وزارة الصحة
Ministry of Health

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National Standard Operating Procedures for Expanded Newborn Screening

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Validated by		Approved by	
Name	**Dr. Qamra Al Sariri**	Name	**Dr. Badriya Al Rashdi**
Designation	**Director General of Quality Assurance Center**	Designation	**Director General of Health Services and Programs**
Signature		Signature	
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National Standard Operating Procedures for Expanded Newborn Screening

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Sultanate of Oman

Ministry of Health

Directorate General for Health Services and Programs

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A. Acknowledgments:

This document was developed as a Standard Operating Procedure (SOP) for Newborn Screening in Oman, that explains the process and the requirements that need to be established prior to implementing the NBS program. We acknowledge with gratitude, all contributors and reviewers for their effort in writing this SOP. Different inherited disorders were extensively discussed during the preparation for the initial proposal for expansion of NBS in Oman.

Department of Woman and Child Health:

- *Dr. Jamila Al-Abri, Director of Woman & Child Health Department – Department of Woman & Child Health*
- *Dr. Moza Humaid Al Abri, Consultant Family Medicine, child health section, Department of Woman & Child Health*
- *Dr. Moza Al Hatmi, Head of Child health Section, Department of Woman & Child Health*

Department of Research and Studies Department,

- *Dr. Sultana Al Sabahi, Head of Research Management and Knowledge Translation Department, General of Planning and Studies, MOH*

The Royal Hospital:

- *Dr. Aisha Al Sinani, Sr. consultant, Pediatric Endocrinology, Royal Hospital*
- *Dr. Huda Al Mahdili, Sr. Consultant Biochemistry, Royal Hospital*
- *Dr. Nadiya Al Hashmi, Sr. Consultant, Metabolic and Genetic Disorders, Royal Hospital*

Sultan Qaboos University Hospital:

- *Dr. Fathiya Al Murshidi, Clinical and Biochemical Geneticist, Sultan Qaboos University Hospital.*
- *Dr. Khalid Al Thihli, Clinical and Biochemical Geneticist, Sultan Qaboos University Hospital*
- *Dr. Hilal Al Manthari, Consultant Neonatologist, Sultan Qaboos University Hospital*
- *Mr. Matar Mohammed Ali Al-Maney, Chief Biomedical Scientist (BMS), Clinical Biochemistry Department, Sultan Qaboos University Hospital*

Edited by: *Juanita Singh Roshmi Albert, Department of Woman and Child Health*

B. Acronyms:

AA	Amino Acids
AC	Acylcarnitine
ASA	Argininosuccinic acidemia
BMS	Biomedical Scientist
C0	free carnitine
C2	Acetylcarnitine
C5	Isovalerylcarnitine
C5-DC	Glutarylcarnitine
C8	Octanoylcarnitine
C10	Decanoylcarnitine
CAH	Congenital Adrenal Hyperplasia
CDC	Centers for Disease Control and prevention
CH	Congenital Hypothyroidism
CLIA	Clinical Laboratory Improvement Amendments
CLIR	Collaborative Laboratory Integrated Reports
CNBS	Central Newborn Screening team
DBS	Dried Blood Spots
DNA	Deoxyribonucleic Acid
EPR	Electronic patient records
EPI	Expanded Programme for Immunization
EQA	External Quality Assurance
GA1	Glutaric aciduria type 1
GAL-1-P	galactose 1-phosphate
GCMC	Gas Chromatography Mass Spectrometry
HCU	Homocystinuria
HIS	Health Information System
HPLC	High Performance Liquid Chromatography.
IQC	Internal Quality Control
IVA	Isovaleric acidaemia
LBW	Low Birth Weight
LCHADD	long-chain hydroxyacyl-CoA dehydrogenase deficiency
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry

LNBS	National Laboratory for Newborn Screening
MCADD	Medium-chain acyl-CoA dehydrogenase deficiency
Met	Methionine
MMA	Methylmalonic acidemia
MRM	Multiple reaction monitoring
MS/MS(TMS)	Tandem mass spectrometry
MSUD	Maple syrup urine disease
NBS	Newborn Screening
NEQAS	UK National External Quality Assessment Service
NICU	Neonatal Intensive Care Unit
NGC	National Genetic Center
NGS	Next-Generation Sequencing
PA	propionic acidemia/ propionic aciduria
Phe	phenylalanine
PKU	phenylketonuria
PPV	positive predictive value
QA	Quality Assurance
R4S	The Region 4 Stork (R4S) Collaborative Project
RH	Royal Hospital
RNBS	Regional Newborn Screening team
SCABU	Special Care Baby Unit
SQUH	Sultan Qaboos University Hospital
Thcy	total homocysteine
TOT	Training of Trainers
TPN	Total Parenteral Nutrition
TSH	thyroid stimulating hormone
TYR	tyrosine
WHO	World Health Organization
EPR	Electronic patient record

C. Purpose

The World Health Organization (WHO) defines newborn screening (NBS) as a public health program, which aims for an early identification of conditions for which early and timely interventions can lead to the elimination or reduction of associated mortality, morbidity, and disabilities. It does not refer to a single screening method but rather is a comprehensive screening that includes clinical examination for birth defect, screening for hearing, vision and congenital heart diseases and blood test for selected metabolic and endocrine disorders. The metabolic and endocrine diseases included in a screening program do not manifest at birth and have a window period of a few days to months. This gives an opportunity to detect the condition by performing a screening test, which if positive needs to be confirmed by a diagnostic test, and to initiate treatment at the pre-symptomatic stage, thereby arguably achieving the best possible outcome. Follow-up testing is typically coordinated between geneticists and pediatrician or primary care physician.

Today, given the anticipated benefits associated with early detection of serious but treatable disorders, NBS, in different forms, has become the standard of care in most modern public health care systems. The conditions included in NBS programs around the world vary greatly, based on the legal requirements for screening, prevalence of certain diseases within a population, political pressure, and the availability of resources for both testing and follow-up of identified patients.

A key element for the success of the NBS program is establishing a standard operating procedure (SOP). The program includes multiple processes and extensive work including reaching out for the newborn for screening, informing family about the screen positive status, bringing back the newborn for the health care facility for confirmatory testing with emergency management measures where applicable, urgent transfer of symptomatic patient to a metabolic/endocrine center, tracking confirmatory testing, updating the health care records and updating the central NBS laboratory with case closure.

Therefore, this document was developed as an SOP for Newborn Screening in Oman, that explains the process and the requirements that need to be established prior to implementing the NBS program.

The purpose of this document is:

a) To ensure timely screening of all newborns for the inborn errors of metabolism in the healthcare institutions and before discharge.
b) To ensure follow-up, referrals and management of screened positive cases.
c) To ensure that all healthcare institutions have the following active components:
   - A dedicated newborn screening team
   - Healthcare personnel trained on newborn screening tools and methods.
   - Management Information System (MIS).
d) To strengthen capacity of healthcare providers on NBS; knowledge, service provision, communication skills with the experts at central level on positive screening test and counselling families of children with positive screening test.
e) To increase community awareness about NBS.

D. Scope

This document provides the standard operating procedures in the screening of newborns for inborn-error of metabolism at all level of care (primary, secondary, tertiary)

E. Definition

S. No	Term	Definition
1	**A newborn**	A child up to 28 days of age
2	**EPR**	Electronic Patient Record
3	**Newborn screening for inborn errors of metabolism**	A screening program for neonates for different congenital metabolic and hormonal life birth defects.
4.	**LBW**	Infants <2500 grams
	VLBW	Infants <1500 grams
	ELBW	Infants < 1000 grams
5	**Preterm**	Babies born alive before 37 weeks of pregnancy are completed.
6	**Primary Health Care (PHC)**	The first and essential entrance to all other health care levels (secondary and tertiary). It acts as a link between the community and service provider, and reflects the development of the health system (MOH site).

S. No	Term	Definition
7	**Governorate Hospital**	A hospital that provides secondary and tertiary cares to inhabitants of the health governorate in which it is located. (Annual Health Report-Department of Health Information and Statistics report 2021)
8	**National NBS team**	The task force responsible for monitoring the implementation of the NBS program (The administrative Qarar 13/2021) that is responsible for making policies, providing technical, financial as well as administrative support to the program, developing program guidelines, assessing the flow of service and conducting audits on service provision
9	**Central NBS team**	The team that is responsible for the functioning in conjunction with the central newborn screening lab and ensuring good communication with the NBS teams at the Governorates levels.
10	**Regional NBS team**	NBS team at the levels of the Governorates hospitals that is in direct contact with the Central NBS team
11.	**Metabolic Centre**	A specialized center that provides metabolic genetic services (RH & SQUH)

F. Procedure

1. Specimen collection at healthcare facility

1.1 Consent:

NBS should be offered for all newborns born in government and private institutions. For Omanis the test is mandatory, and thus no consent is needed. For non-Omanis, a consent will be offered to explain the financial consequences related to the medical care of the affected newborn. In cases where consent for newborn screening is refused by the expatriate parent, it is still essential that congenital hypothyroidism screening is performed separately by collecting serum TSH. A copy of the consent form can be found in Annex 1

1.2 Timing of specimen collection

- Ideally specimen collection should be done between the age of 24 to 48 hours.
- Age of 24 hours is the minimum accepted.
- Mothers should stay as inpatient for a minimum of 24 hours post-delivery.
- Sample must be collected before discharge, samples collected before 24 hours must be repeated.

1.3 Special situations

Preterm baby admitted to NICU/SCBU and Sick babies receiving total parenteral nutrition (TPN)

In preterm infants, enzymes may not mature early on to show biochemical abnormalities. Moreover, use of total parenteral nutrition (TPN) may interfere with diagnosis of amnioacidopathies and carnitine disorders. In these cases, the screening should be done at 24 - 48 hours of life. A repeat screening sample should be sent at discharge or 28 days of life, whichever comes first. For the preterm infants, repeat screening at 28 days or before discharge is not required if they are born ≥ 34 weeks, ≥ 2.0 kg and did not receive TPN.

Sick Newborn admitted to NICU/SCBU not on TPN

Screening should be done at 24-48 hours of life, or prior to discharge from NICU if being discharged before 48 hours. For repeat testing requirement, follow same guidelines as above and below depending on their birth weight and gestational age.

Low Birth weight infants

Very Low birth weight (VLBW) infants (<1500 grams) will also need retesting due to immature enzyme systems or thyroid functioning. If the infant is <1500 grams and was not admitted to an NICU, repeat screening at 3 to 4 weeks of age. If the VLBW infant shows clinical signs consistent with any disorder prior to repeating the NBS, confirmatory testing should be done immediately. With respect to the screening protocol and timing, preterm infants ≥ 34 weeks and weight ≥ 1500 g should NOT be treated differently than term infants. In other words, no repeat sampling is required unless otherwise indicated for a term infant.

Transferred to another institution

In the event of a transfer to another facility, shortly after birth or before screening sample has been collected, the transferring facility must document that the screening was not done and ensure that the next facility is aware of the need for screening. Hospitals transferring a sick neonate to an NICU should document in the medical record whether the first newborn screen sample has been collected or not. The receiving NICU should also note whether newborn screening has been done. If not, the neonate should have a newborn screening sample collected upon admission, and a second screening sample collected again at 24- 48 hours of age.

Transfusion

If a newborn needs to receive transfusion, it is critical to collect a specimen prior to the transfusion. Even small transfusions may invalidate screening test results. A second sample for newborn screening should be collected 120 days (4 months) after the last transfusion. Unscreened infants transfused before admission to the NICU should be screened regardless, but will need re-screening.

Omanis born outside the country

Infants born outside the country may or may not have received NBS in their country of birth. NBS should be done as soon as possible or at the two weeks' routine visit at primary health care facilities. The primary care provider should check if NBS was done or not. If not, the family/caregiver should be directed to attend any nearest health facility which provides the NBS program service. Primary health care provider should notify the regional hospital NBS team.

1.4 Who should collect the specimen for NBS?

The NBS specimens should be collected by staff nurses in the postnatal wards for babies in postnatal wards and by staff nurses in NICU/SCBU for babies admitted to NICU/SCBU.

1.5 Documentation

- It is important to document the collection of NBS specimen in the hospital electronic patient record (EPR) of the baby as well as the baby's Child Health Record (pink card).
- NBS should be ordered as a test item in the newborn's EPR, if delivered in a health facility.
- The order should electronically be linked with the local laboratory where sample reception, coding and packaging will take place. It should also be electronically linked with the central NBS lab through the software acquired and built into the current electronic patients' records systems.
- During discharge of the newborn if the NBS was not done, an alert should be generated to the discharging doctor and the reasons for not doing the screening should be documented. The local NBS team and the Head of Woman and Child Health section should also be alerted. The local NBS team will be responsible to track all these alerts, and organize for the sample to be collected within the first 4 days of life.
- Results of NBS should be updated into the EPR once released.
- The NBS results should also be documented in the baby's pink card as follow:

This part should be completed before discharging the baby from the hospital.

- ***NBS***

  □ ***Done***

  □ ***Not done (reason: ----------)***

At the two weeks routine visit or before for the positive cases

- ***Results of NBS***

  □***Negative***

  □***Positive (specify the positive condition/s)***

- ***Final diagnosis:***

2. Sample Preparation and Packaging and Dispatch:

- Collected NBS cards should be received by the assigned laboratory member of the local/regional NBS team.
- The card is checked for the appropriate filling of the identifying information, and other required clinical data as accepted in the adopted DBS (Dried Blood Spots) for the NBS purpose.
- The DBS card is also checked for the quality of the samples, and appropriate filling of the DBS circles.
- The cards are then allowed to dry before packaging
- Collected samples should be shipped on the same day so that they can be included into the next day run latest, if collected on Saturday to Wednesday. Samples collected on Thursday, but missed same day shipment, and on Friday must be ready for dispatch to the lab on Saturday. The sample collected must reach the central NBS within a maximum of 48 hours from collection.
- The dried blood spots (DBS) samples once collected at the delivery centers by the assigned local/regional NBS team member will be received at the NBS lab 6 days a week except for Friday.

3. Laboratory procedures

The analytical component of the NBS program is crucial for the success of the NBS program. The central NBS laboratory will be receiving all newborn screening samples from all hospitals or delivery sites around the country for testing.

3.1 Samples Reception and Registration

All DBS samples should have been electronically requested for newborn screening through the Health Information System (HIS) system. The automatically created request number/code that will be used for the labeling of the sample, should also be automatically captured by the integrated NBS laboratory software.

Minimum requirements in a the electronic NBS software

The following are minimum requirements in a the electronic NBS software:

1) The electronic NBS item will be integrated into existing HIS across different hospitals and health care facilitates in Oman.

2) The newborn screening software should be capable of efficient high throughput handling of NBS data entries.
3) It should be user-friendly.
4) It should allow for data mining, enabling different queries, like cut offs validation and review for example.
5) Availability of continuous support and sustained licensing.
6) It should allow local super users at the NBS for some software manipulation and customization as specified in the terms of agreement.

DBS samples are registered and a unique sample number is given. Each DBS card/request must contain a minimum of the following information:

- Newborn's hospital Number on a barcoded label if possible
- Newborn's name, date and time of birth
- Gestational age and weight at birth
- Date and time the sample was taken
- Local NBS team contact number
- Documentation of consent for non-Omanis
- Is the baby on nil by mouth, on special formula other than standard formula or on TPN?

Rejection criteria for samples which are not suitable for analysis:

Prior to testing, all DBS cards are inspected for acceptability. The following samples are rejected:

- A DBS with no blood sample, or spotted blood sample does not fill the entire circle (insufficient).
- Unevenly saturated or oversaturated DBS cards
- Inaccurate or contradictory identifying information

3.2 Conditions included in the Current Expanded NBS

Annex 2 lists the conditions included in the expanded newborn screening program. It also shows the primary analytes used for screening of these conditions. Cut off values were adapted to start the pilot phase. During the pilot phase, these cut offs will be validated and revised.

3.3 How the cut off values were developed

The initial cut offs were suggested to be adopted according to the following general framework:

1) The currently available percentile distribution of various amino acids and acylcarnitines among healthy Omani newborns were reviewed as provided by the Clinical Biochemistry lab at Sultan Qaboos University Hospital- SQUH.
2) The disease range of various analytes among known Omani infants affected with the conditions of interest were studied for their range distribution.
3) Published data from The Region 4 Stork (R4S) Collaborative Project, now continued as Collaborative Laboratory Integrated Reports (CLIR), were reviewed for cut off percentile distribution of disease ranges for various analytes (McHugh et al, 2011).
4) Initial cut offs were internally discussed and finally agreed upon so that they are on the conservative end of the 1st percentile of disease range for affected Omani infants or on the 25th percentile of disease range as published in R4S/CLIR, provided the selected cut off does not overlap with the percentile distribution of the analytes among normal Omani infants.
5) All cut offs used will be fixed, including cut offs for biotinidase and Galactose-1-phosphate uridyltransferase (GALT). However, consideration maybe given for floating cut offs if significant inter-run variation was noted during the pilot phase.

3.4 General analytical aspects

- Sample preparation requires a sub-punch to be taken from the DBS to enable extraction of the analytes of interest for quantification (see appendix III for the list of conditions adopted for NBS and corresponding analytes to be targeted for detection). For improved efficiency, the cards should be punched through semi-automated instruments which enable bar code reading, sub-punch location and the distribution of samples into a 96 well plate format to enable sample extraction. A fully automated robotic punching system will be preferred for this purpose.
- Analytes from DBS along with internal quality control samples are extracted into organic solvents.
- All specimens are tested by the primary screening methods including tandem mass-spectrometry (TMS) and fluoroimmunoassay.
- Data are analyzed by using dedicated software and any abnormal results are flagged and confirmed before reporting.

- Second-tier tests may be developed over time for the purpose of reducing false positive rate without additional sample requirements. These may include homocysteine measurement for homocystinuria, single gene sequencing or target mutation testing for founder mutations once established.
- Screening results should be made available within a maximum of 48 hours after the sample is received in the NBS lab.
- For any positive result flagged on a first run, repeat analysis is mandated from the same care.
- Attempts should be made for all confirmatory testing required as described below to be done locally without the need for outsourced external testing.
- Calibration options include simple ratio to an IS or use of a dried blood spot calibration curve on each batch. In-house calibrators or commercial kits may be used.
- Validation of analysis shall include automatic flagging of inadequate IS abundances by the analytical software

3.5 Quality assurance

- For quality assurance (QA), there is a need to participate in a recognized laboratory accreditation process that addresses: structure, process and outcome characteristics
- The laboratory should participate in real time performance monitoring using carefully designed internal quality control procedures with clearly defined batch acceptability criteria and trend analysis.
- The laboratory should participate in an approved regular external quality assurance scheme arrangement with a clearly defined poor performer policy used to identify and address inadequate performance; when available.
- The laboratory should be able to perform population data monitoring and analysis to identify and report performance and trend analysis using real patient data.
- The lab is expected to record and report incidents using this as a learning tool to improve service provision.
- The laboratory should participate in an external quality assurance (EQA) schemes such as the UK National External Quality Assessment Service (NEQAS) and The Centers for Disease Control and Prevention (CDC) newborn screening proficiency program, or the CDC schemes or sample exchange with other laboratories offering NBS when an EQA scheme is not available.
- Internal quality control and performance monitoring
  - It is recommended that relevant levels of dried blood spot internal quality control (IQC) are included at the beginning and end of each plate.
  - When suitable levels are not always available commercially, there may be a need for in-house preparations to be used on each plate.
- External quality assurance of screening metabolites
  - The central NBS should seek membership of the EQA scheme offered by the Centre for Disease Control (CDC)

3.6 Indications for repeat testing in a newly collected DBS sample

1. A first sample collected before the age of 24 hours of life for any reason.
2. A first sample that was rejected for any of the above-listed reasons.
3. The first sample was collected from a preterm newborn less than 34 weeks of gestation
4. The first sample was collected from a very low birth weight newborn (birth weight less than 1500 grams)
5. The first sample was collected from a newborn receiving TPN.

