy services	*At 18 months and continue throughout childhood*
Detailed developmental assessment	*At diagnosis, 6 months, 1 year then yearly*
MRI brain and MRS	*At diagnosis and then if there is neurological deterioration*
Hearing test	*At diagnosis*
Bone age	*At 3 years and then every 2 years*
X-ray of long bones	*When osteoporosis suspected*

e. Management of feeding problems:

1. ***General recommendations:***
   - *Monitor growth and intake of essential nutrients with every 200 gram change in weight.*
   - *Consider overnight tube feeding or limited nocturnal fasting times.*
2. ***Children with mild to moderate feeding problems***
   - *Use a combination of pureed foods and commercial enteral feeds to meet the nutritional prescription in addition to the medical protein and other energy modules.*
   - *Increase the frequency of meals and reduce quantity per feed.*
3. ***Children with severe feeding problems***
   - *G-tube feeding consisting of an enteral product to the natural protein in addition to the medical protein and other energy modules.*
   - *In cases of severe vomiting: use pharmacotherapy, consider fundoplication or J-tube feeding.*
     - ***a. <u>Sick day management:</u>***
       - *At the first sign of illness, start sick day formula.*
       - *Sick-day diet to provide 120-130 kcal/kg/day for neonates or 110 to 120% of normal energy needs in older individuals.*

- *Minor illness like URTI can normally be managed with a sick day formula providing 50% of normal protein intake and high in calories and meeting or exceeding CHO requirements.*
- *Give prophree or polycose as tolerated.*
- *Give ibuprofen (10 mg/kg/dose q 6 h) when fever >38 C.*
- *Give ondansetron (0.15 mg/kg/dose q 8 h) to manage vomiting.*

b. ***Management with immunizations:***
- *Give sick day formula 50% for 24 hours.*
- *Manage fever >38 C with ibuprofen.*
- *Give double dose of carnitine (200 mg/kg/day).*
- *If the condition has not improved within 24 hours, bring to ER.*

c. ***Management with surgical procedure:***
- *Ensure that the patient is at his usual state of health prior to procedure.*
- *Ensure stability of metabolic parameters including ammonia, plasma aminoacids, chem1, urine for ketones prior to procedure.*
- *High caloric intake with IVF D10 and lipids which provide 110 to 120% of normal energy needs starting 12-24 hours prior to procedure.*
- *After surgery, follow the guidelines mentioned in the Transition to Long Term Management (Wards protocol) section of this manual.*

d. ***Liver transplantation:***

The overall experience reported does not clearly demonstrate the effectiveness of this therapy to either prevent further deterioration or to improve survival and quality of life.

7. Long Term Management of Propionic Acidemia (Home and Outpatient Visits) (22, 42-49)

a. Nutritional management:

The aim of dietary management includes:

- *Normal weight gain, linear growth, and head growth.*
- *Normal psychomotor development, as assessed by serial examinations and valid developmental screening tools (e.g., Denver Developmental Screening Test II).*
- *Protein is restricted in the diet to provide limited amounts of isoleucine, methionine, threonine, valine and odd chain fatty acids and further supplemented with aminoacids mixture to provide additional amino acids without the offending amino acids (see Tables 20-22).*
- *Avoidance deficiencies of essential amino acids, fatty acids, and micronutrient*

Metabolic Dietitians should ensure the following:

- *Home formula supplies include aminoacids mixture e.g.: Propimex-1® (for infant and toddler), Propimex-2® (for children; adolescents, and adults) see appendix 1.*
- *For infants: breastmilk or regular formula as a natural protein source. For older children: the source of natural protein is from regular diet.*
- *Compliance of the mother with weighing skills, appropriate preparation of formula and 3-day diet records prior to clinic visit.*
- *Monitoring of amino acid concentrations. The frequency of amino acid monitoring varies by age, metabolic stability, compliance, and regional clinical practice. For rapidly growing infants, monitoring weekly is recommended (see monitoring section).*

Table 20: Treatment ranges for target amino acids in PA:

Amino Acid (µmol/L)	***(2-4 hr postprandial)***			
	Newborn	*1-3m*	*3m-6y*	*6-18y*
GLYCINE (GLY)	*106-254*	*105-222*	*125-318*	*158-302*
ISOLEUCINE (ILE)	*27-80*	*32-87*	*13-81*	*38-95*
METHIONINE (MET)	*6-36*	*4-39*	*5-34*	*16-37*
THREONINE (THR)	*65-147*	*64-225*	*40-139*	*74-202*
VALINE (VAL)	*78-264*	*96-291*	*85-334*	*156-288*

Note: the optimal levels should be near the lower limit of normal ranges. These levels are based on BC Children Hospital reference ranges.