3.7 Reporting results

- The result of the NBS will be recorded in the electronic health record of the screened infant
- Requests for repeat testing and positive newborn screening results are notified verbally immediately to the clinical nurse specialist of the central NBS team and he/she is notified of an electronic report emailed to him/her.
- Positive NBS reports related to disorders in Category A as outlined in category A are also notified verbally immediately to the biochemical geneticist on call.
- All results of samples should be released and made available within a maximum of 48 hours of receiving the DBS sample. All abnormal results should be reported to the appropriate follow-up program team immediately.
- Results may be reported as follows:
  - Positive, notify the clinical nurse specialist and follow disease action plan as outlined
  - Unsatisfactory, repeat testing is needed, notify the clinical nurse specialist
  - Negative, no further testing needed

3.8 Confirmatory Testing (Diagnostic Testing)

The NBS program in Oman should aim for all confirmatory testing to be performed in a local biochemical genetics laboratory or molecular genetics laboratory depending on the nature of the confirmatory test required. The laboratories expected to perform confirmatory testing should be empowered with the technical expertise and equipment capacity needed to perform relevant confirmatory testing. Confirmatory testing required may fall under one of the following categories of diagnostic testing:

1. Urine organic acids analysis using Gas Chromatography Mass Spectrometry (GCMS)
2. Plasma amino acids (quantitative) using High Performance Liquid Chromatography (HPLC) or Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)
3. Quantitative total homocysteine (this maybe outsourced during initial phase of the expansion until it is developed as a second-tier testing)
4. Single gene sequencing
   - The capacity of the National Genetics Center (NGC) in conjunction with the Molecular Genetics and Genomic Lab at SQUH will have to be evaluated for readiness to perform this confirmatory testing for various conditions.

5. Target mutation testing
    - Knowledge about currently known and newly discovered founder mutations is likely to expand over time as more newborns in Oman are screened and characterized molecularly. This should enable further development of founder-mutation based confirmatory testing depending on the population genetic characteristics defining the founder mutation.
    - The NGC with the Molecular Genetics and Genomic Lab at SQUH should collaborate to distribute the load of target mutation testing development. Both laboratories should ensure competency with extraction of DNA from DBS samples for this purpose with the start of NBS expansion.

- Confirmatory testing comes with its added cost and also technical and physical space demands. It is preferable that confirmatory testing is integrated within NBS laboratory. However, outsourcing to other external labs like biochemistry lab at SQUH may be considered. This would entail provision of HPLC/LC-MS/MS and GCMS for biochemical confirmatory testing. Depending on the number of positive cases flagged, and the demand on confirmatory biochemical testing, additional cost maybe implicated.
- Given the cost-effective nature of the current Next-Generation Sequencing (NGS) technologies, it might be advisable to acquire an NGS panel to be developed and run locally at the NGC to cover sequencing and copy number variant analysis of all screened disorders. The pilot phase of the NBS expansion maybe used to initially guide estimating the cost implicated with adopting this approach for confirmation. Given clinic experience, high rates of consanguinity and the endogamous nature of our society, it is likely that over time genetic confirmatory testing would rely more on target mutation testing for founder mutations characterized in our population with less need to rely on NGS.

3.9 Retention, Storage and Uses of Residual Blood Spots

- Residual dried blood spots collected during NBS should be stored for a period of 5 years for QA purposes starting at the date the sample was received in the NBS lab. Once this period is elapsed, the DBS must be destroyed within a period of 6 months.
- Stored DBS samples should be identified with a laboratory code and should not be linked to a medical record number. However, the NBS should possess the ability to link the stored DBS with patient's medical record number when appropriately indicated.

- The stored cards may be used for the following purposes:
    - Laboratory audit, training, and quality improvement-related purposes. This may include re-evaluation of a false negative reporting of a clinically diagnosed patient for example.
    - Screening program development, method development and establishment of normal ranges or reference intervals for new and existing tests.
    - Residual new born blood spots or screening data may be used for research, without seeking individual consent provided the following requirements are met:
        - All identifiers have been removed from samples and data before they are given to researchers.
        - The research has been approved by a recognizable local research ethics committee.
        - The research does not violate the Declaration of Helsinki for medical research involving human subjects.
        - At least one residual blood spot should still be retained to cover the period of retention for QA purposes.
- The retained DBS sample maybe used for forensic or coronial purposes provided that appropriate legal permission (written court order or by instruction of a Coroner or Public Persecution) is obtained, AND the individual is dead or missing and thus cannot be sampled for the coronial or forensic purpose. The Director of the NBS lab should obtain a legal advice from` the Directorate General of Legal Affairs in the Ministry of Health when these requests are received. Samples from individuals who are alive and not missing should only be released for this purpose if written court order is received and the Ministry of Health regulations should be followed in such case.

4. Management of positive results

The management of the positive results will depend on the disease category. The diseases included in the NBS can be categorized into three categories: A summary for the management of the positive results is provided in Algorithm 1.

Category A conditions:

4.1.1 Increased Citrulline

4.1.2 Increased Argininosuccinic Acid (ASA)

4.1.3 Increased Leucine/Isoleucine

4.1.4 Increased 17 Hydroxyprogesterone (17-OHP)

4.1.5 Increased C3 Acylcarnitine

4.1.6 Increased C5 Acylcarnitine

Category B condition:

4.2.1 Decreased C0 (free) Carnitine

4.2.2 Increased C16-OH (+/-C18:1-OH) Acylcarnitine

4.2.3 Increased C0/[C16+C18]

4.2.4 Increased C16 (+/-C18:1) Acylcarnitine

4.2.5 Increased C14:1 Acylcarnitine

4.2.6 Increased C8, C6, C10 Acylcarnitine

4.2.7 Increased C5:OH Acylcarnitine

4.2.8 Decreased Galactose-1-Phosphate Uridyltransferase (GALT) Enzyme Activity 71

4.2.9 Decreased Biotinidase Activity

Category C conditions:

4.3.1 Increased Thyroid Stimulating Hormone (TSH)

4.3.2 Increased C5DC acylcarnitine

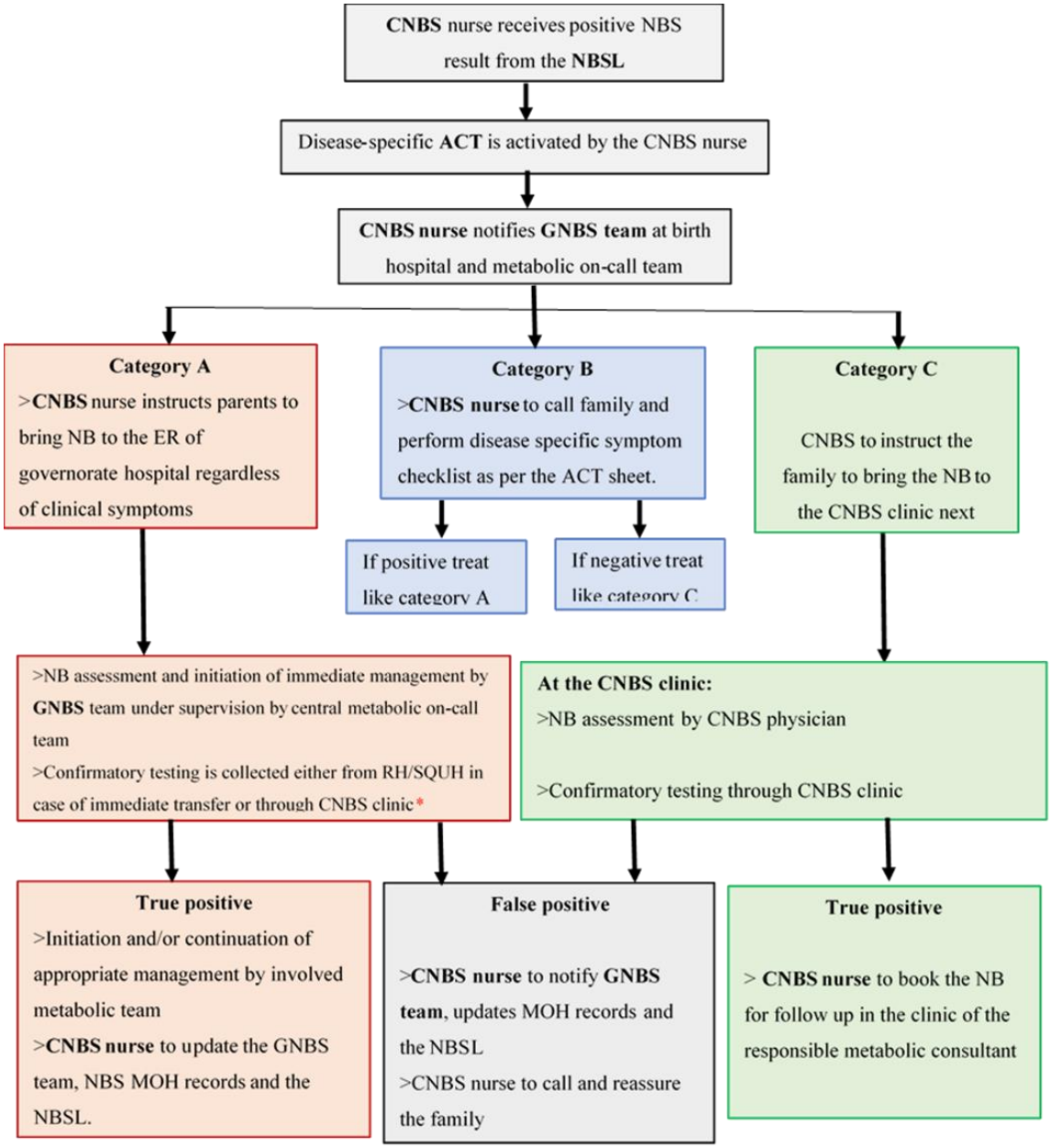
4.3.3 Increased Phenylalanine

4.3.4 Increased Succinylacetone

4.3.5 Increased Methionine

- **An overview of the Newborn Screening Program can be found in Annex 3.**
- **Medications for the various conditions can be found in Annex 4.**

Algorithm 1: Positive Intake Algorithm



*Confirmatory testing should NOT be collected from the Governorate hospital unless under specific exceptional situations

**For congenital hypothyroidism, NS assessment and confirmatory testing to be arranged through the Governorate NBS team.

- All positive results should be communicated to the Central Newborn Screening (CNBS) team. The CNBS should contact the Regional Newborn Screening Team (RNBS) and activate the disease specific ACT.
- The following algorithm summarize the actions that should be taken based on the disease category

4.1. Management of Category A conditions

1. Increased Citrulline
2. Increased Argininosuccinic Acid (ASA)
3. Increased Leucine/Isoleucine
4. Increased 17 Hydroxyprogesterone (17-OHP)
5. Increased C3 Acylcarnitine
6. Increased C5 Acylcarnitine

CATEGORY A

4.1.1 Increased Citrulline

Urea Cycle Disorder

Differential Diagnoses:

- Citrullinemia I
- Argininosuccinic acidemia
- Citrullinemia II (citrin deficiency)
- Pyruvate carboxylase deficiency

Actions:

- CNBS nurse should contact the family to inform them of the newborn screening result
- She/he should ascertain the clinical status of the baby through phone call:
    - poor feeding
    - vomiting
    - lethargy
- She/ he instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital regardless of clinical status
- **At the Governorate hospital,** following should be done:
    - Newborn to be assessed by a neonatologist/pediatrician to evaluate the newborn status for the following: poor feeding, vomiting, lethargy, hypotonia, tachypnea, seizures, and signs of liver disease
    - Immediate measurement of blood ammonia and blood gases. Other investigations as required based on the newborn symptoms/signs.
    - Follow management recommended by the on-call metabolic consultant.
    - Arrange to transfer the neonate to the metabolic center once stabilized

- **At the metabolic center,** following should be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about hyperammonemia/underlying urea cycle disorder.
    - Report final diagnosis to central NBS nurse.

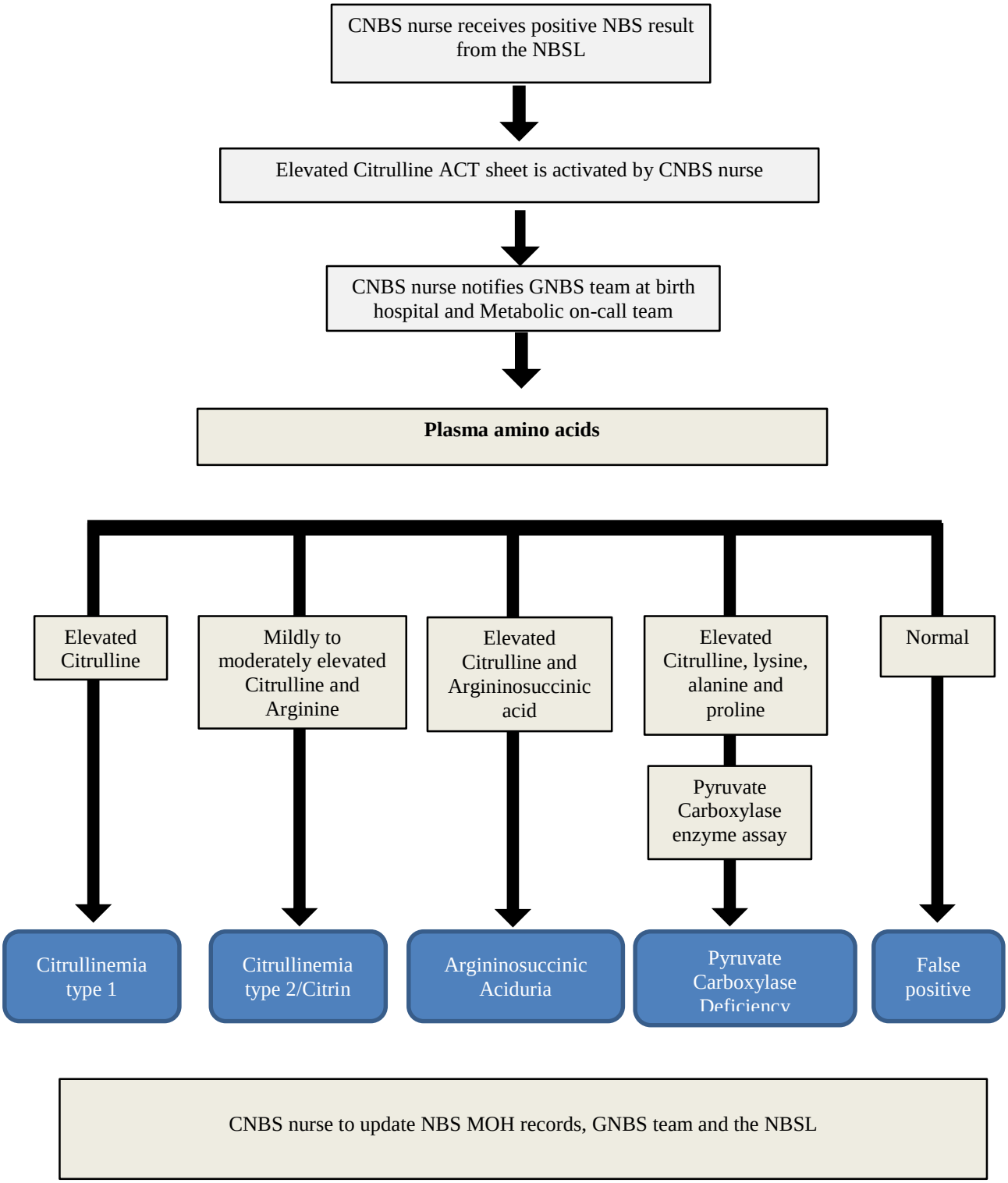
Supportive tests:

- Plasma ammonia to determine presence of hyperammonemia.
- Liver enzymes, lactic acid, bilirubin and alpha fetoprotein may be elevated in citrin deficiency.
- Blood lactate will be elevated in pyruvate carboxylase deficiency.

Diagnostic tests:

- Plasma amino acid analysis:
    - In citrullinemia, increased citrulline
    - In argininosuccinic acidemia, argininosuccinic acid will be present.
    - In citrin deficiency, increased citrulline, arginine, threonine, methionine and tyrosine

Algorithm 2: Category A: Increased Citrulline - Urea Cycle Disorder



CATEGORY A

4.1.2 Increased Argininosuccinic Acid (ASA)

Amino Aciduria/Urea Cycle Disorder

Diagnosis: Argininosuccinic aciduria (100% sensitivity and specificity)

Actions:

- CNBS nurse contacts family to inform them of the newborn screening result
- Ascertain clinical status through phone call:
    - poor feeding
    - vomiting
    - lethargy
    - rapid breathing
    - abnormal movements

- Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital regardless of clinical status

- **At the governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician and evaluate the newborn for the following: poor feeding, vomiting, lethargy, hypotonia, tachypnea, seizures, and signs of liver disease
    - Immediate measurement of blood ammonia. Other investigations as required based on the newborn symptoms/signs.
    - Follow management recommended by the on-call metabolic consultant.
    - Arrange to transfer the neonate to the metabolic center once stabilized

- **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about hyperammonemia/argininosuccinic aciduria
    - Report findings to central NBS program specialty nurse.

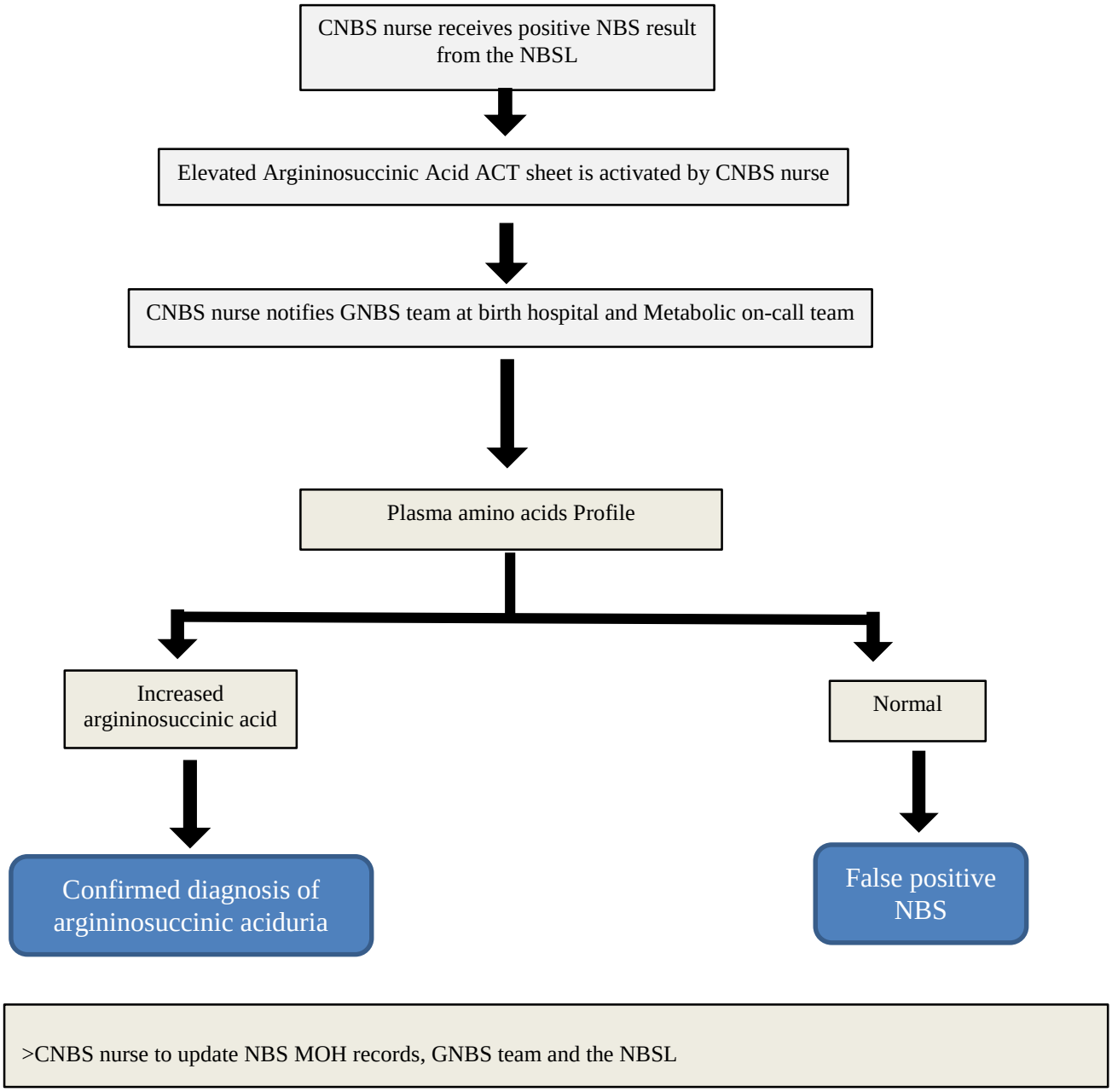
Supportive tests:

- Plasma ammonia to determine presence of hyperammonemia.
- Liver enzymes, bilirubin and urea and electrolytes.

Diagnostic tests:

- Plasma amino acid analysis to confirm the presence of argininosuccinic acid

Algorithm 3: Category A: Increased Arginninosuccinic Acid (ASA) – Urea Cycle Disorder



CATEGORY A

4.1.3 Increased Leucine/Isoleucine

Maple Syrup Urine Disease (MSUD)

Differential Diagnoses:

- Maple Syrup Urine Disease (MSUD)
- Hydroxyprolinemia (a benign condition)

Actions:

- CNBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - Irritability
    - poor feeding
    - vomiting

- Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital regardless of clinical status

- **At the governorate hospital,** following is to be done:
    - Newborn should be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, vomiting, lethargy, hypotonia, tachypnea, seizures, and/or cycling movements. Presence of maple syrup (burnt sugar)-like smell in urine and/or cerumen.
    - Immediate measurement of blood gas, electrolytes and urine or blood ketones.
    - Follow management plan recommended by the on-call metabolic consultant including immediate initiation of branched chain amino acid (BCAA)-free medical formula.
    - Arrange to transfer the neonate to the metabolic center once stabilized.

- **At the metabolic center,** following should be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about MSUD.

- Report findings to central NBS program specialty nurses.

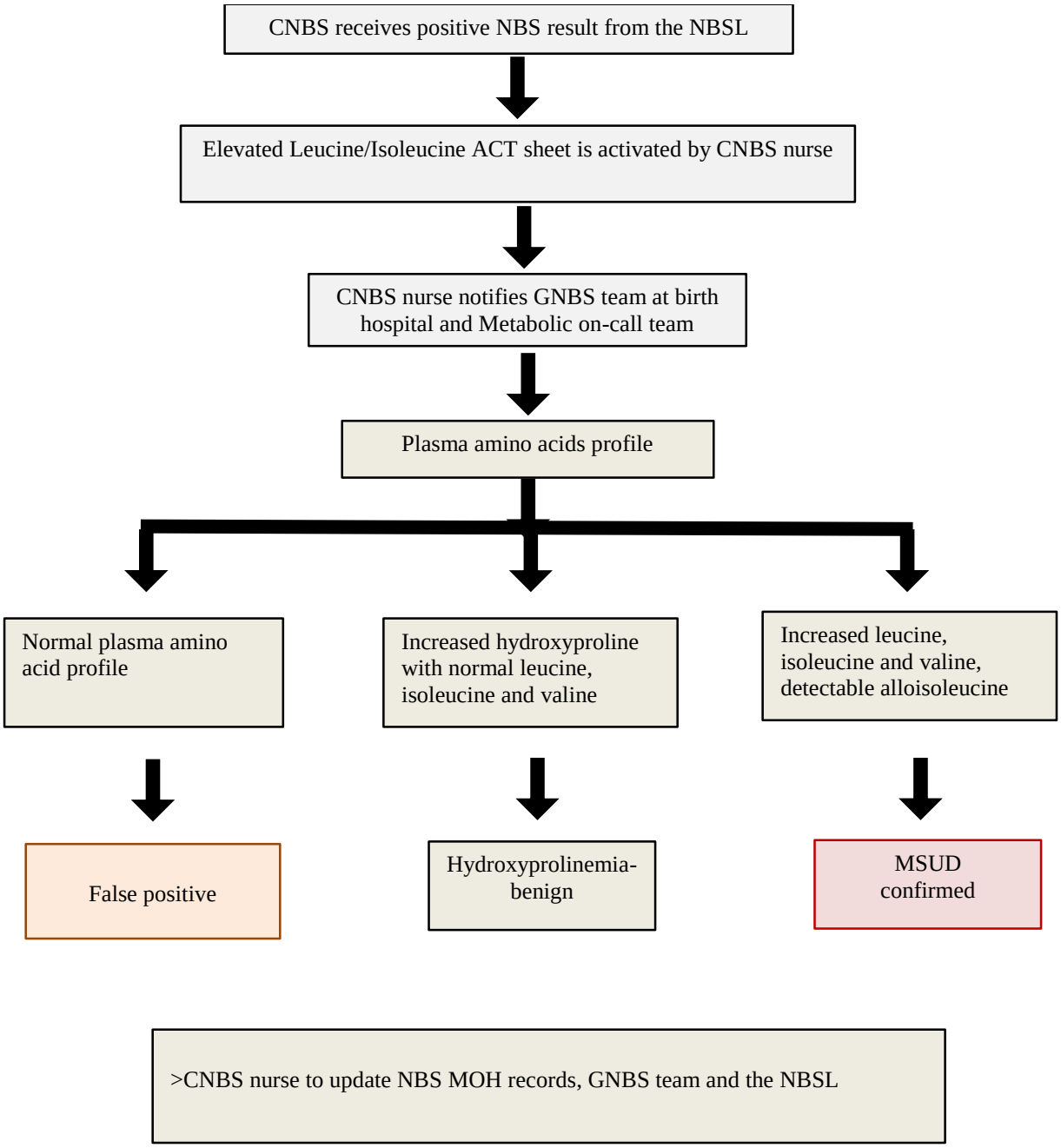
Supportive tests:

- Blood gas and electrolytes: high anion gap metabolic acidosis
- Urine or blood ketones: Ketonuria

Diagnostic tests:

- Plasma amino acid analysis to confirm the presence of alloisoleucine and the elevation of leucine, isoleucine and valine.
- Direct targeted variant analysis/MSUD gene panel as indicated

Algorithm 4: Category A: Increased Leucine / Isoleucine - Maple Syrup Urine Disease (MSUD)



CATEGORY A

4.1.4 Increased 17 Hydroxyprogesterone (17-OHP)

Congenital Adrenal Hyperplasia

Differential Diagnosis:

- Congenital adrenal hyperplasia due to 21-hydroxylase deficiency

Warning:

Infants with Congenital Adrenal Hyperplasia are at risk for life-threatening adrenal crises, shock, and death in males and females.

Actions:

- CNBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - vomiting
    - lethargy

- Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital regardless of clinical status

- **At the Governorate hospital,** following should be done:
    - Newborn should be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, vomiting, lethargy, ambiguous genitalia or non-palpable testes
    - Immediate measurement of serum electrolytes and blood glucose. Other investigations as required based on the newborn symptoms/signs.
    - The Regional team should contact the endocrine team for further management of the case.
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about congenital adrenal hyperplasia/ambiguous genitalia.

- Report findings to the Central NBS program specialty nurse.

Supportive tests:

- Serum electrolytes: low sodium and high potassium
- Blood glucose: low

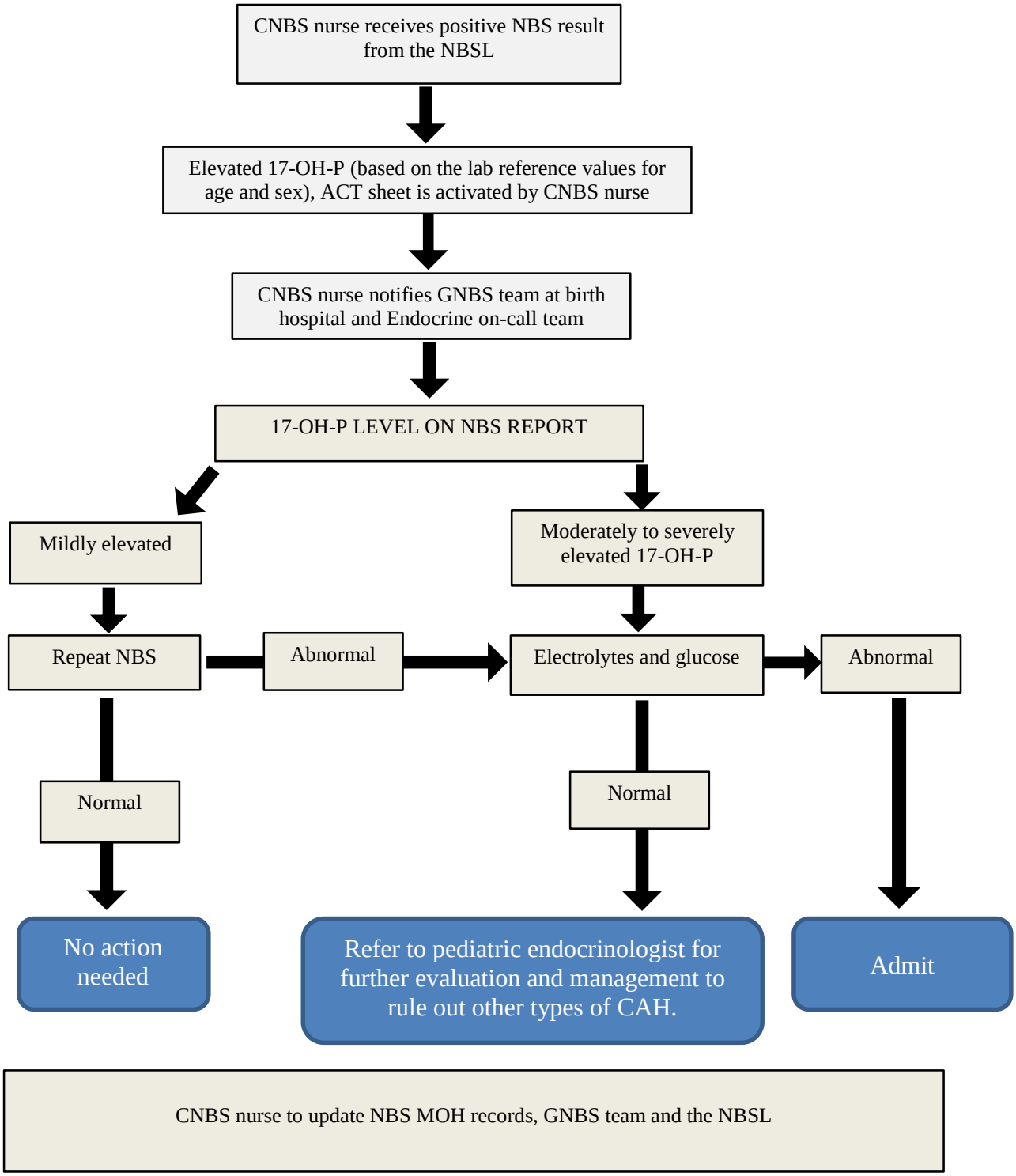
Diagnostic tests:

- If initial 17-hydroxyprogesterone is moderately to severely elevated or if initial level is mildly elevated and the repeat serum 17-hydroxyprogesterone is elevated:
- ACTH stimulation test
- Complete adrenal cortical hormone profile

Special considerations:

False positive NBS is associated with stress or prematurity.

Algorithm 5: Category A: Increased 17-OH-Progesterone - Congenital Adrenal Hyperplasia



CATEGORY A

4.1.5 Increased C3 Acylcarnitine

Organic Acidemia

Differential Diagnoses:

- Propionic Acidemia
- Methylmalonic acidemia
- Cobalamin C deficiency (methylmalonic acidemia with homocystinuria)
- Transcobalamin deficiency
- Maternal vitamin B12 deficiency

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - lethargy
    - poor feeding
    - vomiting
    - rapid breathing
    - abnormal movements

- Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital regardless of clinical status
- **At the governorate hospital,** following should be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, vomiting, lethargy, hypotonia, tachypnea and check urine/blood ketones at the bedside.
    - Immediate measurement of glucose, blood gas, electrolytes, ammonia, lactate and urine/blood dipstick for ketones.
    - Follow management plan recommended by the on-call metabolic consultant including immediate initiation of dextrose containing intravenous fluid with glucose infusion rate of 8-10mg/kg/min and a loading dose of carnitine of 50mg/kg/dose
    - Arrange to transfer the neonate to the metabolic center once stabilized.

- **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about propionic/methylmalonic acidemia and possible underlying metabolic disorders
    - Report findings to the central NBS nurse.

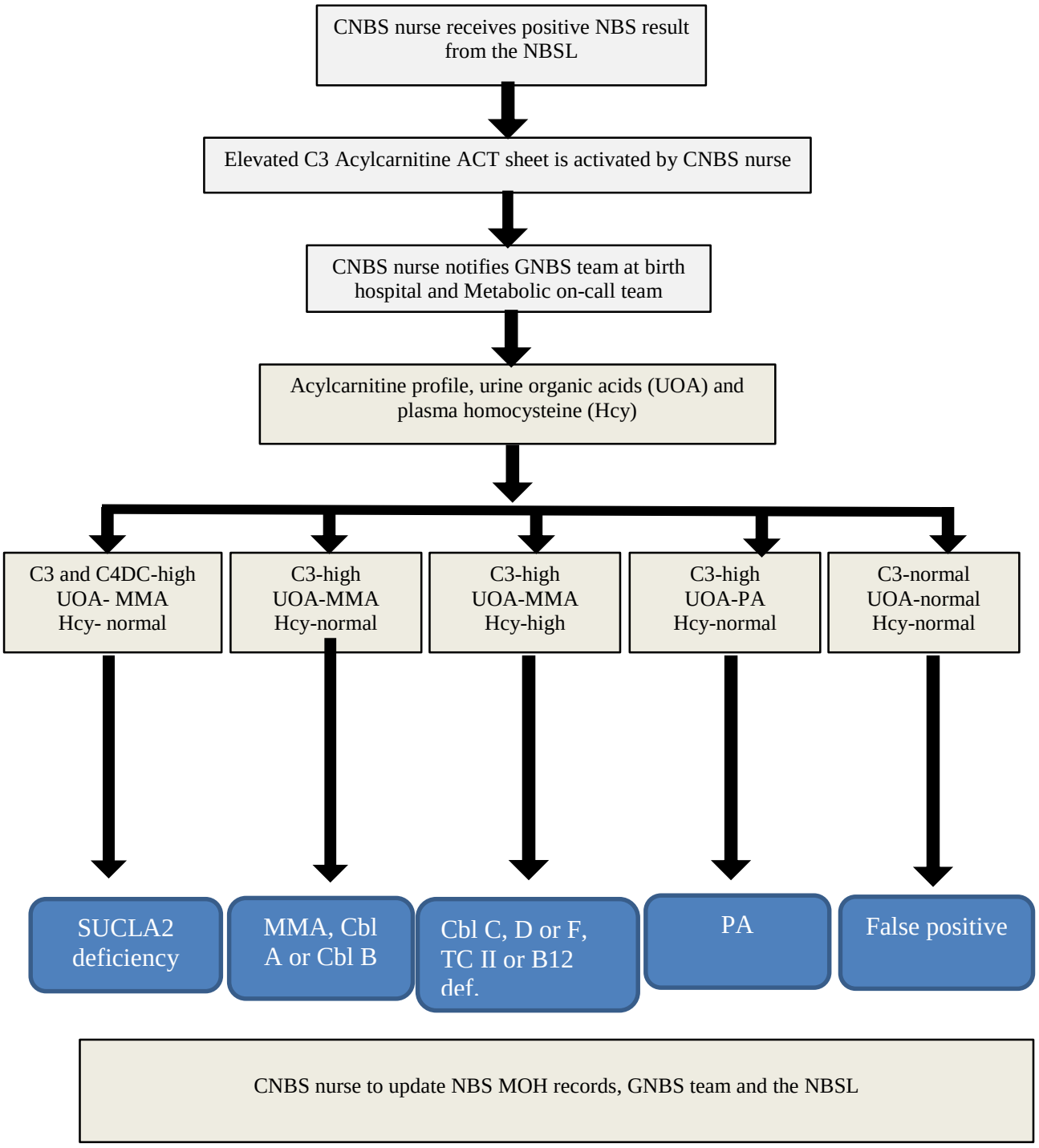
Supportive tests:

- Blood gas and electrolytes: high anion gap metabolic acidosis
  Glucose: hypoglycemia is a common finding
- Urine or blood ketones: Ketonuria
- Lactate: typically, mildly elevated

Diagnostic tests:

- Plasma or DBS acylcarnitine profile: elevated C3 acylcarnitine and C3/C2 ratio
- Plasma amino acid analysis shows elevated glycine
- Urine organic acids
- Plasma total homocysteine: elevated in cobalamin C deficiency
- Serum vitamin B12 level: elevated in cobalamin disorders
- Direct targeted mutation analysis/gene panel as indicated

Algorithm 6: Category A: Increased C3 Acylcarnitine - Organic Acidemia



CATEGORY A

4.1.6 Increased C5 Acylcarnitine

Organic Acidemia

Differential Diagnoses:

- Isovaleric acidemia (IVA)
- 2-Methylbutyrylglycinuria (2MBG)/ short/branched-chain acyl-CoA dehydrogenase-uncertain clinical significance
- False positive: antibiotic-related (pivalic acid derived) artifact.

Actions:

- CNBS contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - lethargy
    - poor feeding
    - vomiting
    - sweaty-feet odor

- Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital regardless of clinical status

- **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, vomiting, lethargy, hypotonia, tachypnea and sweaty feet odor. Check urine/blood ketones at the bedside.
    - Immediate measurement of glucose, blood gas, electrolytes, ammonia, and lactate.
    - If any above investigations are abnormal, follow management plan recommended by the on-call metabolic consultant including immediate initiation of dextrose containing intravenous fluid with glucose infusion rate of 8-10mg/kg/min and a loading dose of carnitine of 50mg/kg/dose.
    - Arrange to transfer the neonate to the metabolic center once stabilized

- **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about organic acidemias and possible underlying metabolic disorders
    - Report findings to the Central NBS program specialty nurse.