Table 21: Recommended daily nutrient intakes:

Age	Natural protein (g/kg/day)	Amino acids mixture(g/kg/day)	Total protein (g/kg/day)
0–12 months	0.7–1 –1.5	1.5–1 –0.7	1.8–2.2
1 year–4 years	1 –1.5	1 –0.5	1.5–2
4–7 years	1 –1.5	0.5–0.2	1.2–1.5
> 7 years	0.8–1.2	0.4–0.0	1.2–1.5

Table 22: Proposed nutrient intake when prescribing nutrition support in patients with Propionic Acidemia (9):

Nutrients	0-5m	5m-1y	1<4y	4<7y	7<11y	11<19y
ILE	110-60/kg	90-40/kg	485–735/day	630–960/day	715–1090/day	956–1470/day
MET	50-20/kg	40–15/kg	275–390/day	360–510/day	410–580/day	550–780/day
THR	125-50/kg	75–20/kg	415–600/day	540–780/day	610–885/day	830–1200/day
VAL	105/60/kg	80–40/kg	550–830/day	720–1080/day	815–1225/day	1105–1655/day
Protein	2.5/kg	2.5/kg	≥30/day	≥35/day	40/day	50/day
Energy	100-125% of RDA for age					

Table 23: Chronic medications for life:

Medication	*Dose*	*Route*	*Comments*
Carnitine	*200-300mg/kg/day divided into 2-3 times /day*	*PO*	
Biotin	*5 -10 mg daily*	*PO*	*Discontinue if no reduction in plasma C_3*
Metronidazole	*10-20 mg/kg/day, one week on drug with three weeks off*	*PO*	
Laxative agents	*Daily use of laxatives at age/weight-appropriate doses*	*PO*	

b. Supportive measures:

- ***Sodium benzoate:*** *There is no evidence to support a role for benzoate in chronic treatment.*
- ***Growth hormone:*** *Only considered if there is a low level of growth hormone/IGF1 and normal nutritional parameters.*
- ***Gastrostomy tube insertion:*** *Recommend consideration be given to the placement of GT tube, particularly in infants and young children with PA at the time of diagnosis.*
- ***Placement of a port-a-cath*** *(totally implantable central venous access device): May be considered when unreliable peripheral venous access is significantly compromising patient care but should be balanced by the increased risk of destabilizing infection.*
- ***Carbaglu®:*** *There is no current published evidence for its use in chronic management although ongoing studies suggest a utility for PA associated chronic hyperammonemia.*

- ***Antiepileptic drugs:*** *As needed.*
- ***Therapy for arrhythmia:*** *As needed.*
- ***Anti-emetics****: Ondansetron may be used on individual bases.*
- ***Alkali therapy:*** *There is no published evidence for the use of alkali therapy like polycitra or sodium bicarbonate on chronic bases.*

c. Look for complications of PA which includes:

1. ***Iatrogenic essential amino acid deficiency****:*
   a. *ILE deficiency: Weight loss or no weight gain; redness of buccal mucosa; fissures at corners of mouth; tremors of extremities; decreased plasma cholesterol and ILE; increased concentrations of plasma lysine, phenylalanine (PHE), serine (SER), tyrosine (TYR), and VAL; skindesquamation, and corneal de-epithelialization.*
   b. *MET deficiency: Decreased plasma MET and cholesterol and increased plasma PHE, proline, SER, THR, and TYR concentrations.*
   c. *THR deficiency: Arrested weight gain; glossitis and redness of buccal mucosa; and decreased plasma THR and globulin concentrations.*
   d. *VAL deficiency: Poor appetite, drowsiness; excessive irritability and crying in infants; weight loss or decrease in weight gain; and decreased plasma albumin concentration.*

2. ***Intellectual and developmental delay:*** *To maximize patients' intellectual-developmental outcome, patients need the following:*
   a. *Early initiation of physical, occupational and speech therapy services to continue throughout childhood.*
   b. *Optimize nutrition.*
   c. *Avoid acute metabolic decompensation.*
   d. *Treat episodes of metabolic decompensation swiftly and aggressively.*

3. ***Stroke like episodes and cerebellar hemorrhage:***
   a. *Ensure adequate fluid and caloric intake during the episode.*
   b. *Symptomatic treatment of focal neurological deficits and altered mental status (supportive care primarily).*