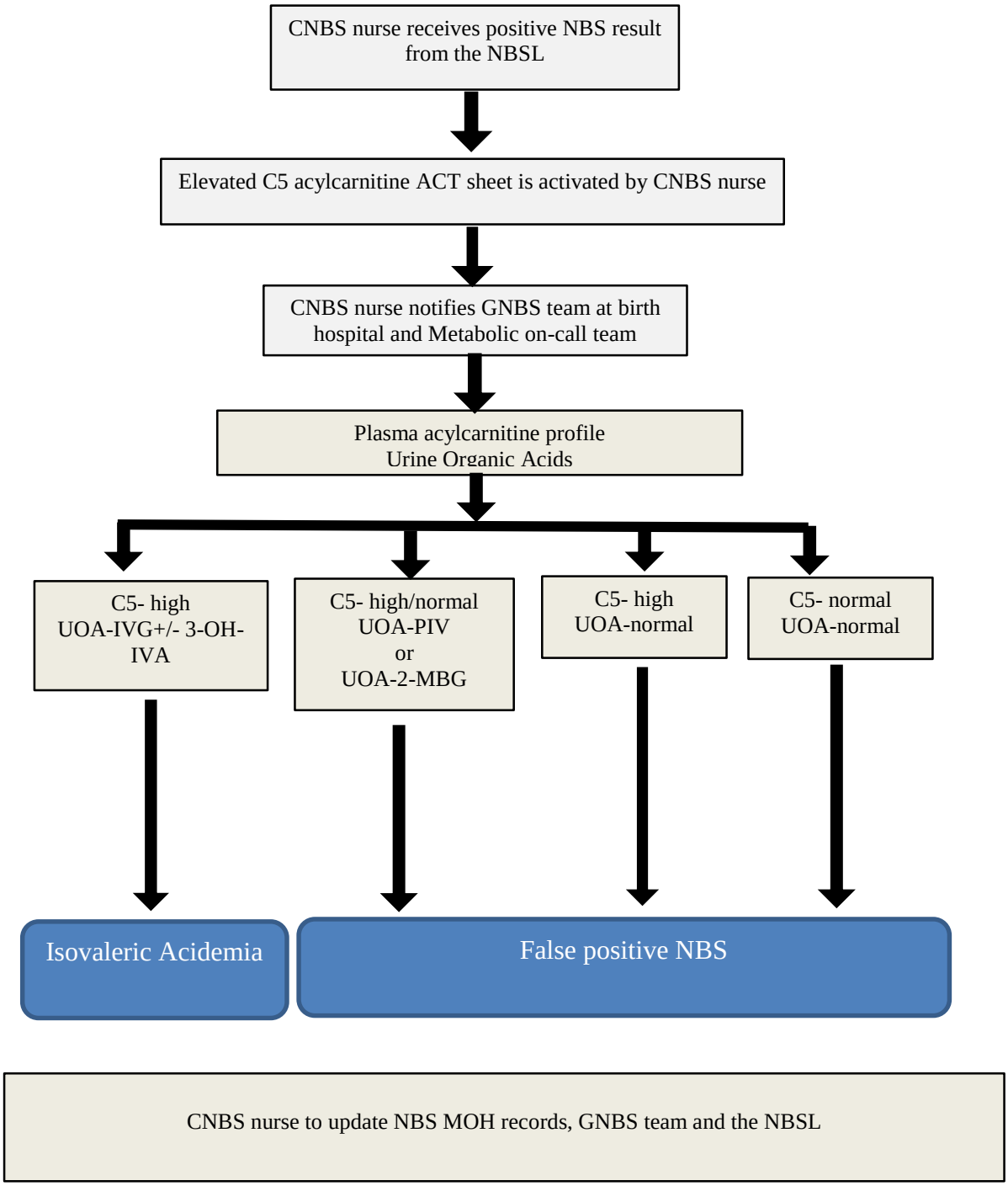
Supportive tests:

- Blood gas and electrolytes: high anion gap metabolic acidosis
- Ammonia: hyperammonemia
- Urine or blood ketones: Ketonuria

Diagnostic tests:

- Plasma or DBS acylcarnitine profile: elevated C5 acylcarnitine and C5/C2 ratio
- Urine organic acids: isovalerylglycine and 3-hydroxyisovaleric acid

Algorithm 7: Category A: Increased C5 Acylcarnitine - An Organic Acidemia



4.2 Management of Category B conditions

1. Decreased C0 (free) Carnitine
2. Increased C16-OH (+/-C18:1-OH) Acylcarnitine
3. Increased C0/[C16+C18]
4. Increased C16 (+/-C18:1) Acylcarnitine
5. Increased C14:1 Acylcarnitine
6. Increased C8, C6, C10 Acylcarnitine
7. Increased C5:OH Acylcarnitine
8. Decreased Galactose-1-Phosphate Uridyltransferase (GALT) Enzyme Activity
9. Decreased Biotinidase Activity

CATEGORY B

4.2.1 Decreased C0 (free) Carnitine

FATTY ACID OXIDATION DISORDER

Differential Diagnosis:

- Systemic Primary Carnitine Deficiency (Carnitine uptake defect (CUD))
- Maternal carnitine deficiency
- Prematurity

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - tachypnea

- **Asymptomatic newborn:**
    - Educate family about signs, symptoms, and need for urgent treatment if infant becomes ill.
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central NBS Clinic,** following is to be done:
    - Newborn is to be assessed for the following: tachycardia, hepatomegaly, reduced muscle tone.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about systemic primary carnitine deficiency.
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: tachycardia, hepatomegaly, reduced muscle tone.
    - Measurement of glucose, blood gas, ammonia, liver enzymes and creatine kinase.
    - If any of the above investigations are abnormal, correct metabolic derangements and initiate treatment with L-carnitine as per recommendations of metabolic consultant oncall.
    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about systemic primary carnitine deficiency.
    - Report findings to the Central NBS nurse.

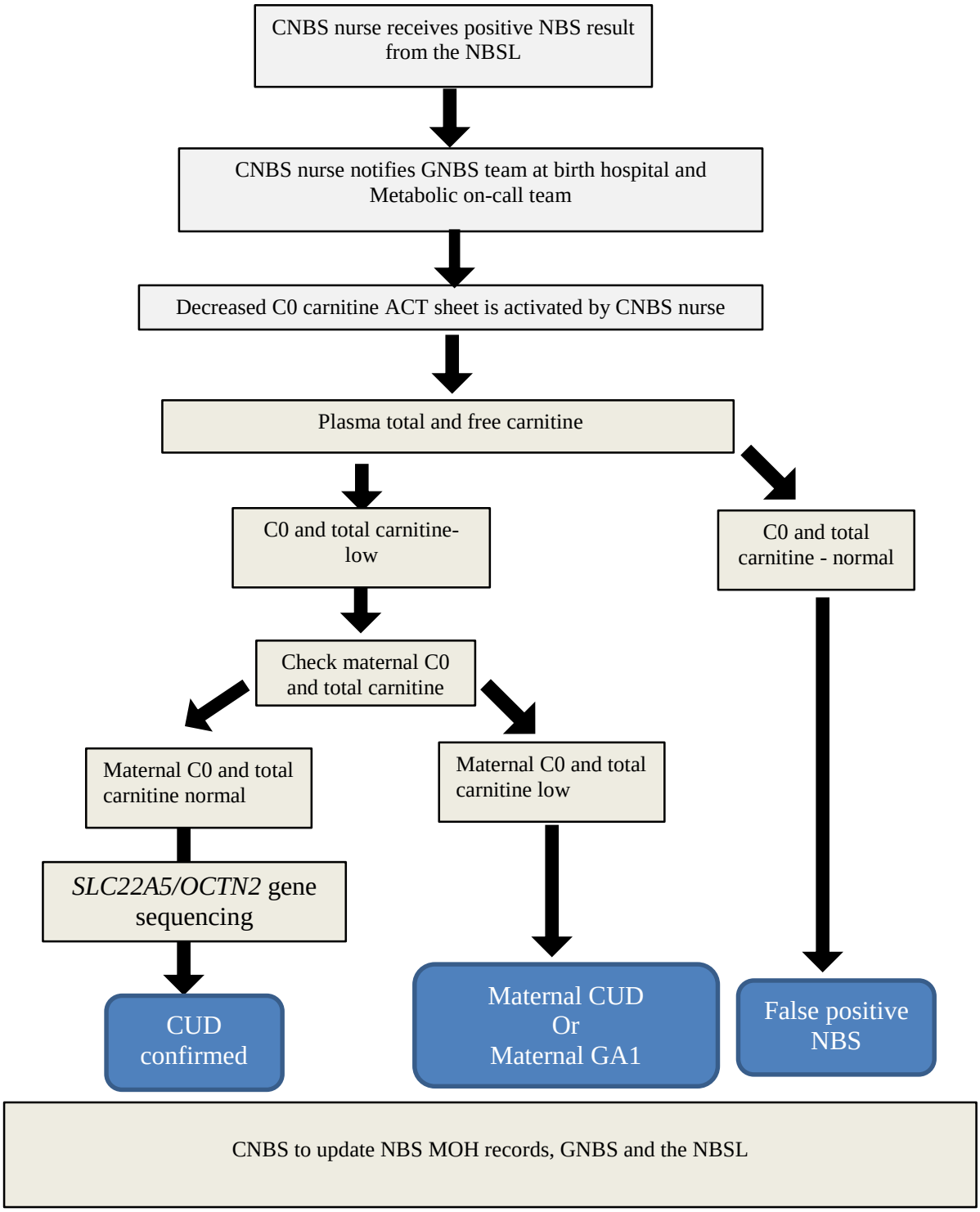
Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation

Diagnostic tests:

- Plasma carnitine analysis will reveal decreased free and total carnitine
- If the total and free carnitine are normal in the infant, it may suggest a maternal carnitine deficiency and plasma carnitine analysis in the mother is indicated.
- SLC22A5 (OCTN2 carnitine transporter) gene sequencing/targeted variant analysis.

Algorithm 8: Category B: Decreased Co (free) carnitine - Fatty Acid Oxidation Disorder



CATEGORY B

4.2.2 Increased C16-OH (+/-C18:1-OH) Acylcarnitine

FATTY ACID OXIDATION DISORDER

Differential Diagnosis:

- Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency
- Trifunctional protein (TFP) deficiency.

Actions:

- Central NBS nurse should contact the family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic
    - At the **Central NBS Clinic**, following should be done:
    - Newborn is to examined be for the following: tachycardia, hepatomegaly, and/or hypotonia.
    - Collect samples for confirmatory testing.
    - Provide the family with basic information about LCHAD/TFP deficiency
    - Central NBS nurse to follow

- **Symptomatic newborn:**
    - Instruct the family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following should be done:
    - Newborn should be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: hypoglycemia, hepatomegaly, cardiac insufficiency; history of sudden unexpected death in a sibling; and history of maternal liver disease during pregnancy.
    - Measurement of glucose, blood gas, lactate, ammonia, liver enzymes and creatine kinase.
    - If infant is symptomatic or any above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant oncall.
    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about LCHAD/TFP deficiency
    - Report findings to newborn screening program specialty nurses.

Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation
- Creatine kinase: elevated
- Lactate: elevated

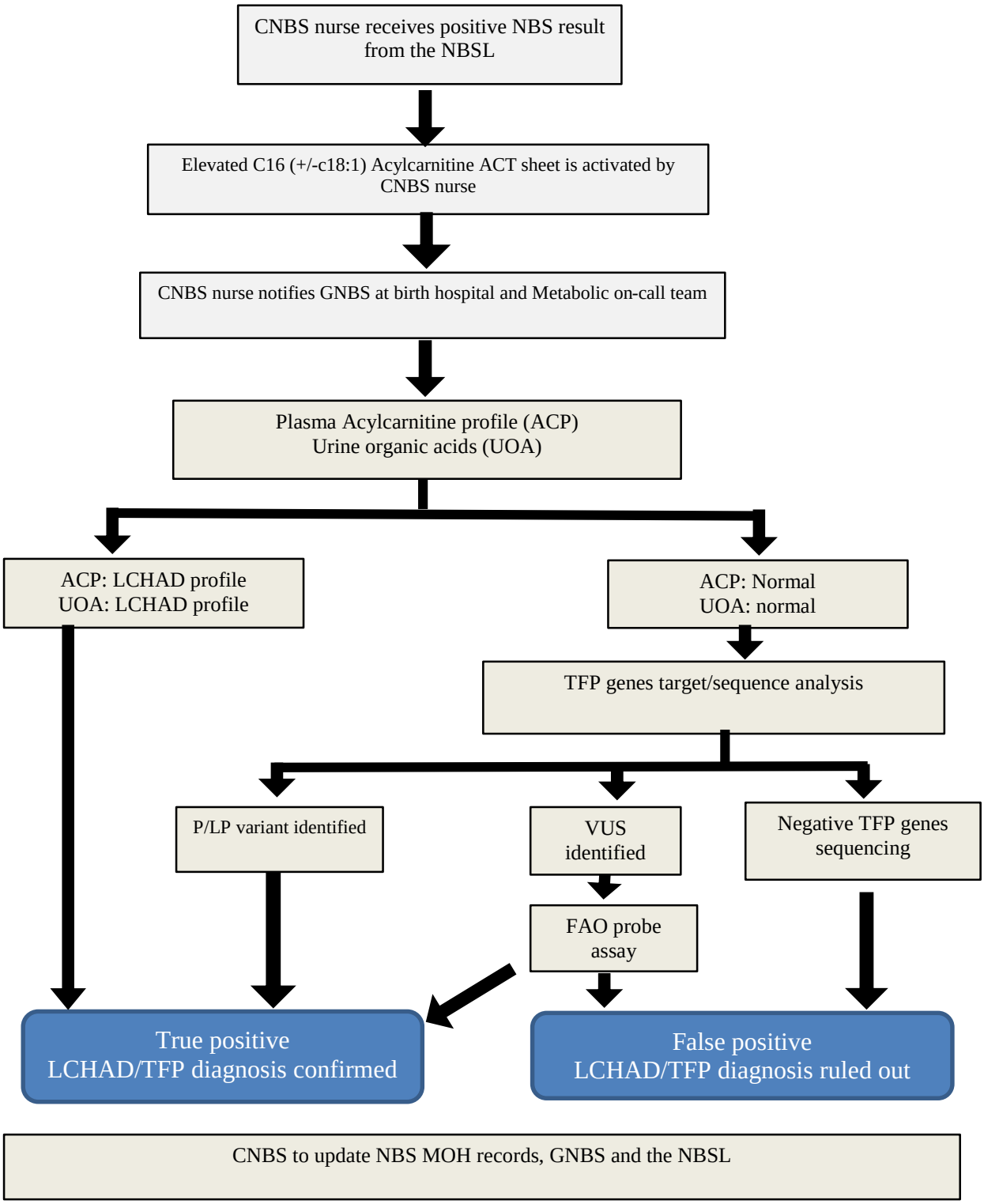
Diagnostic tests:

- Plasma acylcarnitine analysis: typically shows a characteristic pattern consistent with LCHAD or TFP deficiency with increased long chain hydroxyacyl carnitine species (C14-OH, C16-OH, C18-OH, C18:1-OH)
- Urine organic acid analysis may show C6-C14 (hydroxy) dicarboxylic aciduria
- DNA banking for *HADHA* and *HADHB* gene sequencing.

Special consideration:

- Cefotaxime treatment in the baby or mother may result in false positive result.

Algorithm 9: Increased C16-OH (+/-C18:1-OH) Acylcarnitine Fatty Acid Oxidation Disorder



CATEGORY B

4.2.3 Increased C0/[C16+C18]

FATTY ACID OXIDATION DISORDER

Differential Diagnoses:

- Carnitine Palmitoyltransferase 1A Deficiency (CPT1a)/ Hepatic Carnitine Palmitoyltransferase 1 Deficiency.

Actions:

- Central NBS nurse should contact the family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea
    - abnormal movements

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central NBS clinic,** following is to be done:
    - Newborn is to be assessed for the following: hypoglycemia, hepatomegaly, seizures.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about CPT1 deficiency
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: hypoglycemia, hepatomegaly, seizures.
    - Measurement of glucose, blood gas, lactate, ammonia, and liver enzymes.
    - If infant is symptomatic or any of the above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant oncall.
    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about CPT1 deficiency.
    - Report findings to Central NBS nurse.

Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation

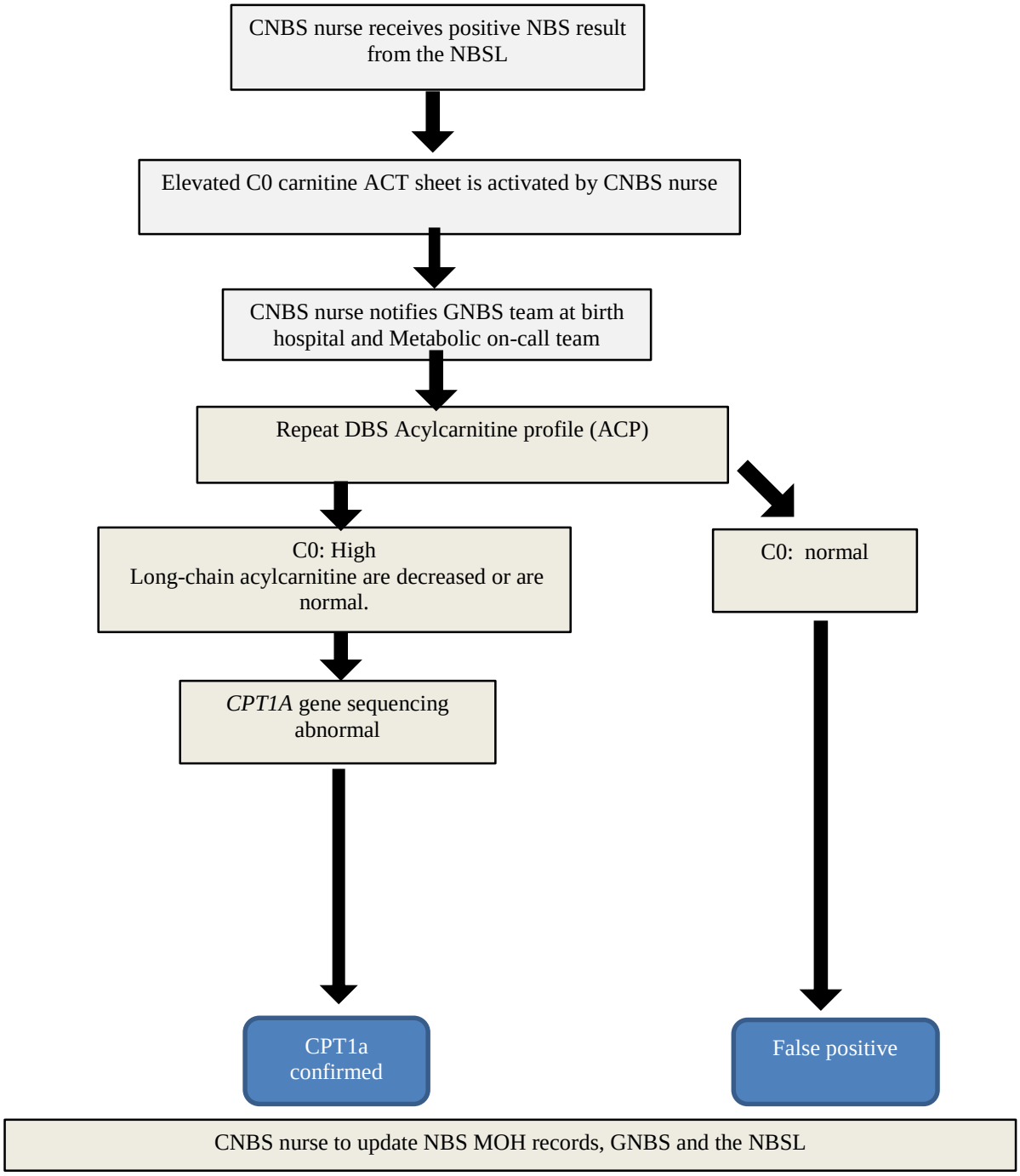
Diagnostic tests:

- Repeated acylcarnitine analysis shows elevated free carnitine C0 with low or normal long-chain acylcarnitines.
- DNA banking for *CPT1A* gene sequencing.

SPECAIL CONSIDERATIONS:

- CPT1 can have a variable presentation. Critical hypoketotic hypoglycemia is a common presenting feature. Newborns may appear asymptomatic but can progress to fasting hypoketotic hypoglycemia, lethargy, hepatomegaly, and seizures, usually precipitated by fasting or acute illness.

Algorithm 10: Category B: Increased C0/[C16+C18] Fatty Acid Oxidation Disorder



CATEGORY B

4.2.4 Increased C16 (+/-C18:1) Acylcarnitine

FATTY ACID OXIDATION DISORDER

Differential Diagnoses:

- Carnitine palmitoyltransferase (CPT2) deficiency
- Carnitine/acylcarnitine translocase (CACT) deficiency

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea
    - abnormal movements

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the newborn to emergency even if the he/she is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central BNS Clinic,** following is to be done:
    - Newborn is to be assessed for the following: dysmorphic facies (microcephaly, high sloping forehead, low-set posteriorly rotated ears, bulbous nose, higharched palate, cataract and widely spaced nipples), hypoglycemia, hepatomegaly, cardiac insufficiency; and history of sudden unexpected death in a sibling.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about CPT2/CACT deficiency
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: dysmorphic facies (microcephaly, high sloping forehead, low-set posteriorly rotated ears, bulbous nose, higharched palate, cataract and widely spaced nipples), hypoglycemia, hepatomegaly, cardiac insufficiency; and history of sudden unexpected death in a sibling.
    - Measurement of glucose, blood gas, lactate, ammonia, liver enzymes and creatine kinase.
    - If infant is symptomatic or any above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant oncall.
    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about CPT2/CACT deficiency
    - Report findings to Central NBS nurse.

Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation
- Creatine kinase: elevated
- Lactate: elevated

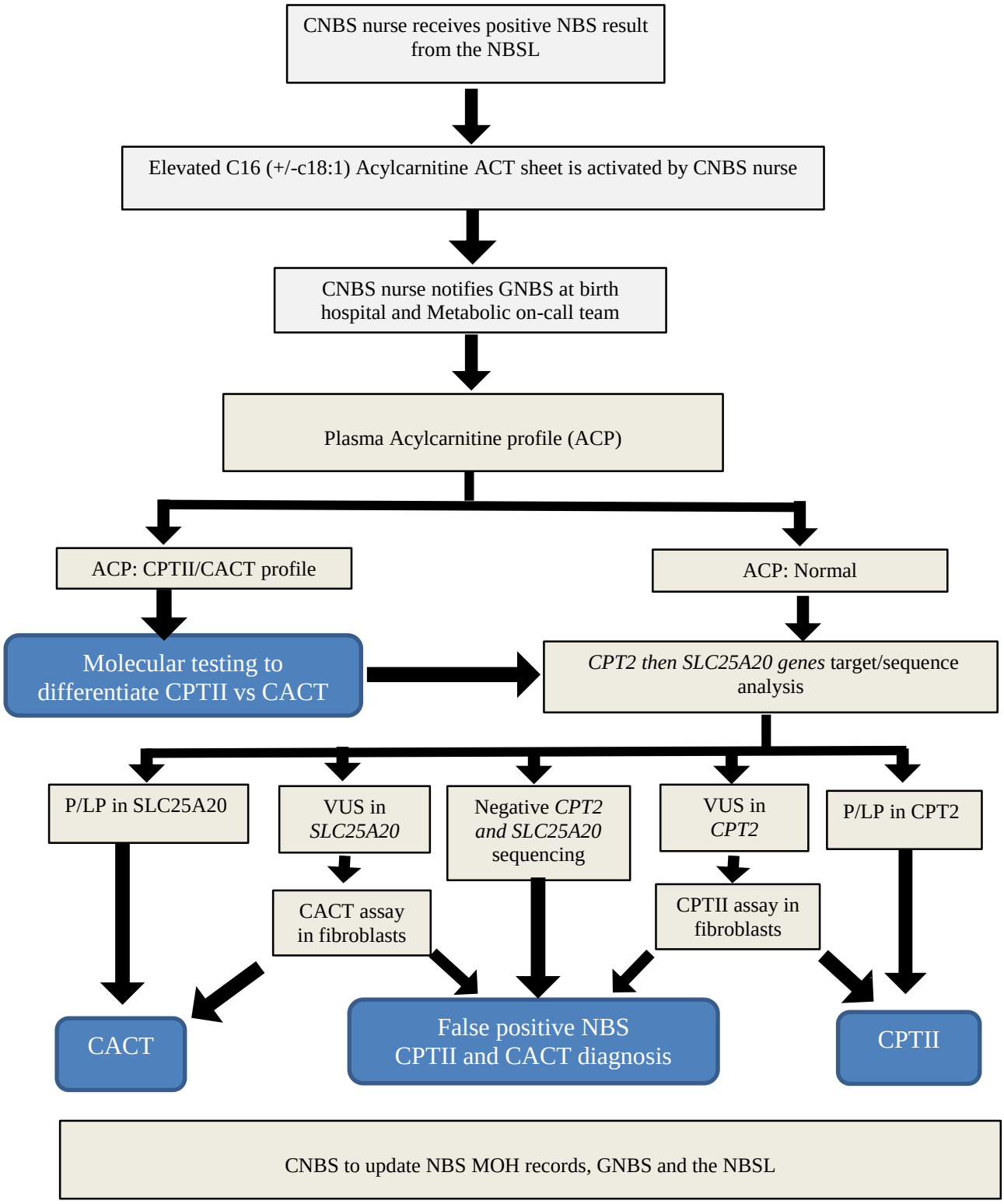
Diagnostic tests:

- Plasma acylcarnitine analysis reveals increased C16 and/or C18:1
- DNA banking for CPT2 and/or SLC25A20 gene sequencing.
- CPTII and CACT enzyme assays on skin fibroblasts (to be done by the metabolic team after first round confirmatory testing)

Special considerations:

- In the lethal neonatal form with dysmorphism and congenital abnormalities, supportive measures may be applied only as rarely will these infants survive.
- In the later-onset muscular form of CPT2 deficiency, the neonate is asymptomatic but muscle disease develops in the adolescent or adult years.
- Translocase deficiency presents similarly to the neonatal form of CPT2 deficiency.

Algorithm 11: Category B: Increased C16 (+/-C18:1) Acylcarnitine Fatty Acid Oxidation Disorder



CATEGORY B

4.2.5 Increased C14:1 Acylcarnitine

FATTY ACID OXIDATION DISORDER

Differential Diagnoses:

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

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - At the Central NBS Clinic, following is to be done:
    - Newborn is to be assessed for the following: poor feeding, lethargy, hypotonia, hepatomegaly, arrhythmia, evidence of cardiac decompensation.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about VLCAD deficiency
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital
    - **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, lethargy, hypotonia, hepatomegaly, arrhythmia, evidence of cardiac decompensation.
    - Measurement of glucose, blood gas, lactate, ammonia, liver enzymes and creatine kinase.
    - If infant is symptomatic or any above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant oncall.

    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - **At the metabolic center,** following is to be done:
    - Initiate and continue appropriate further management of the patient.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about VLCAD deficiency
    - Report findings to the Central NBS nurse.

Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation
- Creatine kinase: elevated
- Lactate: elevated
- Echocardiogram: cardiomyopathy

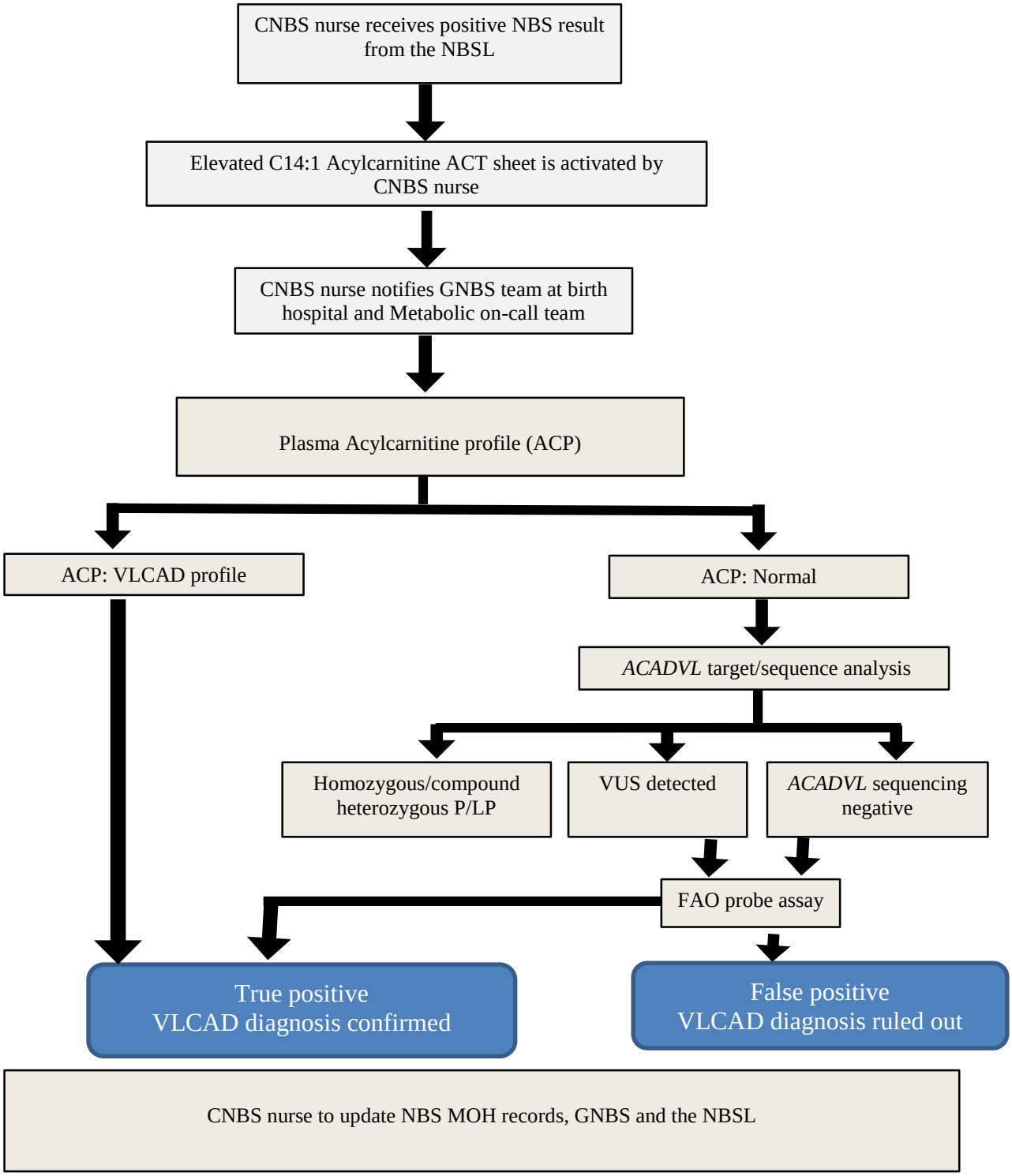
Diagnostic tests:

- Plasma acylcarnitine analysis: profile may show increased C14:1 acylcarnitine (and lesser elevations of other long chain acylcarnitines)
- ACADVL gene sequencing/targeted mutation analysis.
- Fatty acid oxidation probe assay (only by the judgment of the responsible metabolic consultant after first round confirmatory testing).

Special considerations:

- The severe early-onset cardiac and multiorgan failure form typically presents in the first months of life with hypertrophic or dilated cardiomyopathy, pericardial effusion, and arrhythmias, as well as hypotonia, hepatomegaly, and intermittent hypoglycemia. Mortality is high but aggressive and timely initiation of management has good outcome.

Algorithm 12: Category B: Increased C14:1 Acylcarnitine Fatty Acid Oxidation Disorder



CATEGORY B

4.2.6 Increased C8, C6, C10 Acylcarnitine

FATTY ACID OXIDATION DISORDER

Differential Diagnosis:

- Medium Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central NBS clinic,** following is to be done:
    - Newborn is to be assessed for the following: poor feeding, lethargy, hypotonia, and hepatomegaly.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about MCAD deficiency.
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital emergency room.
    - **At the governorate hospital,** following is to be done:

- Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, lethargy, hypotonia, and hepatomegaly.
- Measurement of glucose, blood gas, lactate, ammonia, and liver enzymes.
- If infant is symptomatic or any above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant oncall.
- Arrange to transfer the neonate to the metabolic center once stabilized.
- **At the metabolic center,** following is to be done:
- Initiate and continue appropriate further management of the patient.
- Collect samples for confirmatory testing.
- Provide family with basic information about MCAD deficiency
- Report findings to the Central NBS nurse.

Supportive tests:

- Blood glucose and ketones: may have hypoketotic hypoglycemia
- Ammonia: elevated during metabolic decompensation
- Liver enzymes: elevated during metabolic decompensation
- Lactate: may be elevated during metabolic decompensation

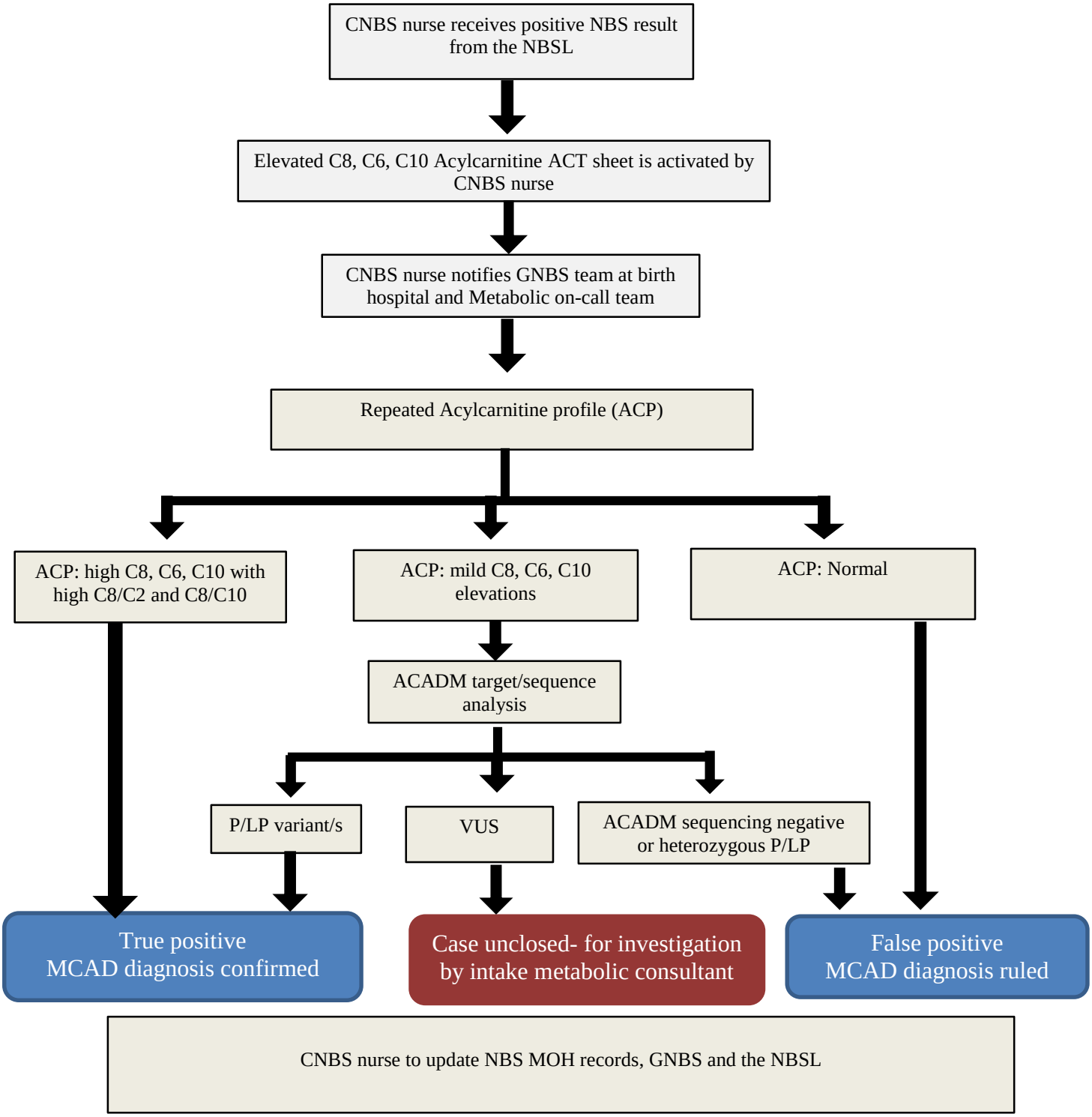
Diagnostic tests:

- Repeated acylcarnitine analysis: will show elevations of C8-acylcarnitine with lesser elevations of C6-, and C10-acylcarnitine
- ACADM gene sequencing/targeted mutation analysis.

Special considerations:

- MCAD deficiency is usually asymptomatic in the newborn although it can present acutely in the neonate with hypoglycemia, metabolic acidosis, hyperammonemia, and hepatomegaly.
- MCAD deficiency is associated with high mortality unless treated promptly.
- Untreated MCAD deficiency is a significant cause of sudden death.
- False positive can be seen in heterozygous carries or in prematurity
- False negative False negatives have been reported in newborns with low free carnitine levels, such as infants born to a mother with low free carnitine levels

Algorithm 13: Category B: Increased C8, C6, C10 Acylcarnitine Fatty Acid Oxidation Disorder



CATEGORY B

4.2.7 Increased C5:OH Acylcarnitine

ORGANIC ACIDEMIAS

Differential Diagnoses:

- 3-methylcrotonyl-CoA carboxylase (3MCC) deficiency (infant or mother).
- **3-hydroxy-3-methylglutaryl (HMG)-CoA lyase deficiency (C5:OH + C6DC)**
- **ß-ketothiolase deficiency (C5:OH + C5:1)**
- Multiple carboxylase deficiency (MCD) including biotinidase deficiency and holocarboxylase synthetase deficiency
- 2- methyl-3-hydroxybutyric acidemia (2M3HBA) due to 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD)/ HSD10 disease
- 3-methylglutaconic aciduria (3MGA) type 1

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for strict avoidance of fasting while well and asymptomatic
        iii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central NBS clinic,** following is to be done:
    - Newborn is to be assessed for the following: poor feeding, lethargy, and hypotonia.