4. ***Seizures:*** *Occur in 50% of patients:*
   a. *EEG at diagnosis and then yearly.*
   b. *Referral to child neurology if epileptiform activity detected.*

5. ***Radiological brain abnormalities:*** *Basal ganglia changes and cerebral volume loss signal abnormalities in the caudate and putamen and delayed myelination. MRS shows increased lactate peak. Routine MRI/MRS is not recommended unless there is neurological deterioration.*

6. ***Cardiomyopathy:***
   a. *Echocardiogram at presentation and yearly afterwards to evaluate for cardiomyopathy.*
   b. *Echocardiogram as needed to evaluate shortness of breath, tachycardia or other signs and symptoms of cardiac failure.*

7. ***Long QT syndrome:***
   a. *ECG yearly.*
   b. *24-hour Holter yearly.*
   c. *ECG and 24-hour Holter for syncope, fainting or other signs and symptoms of Long QT.*

8. ***Immune defects:***
   a. *CBC with differential at diagnosis annually and as needed to follow abnormalities.*
   b. *If neutropenia is present, institute infection control precautions (isolation, gown and glove, etc.) as indicated by hospital policy.*

c. *Expectant management with judicious use of colony stimulating factors only in cases where neutropenia is not resolving or there is evidence of bacterial infection with neutropenia.*

9. ***Pancreatitis:***
   a. *Episodes of acute pancreatitis in PA should be managed like any other case of acute pancreatitis (i.e. fluids, judicial short term bowel rest, jejunal feeds, pain management).*
   b. *When necessary, total parenteral nutrition can be used safely, provided the amount of protein provided is not excessive (at or near the RDA for patient's age).*
   c. *Intravenous carnitine at 200–300 mg/kg/day to maintain propionylcarnitine excretion.*

10. ***Optic neuropathy:***
    a. Annual examination by an ophthalmologist to include visual acuity as well as visual examination of the anterior chamber and dilated evaluation of the fundus.
    b. Treatment of decreased visual acuity, as indicated.

11. ***Dermatologic manifestations:*** *Resembling acrodermatitis enteropathica are frequently associated with deficiency of essential amino acids, particularly isoleucine, which is excessively restricted in the diet of persons with PA.*

12. ***Osteoporosis and osteopenia and failure to thrive.***

13. ***Hearing loss, premature ovarian insufficiency and chronic renal failure (rarely).***

d. Monitoring:

Table 24: Suggested monitoring schedule includes:

Age	*Plasma aminoacids, ammonia, urine dipsticks for ketones, acylcarnitine profile*	*Clinic visit and dietitian visit*
0-3 months	*Weekly*	*Monthly*
3-18 months	*Every 2 weeks*	*Every 3 months*
18 months-10years	*Monthly*	*Every 3 months*
10-18years	*Every 3 months*	*Every 6 months*

Table 25: Suggested other lab and radiological monitoring schedule includes:

Test/action	*Time*
CBC, albumin, prealbumin, Se, Zn, ferritine, Ca, Mg, 25-hydroxy vitamin D, Growth parameters, chem1, bone profile, liver ezymes, urine for organic acids (quantitate Methylcitrate/Citrate ratio), thiamine,lactic acid and cholesterol.	*Prior to each clinic visit*
ECG, Echocardiogram, 24-hour Holter monitoring	*At diagnosis and then yearly*
EEG	*At diagnosis and then yearly*
Ophthalmology exam	*At diagnosis and then yearly*
Referral to physical, occupational and speech therapy services	*At 18 months and continue throughout childhood*
Detailed developmental assessment	*At diagnosis, 6 months, 1 year, then yearly*

MRI brain and MRS	*At diagnosis and then if there is neurological deterioration*
Hearing test	*At diagnosis*
Bone age	*At 3 years and then every 2 years*
X-ray of long bones	*When osteoporosis is suspected*

e. Management of feeding problems:

1. *General recommendations:*
   - *Monitor growth and intake of essential nutrients with every 200 gram change in weight.*
   - *Consider overnight tube feeding or limited nocturnal fasting times.*
2. *Children with mild to moderate feeding problems:*
   - *Use a combination of pureed foods and commercial enteral feeds to meet the nutritional prescription in addition to the medical protein and other energy modules.*
   - *Increase the frequency of meals and reduce quantity per feed.*
3. *Children with severe feeding problems:*
   - *G-tube feeding consisting of an enteral product to the natural protein in addition to the medical protein and other energy modules.*
   - *If there is severe vomiting: use pharmacotherapy, consider fundoplication or J-tube feeding.*

f. Sick day management:

- *At the first sign of illness, start sick day formula.*

- *Sick-day diet to provide 120-130 kcal/kg/day for neonates or 110 to 120% of normal energy needs in older individuals.*
- *Minor illness like URTI can normally be managed with a sick day formula providing 50% of normal protein intake and high in calories and meeting or exceeding CHO requirements.*
- *Give prophree or polycose as tolerated.*
- *Give ibuprofen (10 mg/kg/dose q 6 h) when fever >38 C.*
- *Give granisterone (10-40 m/kg/dose q 8 h) to manage vomiting.*

g. Management with immunizations:

- *Give sick day formula 50% for 24 hours.*
- *Manage fever >38 C with ibuprofen.*
- *Give double dose of carnitine (200 mg/kg/day).*
- *If the condition has not improved within 24 hours, bring to ER.*

h. Management with surgical procedure:

- *Ensure that the patient is at his usual state of health prior to procedure.*
- *Ensure stability of metabolic parameters including ammonia, plasma aminoacids, chem1, urine for ketones prior to procedure.*
- *High caloric intake with IVF D10 and lipids which provide 110 to 120% of normal energy needs starting 12-24 hours prior to procedure.*
- *After surgery, follow the guidelines mentioned in the Transition to Long Term Management (Wards protocol) section of this manual.*

i. Liver transplantation:

- *May be considered in individuals with recurrent episodes of hyperammonemia or acidosis that are not adequately controlled with the medical therapies outlined above.*
- *Recipients of living-related donor livers from carriers (i.e. haploinsufficient livers) seem to have similar results to Orthotopic Liver Transplant (OLT) recipients.*

Appendix 3: The names and brands of special metabolic formula required for management of screened disorders:

Disease	*Metabolic formula (Abbott Nutrition)*	*Metabolic formula (Nutricia)*
PKU	*Phenex™*	*PKU Anamix infant*
MSUD	*Ketonex®*	*MSUD ANAMIX INFANT*
GA-1	*Glutarex®*	*XLYS LOW TRY Maxmaid*
Urea Cycle Disorders	*Cyclinex®*	*UCD2*
PA and MMA	*Propimex®*	*MMA/PA Anamix infant*
IVA	*I-Valex®*	*XLEU MAXMAID*
Galactosemia	*Isomil®.*	*Galactomine 17*

Chapter 9

Criteria for including a disease in NBS programs(50-53):

Introduction

Newborn screening programs for inborn error of metabolism (IEM) have been expanded in the last decade. However, the number of screened disorders ranges from two disorders in some countries to 50 in others. The reasons for this heterogeneity lie under the questions of which and how many diseases shall be included in a newborn screening panel. The newborn screening program is a public health issue, and the funding of such a program in general is subject to the various national and regional health care legislations and considerable differences in the health care infrastructures over the world. However, all are in universal agreement that the aim of newborn screening is to early detect and treat certain medical conditions in order to improve their outcomes in a cost effective manner. Recently, advancement in the screening technology by tandem mass spectrometry and finding of more treatment options like enzyme replacement therapy have raised the question for possibility of population screening for lysosomal storage disorders which were not part of the newborn screening program previously. Continuing growth in the technology and treatment modalities urge us to find a transparent global evaluation to answer the question if a disorder should be included in a newborn screening program or not. In order to answer this question, many questions should be answered as determined by Wilson and Jungner's classical screening criteria. To tackle that question, many newborn screening advisory committees (NSAC) have been formed and many health technology assessments (HTA) have been conducted focusing mainly on health benefits for newborns. Consideration has been of benefits only, without considering the potential harm of falling short of addressing the entire picture, even at the individual level. In addition, organizational issues, as well as ethical, social, and in some instances legal issues, need to be taken into consideration before one can formulate recommendations on the implementation of screening.

Criteria for including a disease in NBS programs:

The most famous set of criteria for NBS panel is Wilson and Jungner's criteria.