- Collect samples for confirmatory testing.
- Provide family with basic information about the diagnostic investigations and the signs and symptoms to that necessitates emergency intervention.
- Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
  - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
  - **At the Governorate hospital,** following is to be done:
  - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: poor feeding, lethargy, and hypotonia.
  - Measurement of glucose, blood gas, lactate, ammonia, liver enzymes and ketones in the urine.
  - If infant is symptomatic or any above investigations is abnormal, start continuous source of intravenous dextrose along with direct consultation of the metabolic consultant on-call.
  - Arrange to transfer the neonate to the metabolic center once stabilized or refer to outpatient metabolic clinic if stable.
  - **At the metabolic center,** following is to be done:
  - Initiate and continue appropriate further management of the patient.
  - Collect samples for confirmatory testing.
  - Provide family with basic information about the diagnostic investigations and the signs and symptoms to that necessitates emergency intervention.
  - Report findings to Central NBS nurse.

Supportive tests:

- Blood glucose and ketones: hypoketotic hypoglycemia in HMG CoA Layse deficiency, ketotic hypoglycemia in beta ketothiolase, 3MCC and MCD.
- Ammonia: may be elevated during metabolic decompensation
- Lactate: may be elevated during metabolic decompensation

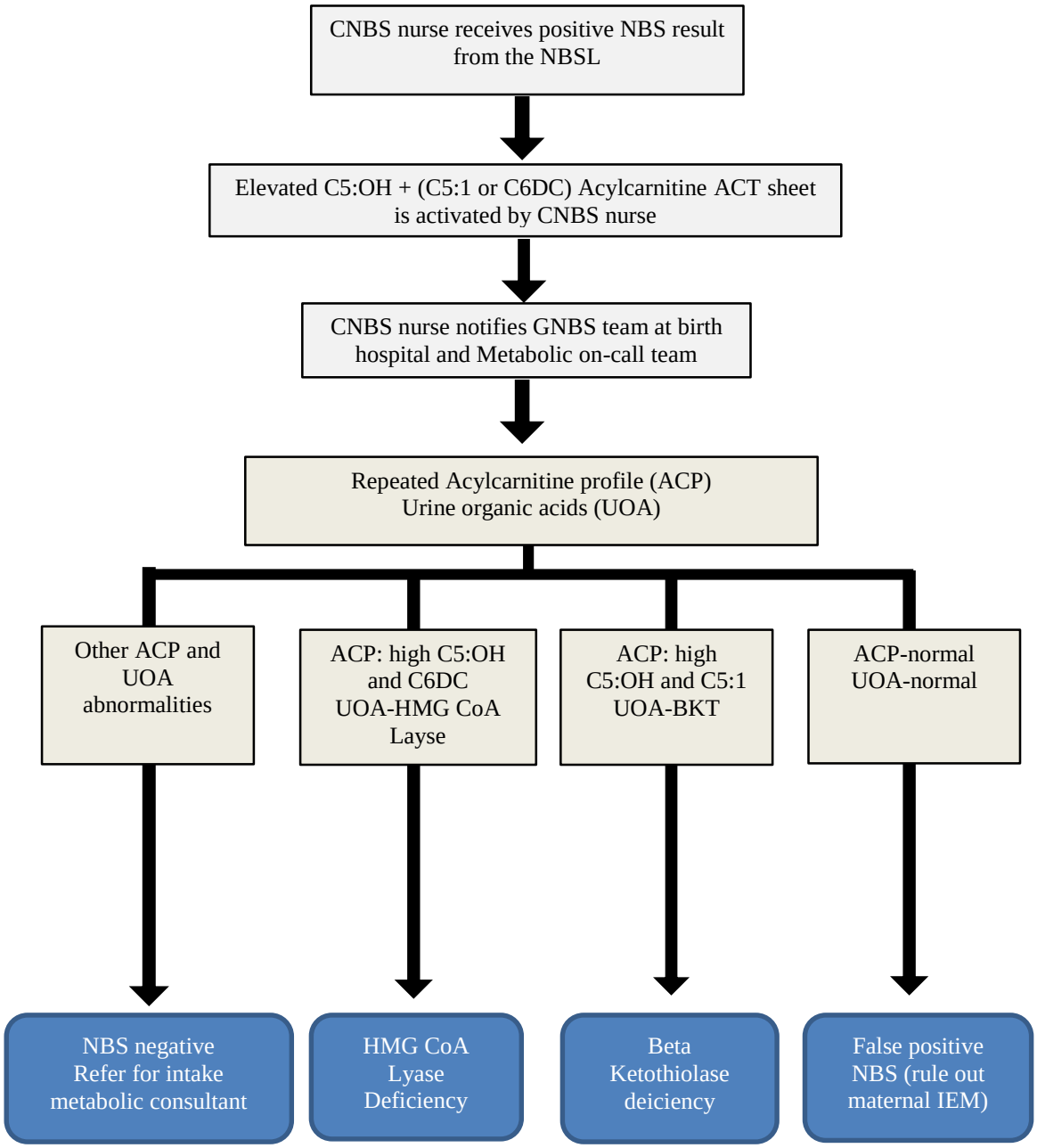
Diagnostic tests:

- Urine organic acids (OA) can clarify all differential diagnoses except similar findings of urine OA in holocarboxylase synthetase deficiency and biotinidase deficiency
- Repeated acylcarnitine profile.
- Serum biotinidase assay (already included in the expanded NBS)

Special considerations:

- In 3MCC deficiency, the undiagnosed condition in the mother can result in an elevation of 3-methylcronotonylglycine in the neonate.
- Emergency treatment protocols is required for HMG CoA Lyase and beta ketothiolase deficiency.

Algorithm 14: Category B: Increased C5:OH Acylcarnitine Organic Acidemia



CATEGORY B

4.2.8 Decreased Galactose-1-Phosphate Uridyltransferase (GALT) Enzyme Activity

Classical Galactosemia

Differential Diagnoses:

- Galactosemia (galactose-1-phosphate uridyltransferase [GALT] deficiency)
- GALT heterozygotes
- GALT variants
- Artifactual reductions due to enzyme inactivation by high temperature and/or humidity.

Actions:

- CNBS nurse contacts family to inform them of the newborn screening result.

- Ascertain clinical status through phone call:
    - poor feeding
    - vomiting
    - irritability
    - jaundice
    - bleeding tendency

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. have a low threshold to bring the baby to emergency even if the infant is mildly ill
    - Instruct family to take the newborn next working day to the Central NBS clinic.
    - **At the Central NBS clinic**, following is to be done:
    - Newborn is to be assessed for the following: poor feeding, vomiting, lethargy, irritability, bulging fontanel, jaundice, hepatomegaly and bleeding tendency.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about classical galactosemia and rational to stop breastfeeding.

- Symptomatic newborns are to be referred to the metabolic center for immediate management.
- Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
  - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital.
  - **At the Governorate hospital**, following is to be done:
  - Newborn is to be assessed by a neonatologist/pediatrician to be evaluated for the following: poor feeding, vomiting, lethargy, irritability, jaundice, hepatomegaly, bulging fontanel, and evidence of bleeding tendency.
  - Immediate measurement of glucose, blood gas, electrolytes, liver function test, full blood count, coagulation profile and urine reducing substances if available.
  - Follow management plan recommended by the on-call metabolic consultant including immediate initiation of galactose/lactose-free infant formula and stopping breast/cow's milk.
  - Arrange to transfer the neonate to the metabolic center once stabilized.
  - **At the metabolic center,** following is to be done:
  - Initiate and continue appropriate further management of the patient.
  - Collect samples for confirmatory testing.
  - Provide family with basic information about Classical Galactosemia
  - Report findings to the Central NBS nurse.

Supportive tests:

- Blood gas and electrolytes: high anion gap metabolic acidosis
- Glucose: hypoglycemia is a common finding
- Urine for reducing substances if positive for chromatography
- Liver function test: elevated transaminases and conjugated hyperbilirubinemia
- Coagulation profile: deranged

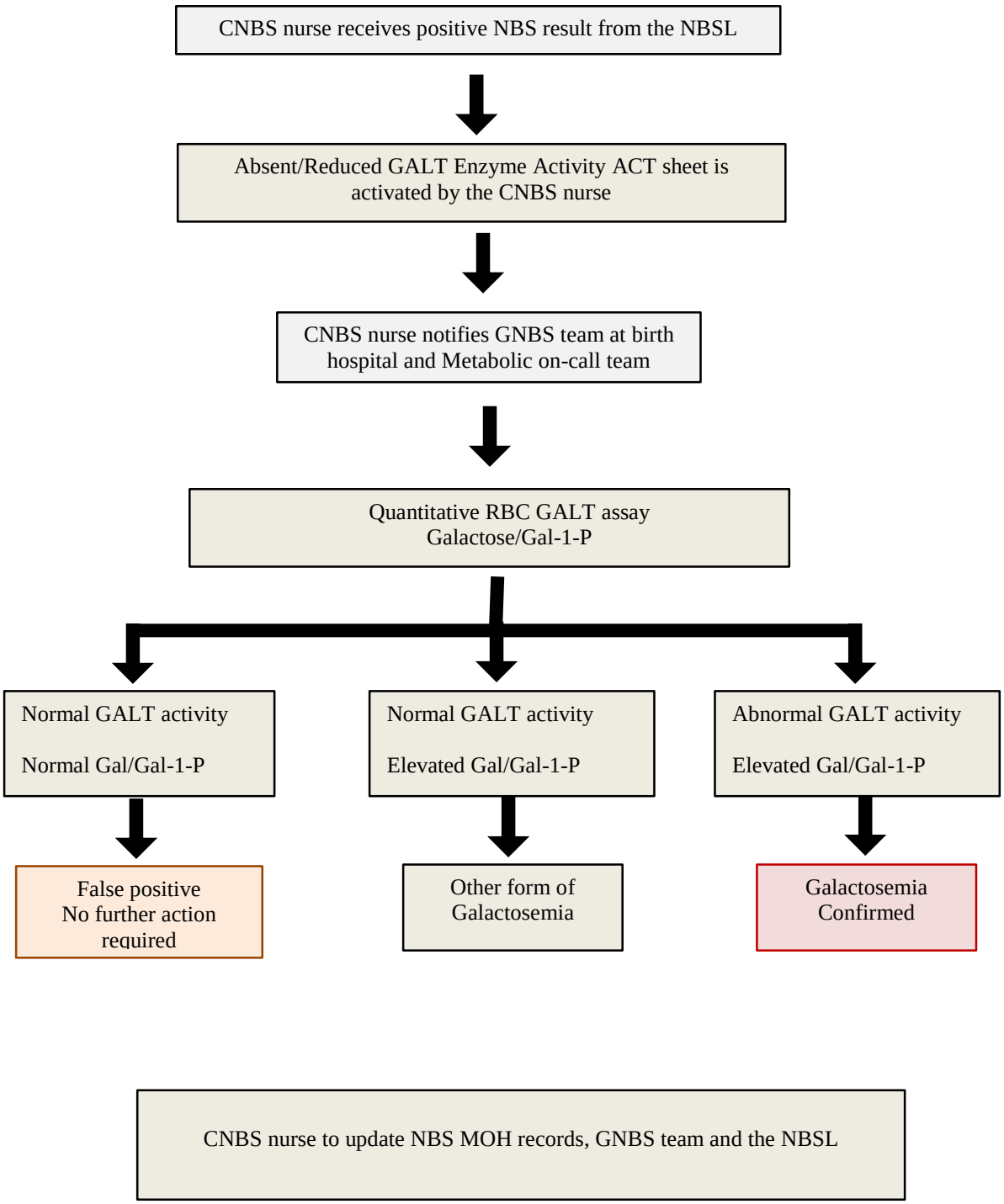
Diagnostic tests:

- Quantification of erythrocyte galactose-1-phosphate (Gal-1-P) and GALT. Classical galactosemia shows GALT<1% and markedly elevated Gal-1-P. Transfusions in infant can invalidate the results of erythrocyte enzyme assays. Enzyme variants may be distinguished by GALT electrophoresis or mutation analysis.
- Direct targeted mutation analysis/gene panel as indicated

Clinical consideration:

- In classical galactosemia, GALT deficiency results in accumulation of galactose-1-phosphate (Gal-1-P) and galactose that can result in life-threatening complications including feeding problems, failure to thrive, hepatocellular damage, bleeding, and *E coli* sepsis in untreated infants among other long-term complications.
- Immediate management is through withdrawal of breast/cow's milk, replacing it with galactose free infant formula, and if symptomatic, emergency measures as indicated.

Algorithm 15: Category B - Decreased Galactose-1-Phosphate Uridyltransferase (GALT) Enzyme Activity



CATEGORY B

4.2.9 Decreased Biotinidase Activity

Biotinidase Deficiency

Differential Diagnoses:

- Biotinidase deficiency (complete and partial)

Actions:

- Contact family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - Poor feeding
    - Lethargy
    - Hypotonia
    - Abnormal movements
    - Alopecia/skin rash

- **Asymptomatic newborn:**
    - Educate family about:
        i. signs, symptoms that should alert family to bring the infant to hospital
        ii. the need for close observation of abnormal movement
    - Instruct family to take the newborn next working day to the central NBS clinic.
    - **At the central NBS clinic,** following is to be done:
    - Newborn is to be assessed for hypotonia, lethargy, and/or seizures.
    - Collect samples for confirmatory testing.
    - Provide family with basic information about biotinides deficiency
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest governorate hospital.
    - **At the Governorate hospital**, following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate for hypotonia, lethargy, and/or seizures.

- Measurement of glucose, blood gas, lactate, and ammonia,
- If the NB is symptomatic, start biotin 10mg once a day after consulting the oncall metabolic team.
- Arrange to transfer the newborn to the metabolic center once stabilized.
- **At the metabolic center,** following is to be done:
- Initiate and continue appropriate further management of the patient.
- Collect samples for confirmatory testing.
- Provide family with basic information about Biotinidase deficiency
- Report findings to the Central NBS nurse.

Supportive tests:

- Blood gas, lactate, electrolytes and urine ketones: metabolic ketolactic acidosis
- Ammonia: hyperammonemia (usually mildly elevated up to several hundred µmol/L of ammonia in plasma)

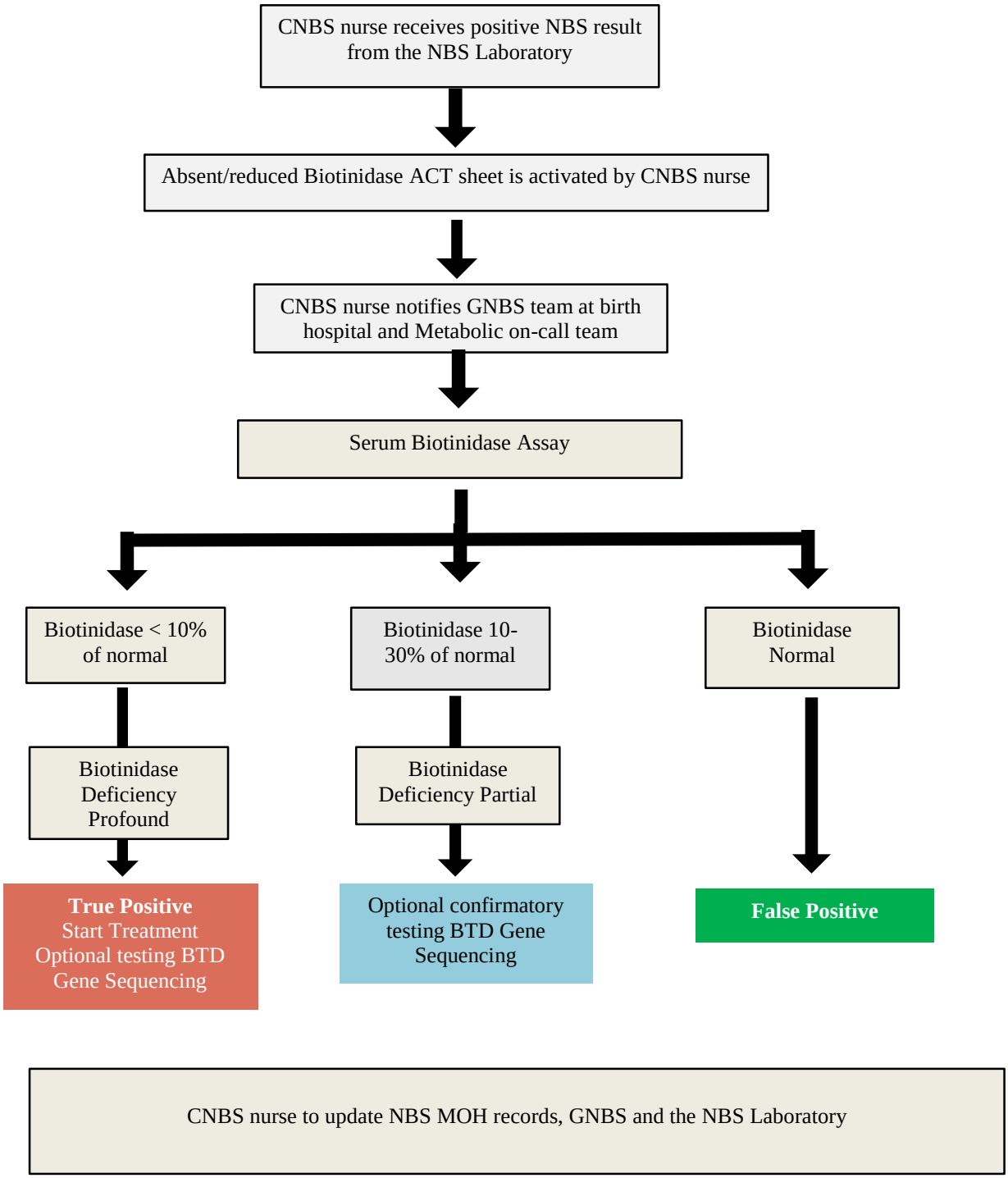
Diagnostic tests:

- Enzyme assay for biotinides in serum or plasma reveals low activity. False positive findings are usually a processing/shipping problem. Urine organic acid analysis may show normal or increased 3-hydroxyisovaleric acid and 3-methylcrotonylglycine. Plasma acylcarnitine analysis may show normal or increased C5-OH acylcarnitine.

Special considerations:

- The neonate is usually asymptomatic but may have episodic hypoglycemia, lethargy, hypotonia, and seizures. Untreated biotinidase deficiency leads to developmental delay, seizures, alopecia, and hearing deficits. Biotin treatment is available and highly effective.

Algorithm 16: Category B: Biotinidase Decreased Biotinidase Deficiency



4.3 Management of Category C conditions

1. Increased Thyroid Stimulating Hormone (TSH) Congenital Hypothyroidism
2. Increased C5DC acylcarnitine
3. Increased Phenylalanine
4. Increased Succinylacetone Tyrosinemia Type 1 (Hepatorenal)
5. Increased Methionine - Homocystinuria

CATEGORY C

4.3.1 Increased Thyroid Stimulating Hormone (TSH)

Congenital Hypothyroidism

Differential Diagnoses:

- Primary congenital hypothyroidism (CH); transient CH

Actions:

- Central NBS nurse contacts the governorate NBS team to inform them of the newborn screening result

Governorate NBS team to call the family:

  - Educate family about signs, symptoms that should alert family to bring the newborn to hospital including decreased activity, poor feeding, jaundice constipation and/or hoarse cry.
  - Instruct family to take the newborn next working day to the Governorate hospital.