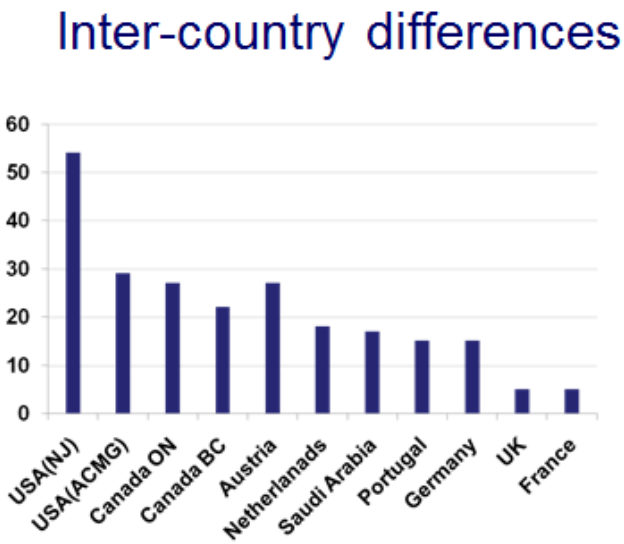
WHO Criteria for newborn screening panel (Wilson and Jungner):

1. *Constitutes an important health problem.*
2. *Available treatment for patients with recognized disease.*
3. *Facilities for diagnosis should be available.*

4. *A recognizable latent or early symptomatic stage.*
5. *Availability of suitable test or examination.*
6. *The test should be acceptable.*
7. *The natural history of the condition is adequately understood.*
8. *Accepted policy of whom to treat as patients.*
9. *The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.*
10. *Case-finding should be a continuous process and not a "once and for all" project.*

Modified WHO criteria 2008 (Andermann et. al) for NBS panels:

1. *The screening program should respond to a recognized need.*
2. *The objectives of screening should be defined at the outset.*
3. *There should be a defined target population.*
4. *There should be scientific evidence of screening program effectiveness.*
5. *The program should integrate education, testing, clinical services and program management.*
6. *There should be quality assurance with mechanisms to minimize potential risks of screening.*
7. *The program should ensure informed choice, confidentiality and respect for autonomy.*
8. *The program should promote equity and access to screening for the entire target population.*
9. *Program evaluation should be planned from the outset.*
10. *The overall benefits of screening should outweigh the harm.*



Since there are criteria about which disorders should be included in NBS program, then the question should be raised as to why this inter and intra country difference with regards to the panel is included. This could be explained by several reasons:

- *The criteria are too vague or theoretical.*
- *Criteria are difficult to assess in a consistent manner.*
- *Technical feasibility to screen for conditions is outpacing efficacy and availability of treatment.*
- *Newborn screening is not solely beneficial; it could produce harm.*
- *Lack of evaluation of benefit minus harm.*
- *RCT and high ranking evidence is lacking in the IEM community. Therefore, the decision of whether to add new tests to the program is generally based on insufficient studies or experts' opinion / advice.*

Therefore, assessing the benefit and harm of including certain diseases in an NBS program is the gold standard way in order to reach the final decision whether to include that disease or not.

Chapter 10

Key Performance Indicators for NBS programs:

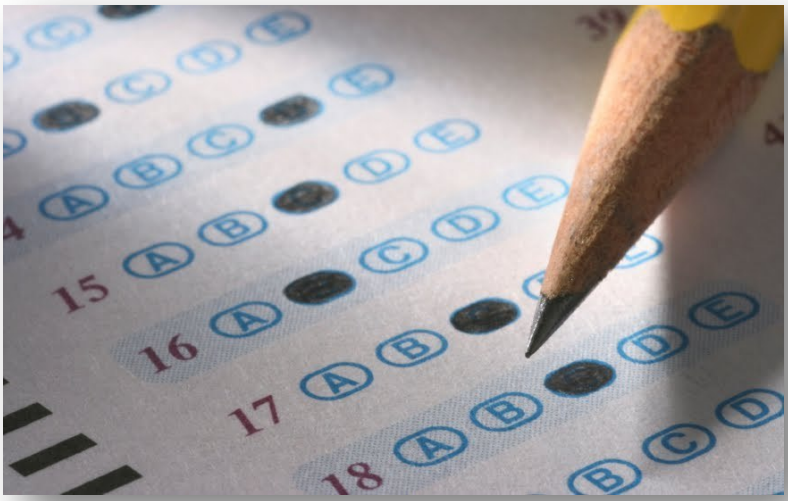
Key Performance Indicators (KPI) for NBS program (54):

An NBS program is a continuous process; therefore, it needs periodic evaluation in order to achieve its vital goal of early detection to improve outcomes. In view of this, several countries have developed key performance indicators (KPIs) for NBS programs to provide a way of measuring how well the screening programs are doing in certain areas. Examples of these KPIs include:

1) *The percentage of newborn screening coverage should be at least 95%.*
2) *The timing of sample collection of newborn screenings should be between 24-72 hours.*
3) *The percentage of invalid dried blood spot specimens/cards due to improper collection and transport should not exceed 5%.*
4) *Reporting time for results.*
5) *The true positive of the screening test.*
6) *Communication with families.*

Chapter 11

Multiple Choice Questions



Please read each question and select the single best answer. Upon completion, you may find the answers available at the end of questions.

1. *The national NBS program was launched in Saudi Arabia in:*
    a. *2005*
    b. *2006*
    c. *2007*
    d. *2008*

2. *The national NBS program currently covers the following of ____% of the Kingdom of Saudi Arabia:*
    a. *100%*
    b. *75%*
    c. *50%*
    d. *30%*

3. *The overall incidence of screened disorders in Saudi Arabia is:*
    a. *1:10000*
    b. *1:100000*
    c. *1:1000*
    d. *1:100*

4. *Which of the following is included in NBS programs in Saudi Arabia?:*
    a. *3-methylglutaconic aciduria*
    b. *Holocarboxylase synthetase deficiency*
    c. *Fumerase deficiency*
    d. *HMG CoA Lyase Deficiency*

5. *Which of the following is NOT included in NBS programs in Saudi Arabia?:*
    a. *Congenital Hypothyroidism*
    b. *Malonic Aciduria*
    c. *Maple Syrup Urine Disease(MSUD)*
    d. *Biotinidase Defciency*

6. *Which of the following can be considered as one of the NBS program stakeholders?:*
    a. *Nurse Coordinator*
    b. *Lab scientist*
    c. *Gastroenterologist*
    d. *Blood bank*

7. *The appropriate time for blood sampling for the term babies is between:*
    a. *12-24 hours*
    b. *24-72 hours*
    c. *72-96 hours*
    d. *96-120 hours*

8. *The most common site for sampling procedure will be:*
    a. *One of the arms*
    b. *One of the legs*
    c. *Heel prick*
    d. *Finger prick*

9. *The venous blood sampling is not preferred because:*
    a. *It is painful.*
    b. *The tube may contain additives like heparin and or EDTA.*
    c. *The amount is not enough.*
    d. *It can cause a false negative result.*

10. *The appearance of such sample is defected because of:*

    a. *applying blood with a capillary tube or other device.*
    b. *removing filter paper before blood has completely filled circle or before blood has soaked through to the second side.*

c. *sending specimen before drying for a minimum of four hours.*
d. *touching filter paper before or after blood specimen collection.*

11. *An increased phenylalanine (Phe) and phenylalanine/Tyrosine (Tyr) ratio is a positive NBS for which one of the following disorders?:*
    a. *Phenylketonuria (PKU)*
    b. *3-methylcrotonyl CoA Carboxylase Deficiency*
    c. *Propionic Acidemia*
    d. *Maple Syrup Urine Disease*

12. *An increased C5 (OH) and C6DC ratio is a positive NBS for which of the following disorders?:*
    a. *Isovaleric Acidemia*
    b. *3-methylcrotonyl CoA Carboxylase Deficiency*
    c. *HMG-CoA Lyase Deficiency*
    d. *Beta-ketothiolase Deficiency (BKT)*

13. *Increased citrulline is a positive NBS for which of the following disorders?:*
    a. *Ornithine transcarbamylase deficiency (OTC)*
    b. *Argininosuccinate synthetase deficiency (ASS)*
    c. *N-acetylglutamate synthase deficiency (NAGS)*
    d. *Carbamoyl phosphate synthetase I deficiency (CPS)*

14. *Increased C3 and C3/C2 is a positive NBS for which of the following disorders?:*
    a. *Maple Syrup Urine Disease (MSUD)*
    b. *Isovaleric acidemia*
    c. *HMG-CoA Lyase Deficiency*
    d. *Methylmalonic acidemia*

15. *In patients with Maple Syrup Urine Disease, the NBS will show:*
    a. *Increased leucine/isoleucine ratios*
    b. *Increased C3/C2 ratios*
    c. *Increased C8/C10 ratios*
    d. *Increased C16/C16 (OH) ratios*

16. *In patients with B-ketothiolase deficiency, the NBS will show:*
    a. *Increased C5*
    b. *Increased C5(OH), C5:1*
    c. *Increased C8 , C6, C10 and C8/C10*
    d. *Increased C14:1, C14:1/C2, C14:1/C12*