At the Governorate hospital, following is to be done:

  - Newborn is to be assessed by a neonatologist/pediatrician
  - Consult pediatric endocrinologist; refer to endocrinologist, if considered appropriate.
  - Collect samples for confirmatory testing.
  - Initiate treatment as recommended by governorate hospital consultant pediatrician/endocrinologist as soon as possible
  - Report results of the confirmatory testing to the Central NBS nurse.

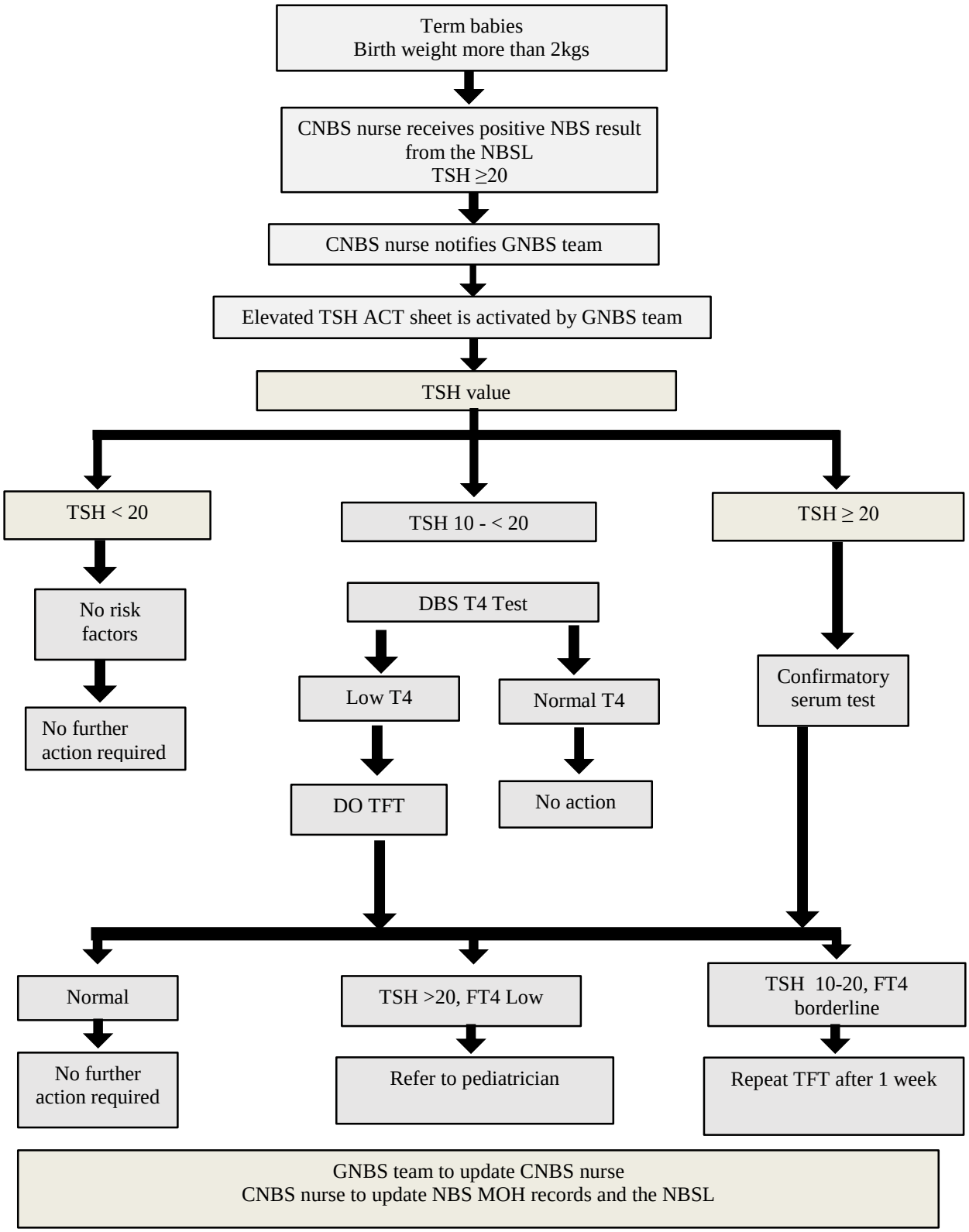
Diagnostic tests:

- Serum free T4 and TSH: low free T4 and elevated TSH in primary hypothyroidism
- Endocrinologist may also recommend total T4 and T3 resin uptake: reduced total T4 and low or normal T3 resin uptake.

Special considerations:

- Most neonates are asymptomatic, though a few can manifest some clinical features, such as prolonged jaundice, puffy facies, large fontanels, macroglossia and umbilical hernia, constipation, poor feeding and poor weight gain among others. Untreated congenital hypothyroidism results in developmental delay and poor growth.

Algorithm 17: Category C: Increased Thyroid Stimulating Hormone (TSH) - Congenital Hypothyroidism



CATEGORY C

4.3.2 Increased C5DC acylcarnitine

An Organic Aciduria

Differential Diagnosis:

- Glutaric aciduria (GA-1)
- Multiple Acyl CoA Dehydrogenase (MAD) deficiency
- Renal insufficiency
- Maternal asymptomatic GA-1

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result
- Instruct family to take the newborn next working day to the Central NBS clinic.

At the Central NBS clinic, following is to be done:

  - Newborn is to be assessed for the presence of macrocephaly and muscle hypotonia.
  - Collect samples for confirmatory testing.
  - Central NBS nurse to follow up confirmatory testing.
  - Results of urine organic acids to be reviewed with the responsible metabolic consultant.
  - If negative and requires further testing, educate family about diagnostic possibilities, complexity of diagnostic work-up and the possibility of neurodegenerative crisis with an intercurrent infectious illness. Decision to start treatment will be left to the responsible metabolic consultant.
  - If confirmed positive, care is transferred to the responsible metabolic consultant hospital to initiate medical and dietary treatment, provide emergency medical letters and appropriate genetic counseling.

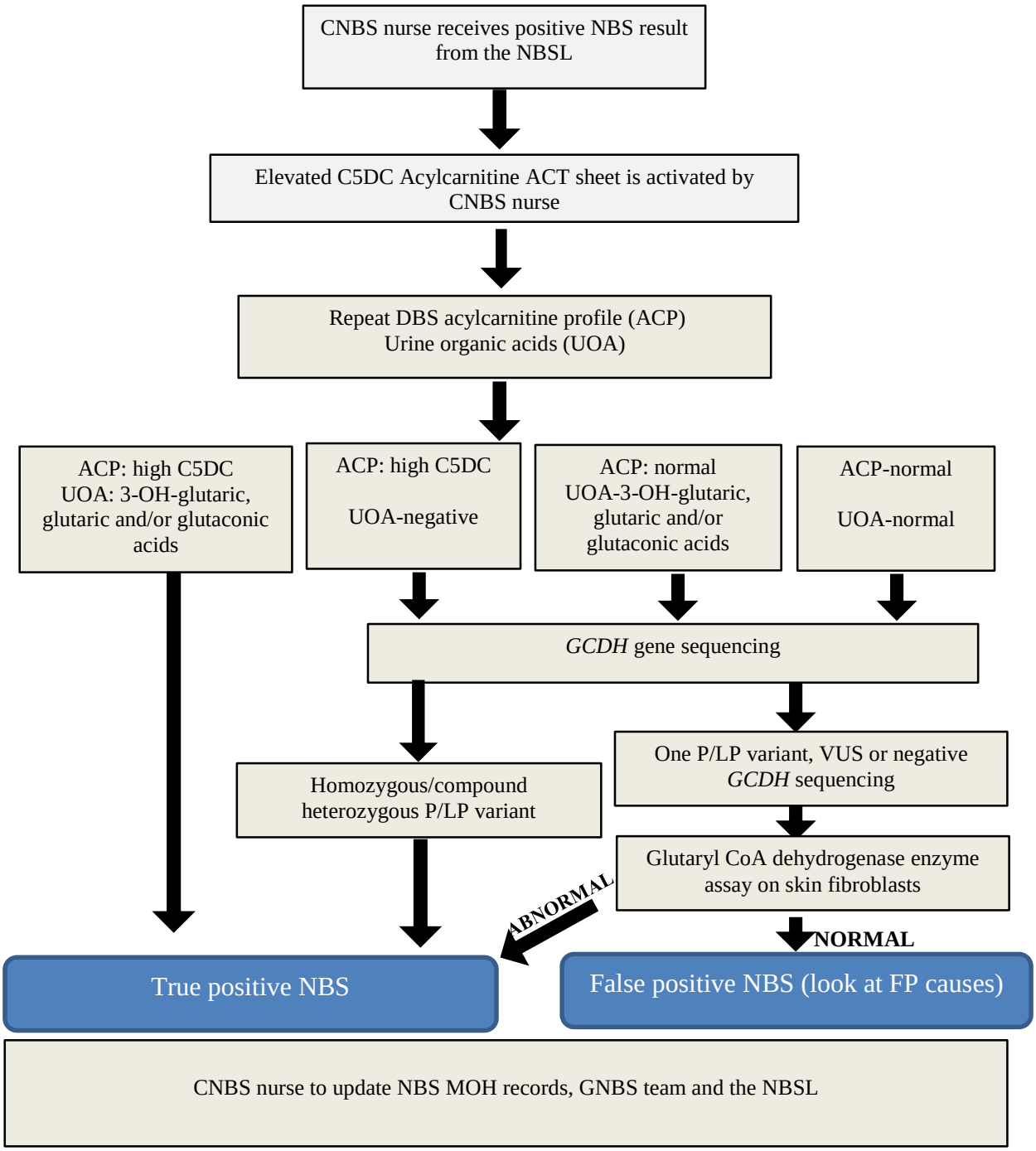
Diagnostic tests:

- Urine organic acid analysis: is diagnostic if it shows increased 3-hydroxyglutaric acid with or without increased glutaric and glutaconic acid.
- DNA banking for GCDH gene sequencing/targeted mutation analysis.
- Further testing including analyzing glutarylcarnitine in urine and 3- hydroxyglutaric acid in blood and CSF, and/or enzyme assay in fibroblasts is left to the decision of the responsible metabolic consultant.

Special considerations:

- The neonate with glutaric acidemia type I is usually macrocephalic but otherwise asymptomatic.
- Later signs include metabolic ketoacidosis, failure to thrive, and sudden onset of dystonia and athetosis due to irreversible striatal damage.
- With appropriate treatment, 60-70% of patients can be protected from neurodegenerative disease.

Algorithm 18: Category C - Increased C5DC acylcarnitine - An Organic Aciduria



CATEGORY C

4.3.3 Increased Phenylalanine

Aminoacidopathy

Differential Diagnosis:

- Phenylketonuria (Classical PKU)
- Non-PKU mild hyperphenylalaninemia
- Tetrahydrobiopterin (BH_4) deficiency
- Transient hyperphenylalaninemia.

Actions:

- Central NBS nurse contacts family to inform them of the NBS result and ascertain the clinical status through phone call:
    - poor feeding
    - lethargy
    - irritability
    - abnormal movements

- **Asymptomatic newborn:**
    - Educate family about the signs, symptoms that should alert them to bring the newborn to hospital
    - Instruct family to take the newborn next working day to the Central NBS clinic
    - **At the Central NBS clinic,** following is to be done:
    - Newborn is to be assessed for the following: lighter skin and hair color compared to parents, microcephaly, hypotonia, oculogyric crises, and dystonia.
    - Collect samples for confirmatory testing.
    - Refer to the metabolic center if symptomatic or if quantitative plasma amino acids necessitate BH4 loading test.
    - Provide the family with basic information about PKU and dietary management.
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following is to be done:
    - Newborn is to be assessed by a neonatologist/pediatrician to evaluate the newborn for the following: microcephaly, hypotonia, oculogyric crises, dystonia
    - Arrange to transfer the neonate to the metabolic center once stabilized.
    - Confirmatory testing will be collected at the metabolic center.
    - Report findings to the Central NBS nurse.

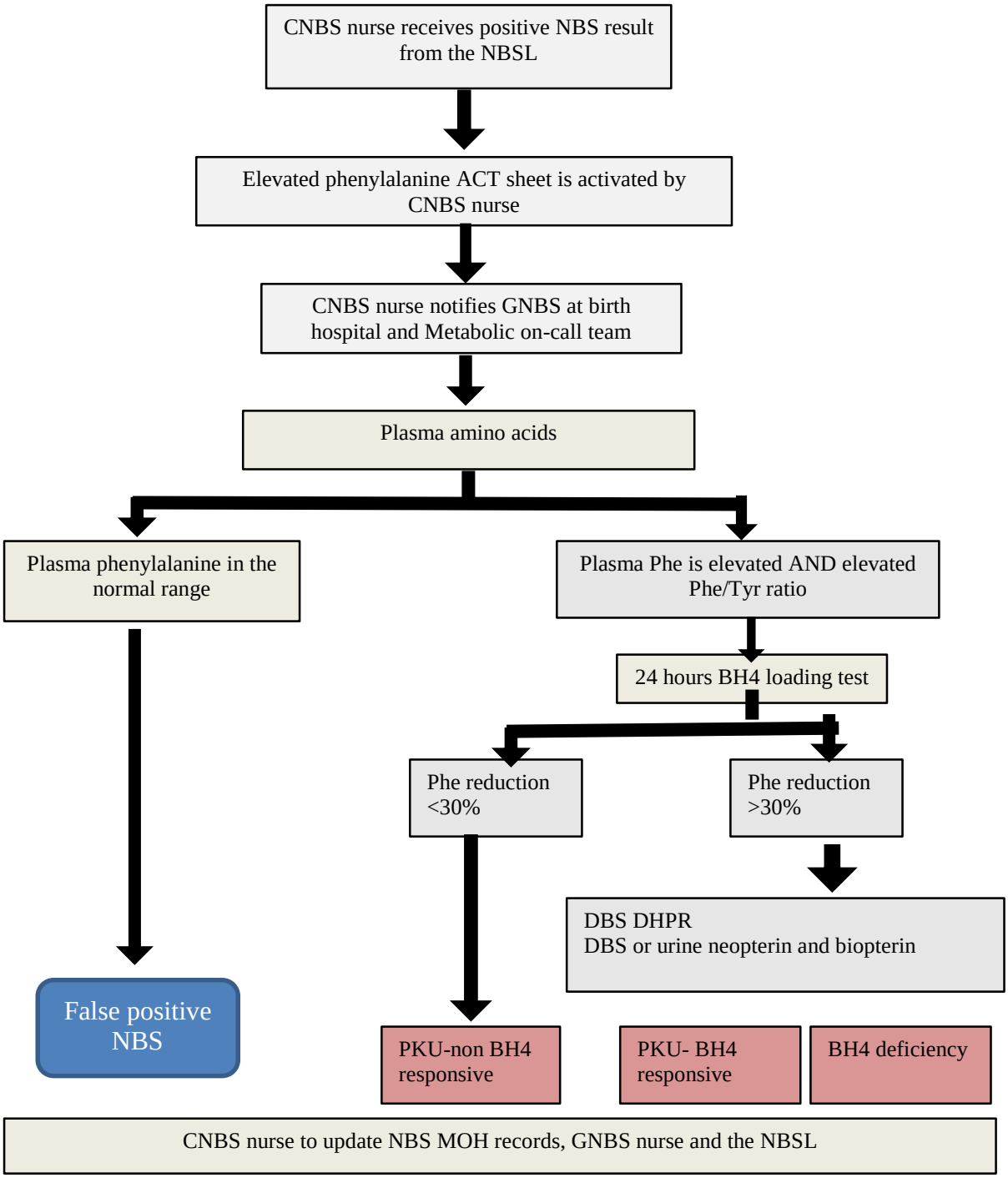
Diagnostic tests:

- Plasma amino acid analysis: increased phenylalanine without increased tyrosine (increased phenylalanine:tyrosine ratio).
- BH4 loading test
- Urine pterin analysis and red blood cell DHPR assay
- Consider PAH gene sequencing/targeted variant analysis.

Disease considerations:

- Classical PKU disease is asymptomatic in the neonate. Untreated, PKU will cause irreversible developmental delay, hyperactivity, autistic-like features, and seizures. Initiation of treatment in the neonatal period leads to normal/near normal outcome.
- BH4 deficiency syndromes can cause early severe neurologic disease (developmental delay/seizures/extrapyramidal movements), early lethality and requires specific therapy including neurotransmitter replacement.

Algorithm 19: Category C: Increased Phenylalanine Aminocidopathy



CATEGORY C

4.3.4 Increased Succinylacetone

Tyrosinemia Type 1 (Hepatorenal)

Differential Diagnosis:

- Tyrosinemia type 1 (hepatorenal)

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result

- Ascertain clinical status through phone call:
    - poor feeding
    - lethargy
    - vomiting
    - tachypnea
    - jaundice
    - irritability

- **Asymptomatic newborn:**
    - Educate family about signs, symptoms that should alert family to bring the infant to hospital
    - Instruct family to take the newborn next working day to the Central NBS clinic
    - **At the Central NBS clinic,** following is to be done:
    - Assess the newborn for poor weight gain, jaundice, hepatomegaly, edema and ascites.
    - Collect samples for confirmatory testing.
    - Provide the family with basic information about Tyrosinemia and dietary management.
    - Central NBS nurse to follow up results of confirmatory testing.

- **Symptomatic newborn:**
    - Instruct family to take the newborn **IMMEDIATELY** to the closest Governorate hospital
    - **At the Governorate hospital,** following is to be done:

- Newborn is to be assessed by a neonatologist/pediatrician for poor weight gain, jaundice, hepatomegaly, edema, ascites and evidence of bleeding tendency.
- Collect related investigations: urea and electrolytes, liver function test, CBC, coagulation screen and other investigations as necessary.
- Contact the metabolic oncall and take directions about management.
- Transfer the newborn to the metabolic center once stabilized.
- **At the metabolic center,** following is to be done:
- Initiate and continue appropriate further management of the patient.
- Collect samples for confirmatory testing.
- Provide family with basic information about Tyrosinemia deficiency
- Report findings to the Central NBS nurse.