17. *NICU called you because the NBS result showed low total carnitine in a 6 day old preterm baby. The most likely diagnosis is:*
    a. *Carnitine uptake defect*
    b. *Propionic acidemia*
    c. *Creatine synthesis defect*
    d. *Prematurity*

18. *High tyrosine, methionine and phenylalanine could be seen in:*
    a. *MCAD deficiency*
    b. *VLCAD deficiency*
    c. *Propionic acidemia*
    d. *Impaired liver enzymes*

19. *NICU called you because of a positive NBS for a sick baby that showed increased leucine, valine, isoleucine, cystine, methionine, phenylalanine, and lysine. The most likely diagnosis is:*
    a. *Baby received TPN*
    b. *Cystinuria*
    c. *Hartnup disease*
    d. *Lysinuric protein intolerance*

20. *NICU called you because of a positive NBS for a sick baby who suffered from intractable seizure that showed increased C8 and C10 but normal ratio. The most likely diagnosis is:*
    a. *Baby received valproic acid*
    b. *Non-ketotic hyperglycinemia*
    c. *MCAD deficiency*
    d. *SCAD deficiency*

21. *In case of blood transfusion, the NBS should be repeated after:*
    a. *7 days*
    b. *30 days*
    c. *60 days*
    d. *120 days*

22. *A 2 month old baby boy presented to your clinic with microcephaly, eczema, and reduced hair and skin pigmentations. The most likely diagnosis is:*
    a. *PKU*
    b. *MSUD*
    c. *Propionic acidemia*
    d. *Methylmalonic acidemia*

23. *Patients with PKU and phenylalanine level = 1400µmol/l will be classified as:*
    a. *Classical PKU*
    b. *Moderate PKU*
    c. *Mild PU*
    d. *Non-PKU hyperphenylalaninemia*

24. *A three day old girl presented to the ER department with vomiting and poor feeding. On examination, she is lethargic and smells like sweaty feet. What is the most likely diagnosis?*
    a. *Methylmalonic acidemia*
    b. *Isovaleric acidemia*
    c. *Propionic acidemia*
    d. *Glutaric aciduria*

25. *A five month old boy presented to ER with hypoketotic hypoglycemia and metabolic acidosis triggered by an illness. The most likely diagnosis is:*
    a. *Isovaleric acidemia*
    b. *3-methylcrotonyl CoA carboxylase deficiency*
    c. *HMG CoA Lyase deficiency*
    d. *Glutaric Aciduria*

26. *Of the following disorders screened by NBS program, the most benign condition is:*
    a. *3-methylcrotonyl CoA Carboxylase Deficiency*
    b. *Glutaric acidemia*
    c. *Propionic acidemia*
    d. *VLCAD deficiency*

27. *Which of the following causes high methylmalonic acid:*
    a. *Liver disease*
    b. *Carnitine supplementation*
    c. *MCT oil supplementation*
    d. *Vitamin B12 deficiency*

28. *A one year old boy presented to ER with loss of consciousness. Urine analysis showed excessive ketosis. The most likely diagnosis is:*
    a. *B-ketothiolase deficiency*
    b. *MCAD deficiency*
    c. *VLCAD deficiency*
    d. *SCAD deficiency*

29. *NBS programs lead to increase detection of ALL but ONE of the following:*
    a. *Duarte variant Galactosemia*
    b. *Non-PKU hyperphenylalaninemia*
    c. *CPT1 deficiency*
    d. *Classical PKU*

30. *Medium Chain fatty acids are between:*
    a. *2-6 carbons long*
    b. *8-12 carbons long*
    c. *14-20 carbons long*
    d. *22-26 carbons long*

31. *While the management of endocrinopathies screened by NBS is standard, the management of inborn errors of metabolism (IEM) screened by NBS is more complex and challenging because of which of the following:*
    a. *Endocrinopathies present earlier.*
    b. *Lack of educational resources about the management of IEM disorders.*
    c. *The administration of the hospitals are not interested in management of IEM disorders.*
    d. *Unknown causes.*

32. *The management of IEM could be subdivided into these parts:*
    a. *Management of acute crises, transition to long term management, long term management and nutritional management*
    b. *Management of acute crises, long term management and nutritional management*
    c. *Nutritional management and chronic medications*
    d. *Chronic medications only*

33. *In emergency management of acute metabolic crises, you should give high caloric intake through :*
    a. *IVF D10% NS@ double maintenance fluid*
    b. *IVF D15% @ double maintenance fluid*
    c. *IVF D10% 0.45 NS@ 1 ½ maintenance fluid rate + intralipid 20% after exclusion of fatty acids oxidation defects*
    d. *IVF D20% @ double maintenance fluid*

34. *Mohammed, a 7 month old boy with Argininosuccinate Lyase deficiency, is admitted to PICU while you are luckily on call that night. He is improving with hyperammonemia scavengers and high caloric intake. You are monitoring electrolytes, ammonia and blood gas q4 hourly. His nurse called you with a glucose result of 20 mmol/l. What will you do?:*
    a. *Decrease Dextrose from D10% to D5%*
    b. *Start intralipid 20%*
    c. *Increase the dose of arginine*
    d. *Start insulin infusion*

35. *If the patient is on combined sodium benzoate and phenylacetate (AMMONUL®) or arginine, you should add to IVF:*
    a. *KCL 40meq/l*
    b. *NaHCO3*
    c. *Magnesium sulfate*
    d. *D15%*

36. *In case of acute hyperammonemia, you should consider dialysis when:*
    a. *Ammonia refractory to medical therapy >2hours or ammonia level >300µmol/l*
    b. *Ammonia refractory to medical therapy >4 hours or ammonia level >500µmol/l*
    c. *Ammonia refractory to medical therapy >6 hours or ammonia level >1000µmol/l*
    d. *Ammonia refractory to medical therapy >8 hours or ammonia level >600 µmol/l*

37. *The dose of sodium benzoate and sodium phenylacetate is:*
    a. *250mg/kg as loading dose over 90 minutes and stop it*
    b. *250mg/kg as loading dose over 90 minutes followed by 250-500 mg/kg/day*
    c. *250mg/kg as loading dose over 90 minutes and then 1gram/kg /day*
    d. *250mg/kg as loading dose over 90 minutes and then 2 gram/kg/day*

38. *The chronic management of propionic acidemaia includes the following:*
    a. *Carnitine, Flagyl, biotin, and protein restricted diet*
    b. *Carnitine, biotin*
    c. *Carnitine, sodium benzoate*
    d. *Carnitine, arginine*

39. *The chronic management of methylmalonic acidemaia includes the following:*
    a. *Carnitine, arginine, sodium benzoate*
    b. *Arginine, sodium phenylbutyrate, and protein restricted diet*
    c. *Carnitine, Flagyl, hydroxocobalamine, and protein restricted diet*
    d. *Phenylbutyrate, hydroxocobalamine, and protein restricted diet*

40. *The chronic management of Glutaric Aciduria type 1 includes the following:*
    a. *Carnitine, riboflavin, and protein restricted diet*
    b. *Protein restricted diet only*
    c. *Carnitine only*
    d. *Riboflavin, biotin, thiamine, and protein restricted diet*

41. *Which of the following is PKU formula?:*
    a. *Ketonex*
    b. *Phenex™*
    c. *XLYS LOW TRY Maxmaid*
    d. *XLEU MAXMAID*

42. *Which of the following is NOT a common complication of propionic acidemia?:*
    a. *Chronic renal failure*
    b. *Cardiomyopathy*
    c. *Skin rash*
    d. *Neutropenia*

43. *Which of the following is NOT part of Wilson and Jungner's criteria for NBS programs?:*
    a. *Treatment for patients with recognized disease.*
    b. *Facilities for diagnosis and treatment should be available.*
    c. *A recognizable latent or early symptomatic stage.*
    d. *Common health problems.*

44. *Which of the following is NOT part of Andermann's criteria for NBS programs?:*
    a. *The screening program should respond to a recognized need.*
    b. *The objectives of screening should be defined at the outset.*
    c. *There should be a defined target population.*
    d. *There should be an agreed policy on whom to treat as patients.*

45. *Which of the following could be used as key performance indicators (KPIs) for NBS programs in Saudi Arabia?:*
    a. *The language.*
    b. *The cost of the program.*
    c. *The percentage of newborn screening coverage.*
    d. *The cities included.*

Answers to the multiple choice questions can be found in the following table:

1.	A	16.	B	31.	B
2.	D	17.	D	32.	A
3.	C	18.	D	33.	C
4.	D	19.	A	34.	D
5.	B	20.	A	35.	A
6.	A	21.	D	36.	B
7.	B	22.	A	37.	B
8.	C	23.	A	38.	A
9.	B	24.	B	39.	C
10.	C	25.	C	40.	A
11.	A	26.	A	41.	B
12.	C	27.	D	42.	A
13.	B	28.	A	43.	D
14.	D	29.	C	44.	D
15.	A	30.	B	45.	C

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