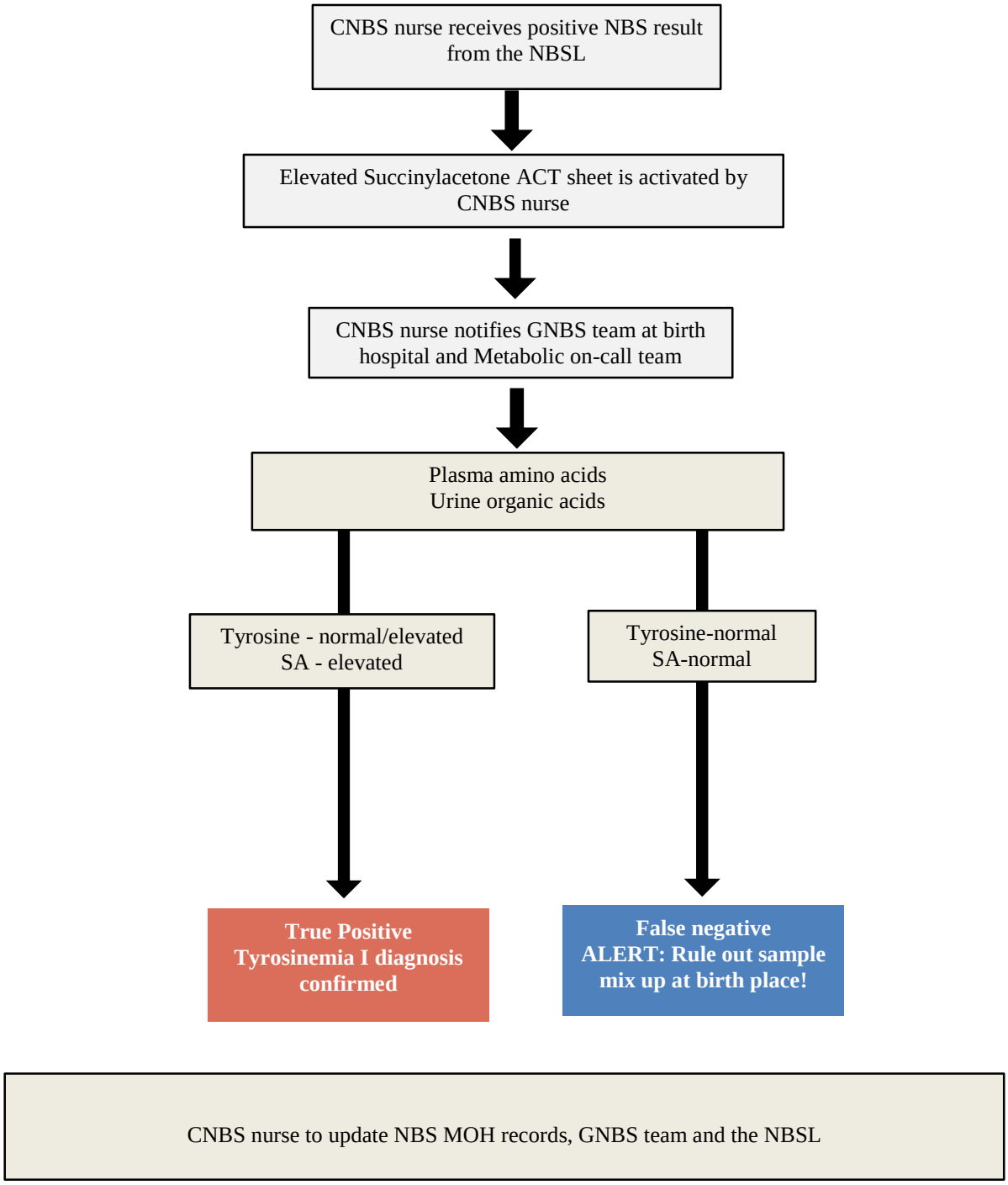
Diagnostic tests:

- Plasma amino acid analysis: increased tyrosine in all of the tyrosinemias.
- Urine organic acid analysis: increased succinylacetone in tyrosinemia I.

Clinical considerations:

- Tyrosinemia I is usually asymptomatic in the neonate. If untreated, patient will progress to liver disease and cirrhosis early in infancy. Nitisinone (NTBC) treatment is highly effective in prevention of disease short term and long-term complications.
- Tyrosinemia II is asymptomatic in the neonate but will cause hyperkeratosis of the skin, corneal ulcers, and in some cases, developmental delay unless treated with a tyrosine restricted diet.
- Tyrosinemia III is probably a benign condition where the majority of individuals remain asymptomatic.

Algorithm 20: Category C: Elevated Succinylacetone (SA) Tyrosinemia



CATEGORY C

4.3.5 Increased Methionine

Homocystinuria (CBS Deficiency)

Differential Diagnosis:

- Classical homocystinuria (cystathionine ß-synthase (CBS) deficiency)
- Hypermethioninemia due to methionine adenosyltransferase I/III (MAT I/III) deficiency
- Glycine n-methyltransferase (GNMT) deficiency
- S-adenosylhomocysteine hydrolase deficiency
- Liver disease
- Parenteral nutrition.

Actions:

- Central NBS nurse contacts family to inform them of the newborn screening result.
- Instruct family to take the newborn next working day to the to the Central NBS clinic.
- **At the Central NBS Clinic,** following is to be done:
  - Evaluate the newborn with attention to liver disease
  - Collect samples for confirmatory testing.
  - Provide family with basic information about classical homocystinuria (other differential diagnoses if indicated).
  - Central NBS nurse to follow up results of confirmatory testing.

Diagnostic tests:

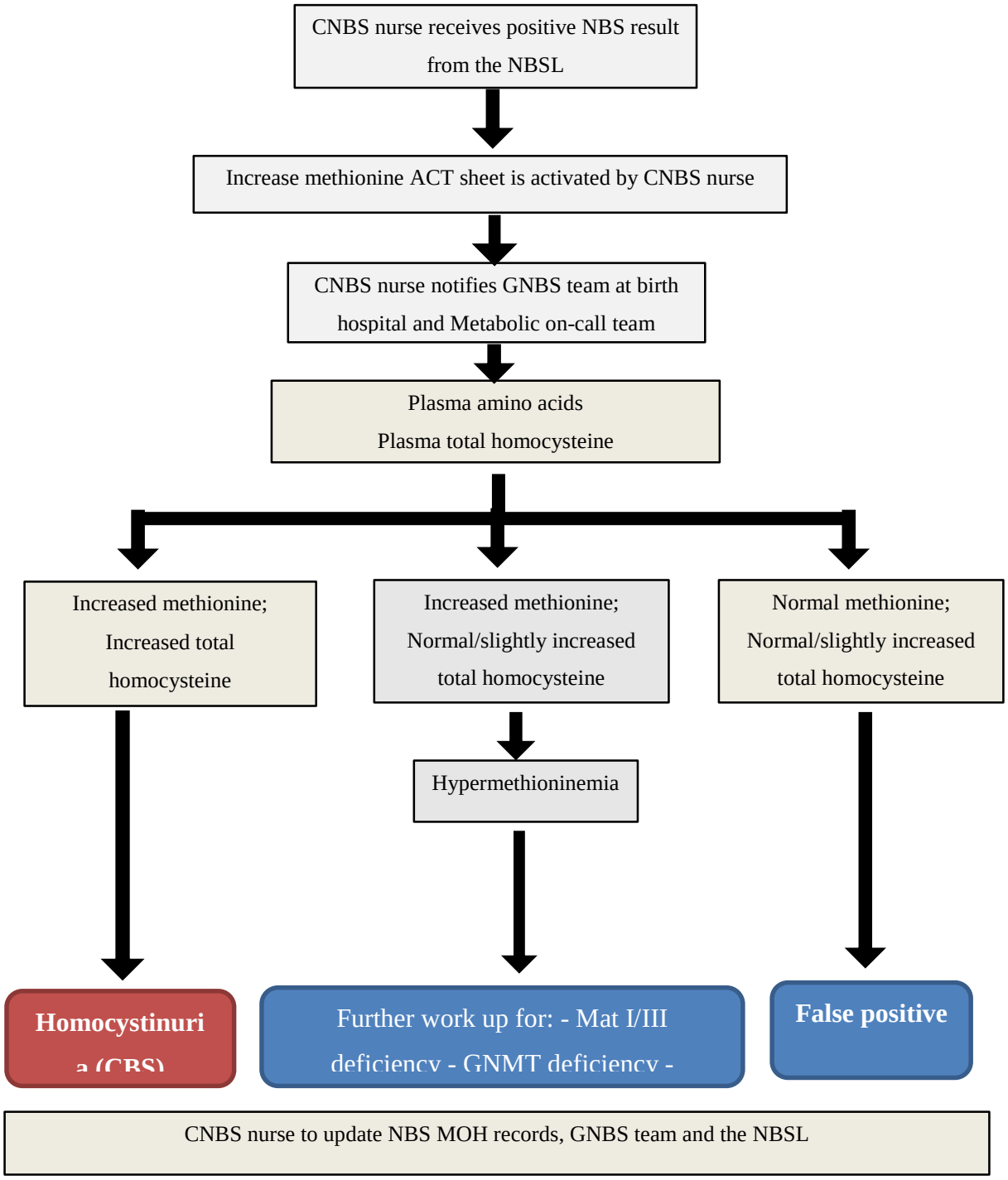
- Quantitative plasma amino acids: increased methionine.
- Plasma total homocysteine: markedly increased.

Clinical considerations:

Neonates with classic homocystinuria are usually asymptomatic. Treatment includes a protein restricted diet and vitamin supplementation. If untreated, later features may include ectopia lentis, developmental delay, abnormalities in long bone formation and an increased risk of thrombosis. MAT I/III deficiency may be benign. Adenosylhomocysteine hydrolase deficiency is a very rare condition associated with developmental delay and hypotonia, and both this disorder and GNMT deficiency can cause liver abnormalities.

The screening program detects high blood levels of methionine. Using methionine as a primary analyte is associated with low sensitivity as methionine is low in most baby formulas, particularly in breast milk. The screening program may also miss a milder form of this condition. this condition. Consequently, all infants who present clinically in later life with signs and symptoms suggestive of homocystinuria, such as dislocation of lenses, osteoporosis and inappropriate tall stature should have the disorder excluded formally by measuring plasma levels of total homocysteine.

Algorithm 21: Category C: Elevated Methionine Homocystinuria (CBS Deficiency)



G. Responsibilities

1. National NBS team

- Knowledge dissemination and awareness
- Building capacities of health care providers
- Allocation and Organization of the Central Newborn Screening Lab
- Equipment acquisition

2. Central NBS Team

2.1 Director of the NBS lab:

- Organization, overall supervision, documentation, quality assurance (QA) and administrative representation of the lab.
- Supervises, revises and authorizes newborn screening testing procedures and protocols.
- Participates in planning, organizing, and implementing laboratory programs and changes.
- Overseas communication between the central NBS lab and birth hospitals, the laboratory personnel, and the follow-up teams.
- Manages the newborn screening laboratory information system
- Coordinates lab integration and implementation of administrative and technical changes, including the implementation of new testing technologies and screening for additional conditions.
- Handles special assignments and projects as directed.
- Conducts annual surveillance to ensure that laboratory standards are met, and ensures attainment and maintenance of laboratory accreditation.
- Facilitates ongoing education and development of the staff.
- Directs and assists in evaluation of new test procedures.
- Attends and participates in professional and scientific workshops, seminars and meetings in a way to foster continuing professional development.
- Shares information with administration and management team on professional and scientific activities.
- Shares information with Lab Managers and staff regarding professional and scientific activities.

2.2 An administrative coordinator

- To carry out administrative tasks related to the NBS as detailed in the job description
- To timely direct communications to the director, chief (senior) BMS or clinical nurse specialists as appropriate.

2.3 Clinical nurse specialists

- Receive immediate notification of positive NBS results
- Timely communicate with the regional NBS team with respect to the positive results and revise sequential steps to be implemented according to the disease action plan.
- Ensure the disease action plan is timely implemented following the first communication with the regional NBS team
- Follow and document the outcome of the disease action plan implemented via the regional NBS team
- Communicate with the regional NBS for repeat DBS sample collection where indicated
- Arrangement appointments for outpatient NBS clinic visits for screen positive patients according to the disease action plan
- Immediately communicate with the metabolic (clinical biochemical genetics) on-call with regard to any symptomatic patient, patients with red flags on initial clinical assessment or patients in need of immediate transfer to specialized care according to the disease action plan
- Ensure all communications and final outcomes of screen positive patients are documented.
- Receiving patients from category B in the clinic
- Primary assessment, and anthropometric measurements
- Filling disease specific symptom check list

- Counseling parents and providing disease-specific instructions
- Collecting blood and/or urine for confirmatory testing
- Sending all samples to confirmatory lab and filling all related paper/online documents
- Tracking the results of confirmatory testing from central confirmatory lab or regional hospitals labs for congenital hypothyroidism
- Updating the final status of the patient in the MOH EPR and the NBS lab
- Recording and providing statistics about different aspects related to NBS

2.4 A chief (senior) biomedical scientist (BMS)

- Supervise the overall technical performance of the NBS
- Facilitate the technical tasks dedicated to BMS
- Organize the rotation, allocation and technical task distribution among all BMS
- Perform immediate troubleshooting measures where indicated
- Implement Quality Assurance measures adopted in the NBS lab in liaison with the director of the NBS
- Aid the director of the NBS lab in all tasks related to the adopted internal standard laboratory accreditation procedures
- Handle/ supervise tasks related to external proficiency testing cycles
- Support the director in ensuring appropriate documentation related to the lab
- Regularly revise and update standard operating procedures (SOPs) in liaison with the lab director

2.5) Biomedical Scientist (BMS)

- Perform technical duties assigned by the chief (senior) BMS
- Adhere to the laboratory SOP
- Adhere to the rotation and distribution of tasks as instructed by the senior BMS
- Comply with the duty and schedule distribution

2.6 Metabolic Dietitian Dieticians

- Respond to and assess the acute dietary interventions required for in-patient newborn screening (NBS) positive cases who are admitted for management and confirmation of diagnosis.
- Provide the basic dietary recommendations and counseling for patients attending the central NBS clinic where dietary management is initiated before confirmation of diagnosis.
- Provide nutrition therapy for all patients with a confirmed diagnosis of an inborn errors of metabolism that requires dietary intervention.
- Assess, review and monitor the laboratory data, food records, and growth patterns for determining dietary recommendations.
- Interpret laboratory and dietary parameters in order to calculate prescriptions for special diets and medical foods.

- Maintain records of patients' dietary prescriptions, analyses, growth and nutritional status, and education/counseling sessions.
- Participate as an interdisciplinary team member of the Metabolic Team as required. Provide patient education and counseling on both an inpatient and outpatient service.
- Teaching nutrition student/Medical student about diet therapy of genetic metabolic disorders.
- Development /update of teaching Materials and educational resources
- Provide on-call cover for weekends and public holidays.
- Continuously liaise with the metabolic medical team on the dietary management of unwell patients with inherited metabolic disorders at home or in outlying hospitals.

2.7 Clinical Pharmacists

- Dispensing medications as prescribed by doctors and other medical professionals
- Reviewing prescriptions to ensure accuracy and to evaluate their suitability for the patient.
- Providing information and advice about drugs, their side effects, correct dosage and proper storage
- Keeping records such as pharmacy files, patient profiles, charge system files, inventories, and registries of poisons, narcotics or controlled drugs
- Planning, implementing and maintaining procedures for mixing, packaging and labelling pharmaceuticals to ensure they meet legal requirements
- Assessing the identity, strength or purity of medications
- Working with other healthcare professionals to plan, monitor, review, or evaluate the quality or effectiveness of drugs
- Ordering and purchasing pharmaceutical supplies, medical supplies, or drugs.
- Maintaining stock and storing and handling it properly
- Analysing prescribing trends to monitor patient compliance and to prevent excessive usage or harmful interactions
- Advising patients on medication brands, medical equipment or healthcare supplies.
- Preparing statistics as requested from the national/central teams.
- Attend training courses as directed by the head of the team.

3. Hospital NBS team

It is recommended that for every delivery hospital or maternity center to have a core team composed of:

- Neonatologist (Or senior pediatrician if not available)
- EPI Nurse
- Maternity ward nurse
- SCBU nurse
- Laboratory technician

The responsibilities of each Regional NBS site team include the following:

- Ensuring completion of newborn records and contact information
- Liaison with the medical supply unit at the hospital to ensure sustained availability of valid DBS cards for the purpose of NBS
- Collection of dried blood spots samples using the supplied DBS cards, and organization for timely recollection of samples as instructed by the central NBS lab.
- Preparation and packaging of samples for transport and ensuring specimens transport within the allowed time-frame.
- Receiving calls from the National NBS in relation to screen positive cases
- Initiation and implementation of the disease-specific action plan as instructed.
- Contacting families with positive results and arranging to call the patient back for clinical assessment and confirmatory testing according to each disorder-specific action plan.

Regional Hospital laboratory:

- The biochemical laboratory personal shall:
- Receive the collected dry blood spots cards.
- Insure that dry spot cards are appropriate filled and labelled
- Pile the cards and arrange for shipment to NBS program laboratory on daily basis (except Friday) with the goal that samples reach the NBS program lab within 24 hours of collection.

4. Department of Women & Child health in Ministry of Health:

- Review and update the SOP based on available new evidence and according to best practices of an expert group in the country.
- Disseminate the SOP to all Women and Child health sections in governorates
- Plan and organize training workshops to train doctors and nurses on how to use the SOP.
- Monitor and evaluate service provision in all health institutions

5. Sections of Women & Child health in the Governorates

- Disseminate the SOP to all health care institutions in the governorate.
- Participate in training of the concerned health care workers on how to use the guideline.
- Follow up the program functioning at the level of the Governorate.
- Cooperate with the WCH department in solving any obstacles in the program.
- Prepare the required statistics requested at the national level.

6. Directorate General of Medical Supplies

- Maintain continuous supply of the recommended medications in all health institutions

H. Document History and Version Control

Document History and Version Control			
01	Initial Release – 1st Edition	Department of Woman and Child Healt	2025
Written by	Reviewed by	Approved by	
Taskforce for Initiating the Pilot phase of the Newborn Screening Programme	Taskforce for Initiating the Pilot phase of the Newborn Screening Programme	Dr. Badriya Al Rashdi Director General for Health Services and Programs	

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J. Annexes:

Annex 1: Consent Form for the NBS Program

Sultanate of Oman
Ministry of Health
Directorate General for Health Services and Programs
Department of Woman and Child Health

NEWBORN SCREENING CONSENT FORM

The Ministry of Health at Sultanate of Oman now mandates expanded newborn screening for all newborns delivered in Oman. This involves taking a few drops of blood collected in a small paper card and used to test for a number of conditions that can be treated early so that serious complications can be prevented.

Child's Name _____
Date of Birth _____ Name of Delivery Hospital: _____
Parent/Legal Guardian _____
My child's medical provider,_____ , has advised me that my child (named above) should participate in the newborn screening program.

As the parent or legal guardian of my child (named above), I choose to:
❑ decline participation in Oman's expanded newborn screening program mandated by the Ministry of Health for all newborns in Oman.
❑ agree to participate in Oman's expanded newborn screening program mandated by the Ministry of Health for all newborns in Oman.

I have been provided information about newborn screening in Oman and the importance of early identification of the disorders. I had the opportunity to discuss these with my child's care provider, who has answered my questions regarding the recommended screening.

I understand the following:

- The purpose and need for newborn screening to include bloodspot screening, and hearing screening.
- If my child does not participate in newborn screening, the consequences of a late diagnosis may include delayed development, intellectual disability, or death.
- The Ministry of Health in Oman strongly recommends that all newborns be screened for certain disorders.
- If my child has one of the screened conditions, failure to participate in newborn screening may endanger the health or life of my child. I had the opportunity to discuss my decision with the care provider.
- If a screening test is positive, this does not necessarily mean the baby is affected, and further testing will be required for confirmation. The cost of these confirmatory investigations is not covered under the original cost of NBS.
- Once confirmed, treatment of some of the conditions included is relatively cheap. However, treatment of others may include medical formula and life-long medications that can be expensive and may not be affordable for low to average income families.

I acknowledge that I have read this document or it has been read to me in its entirety, and I fully understand it.
Parent/Legal Guardian Signature _____Date _____
Witness: _____

Annex 2: Disorders

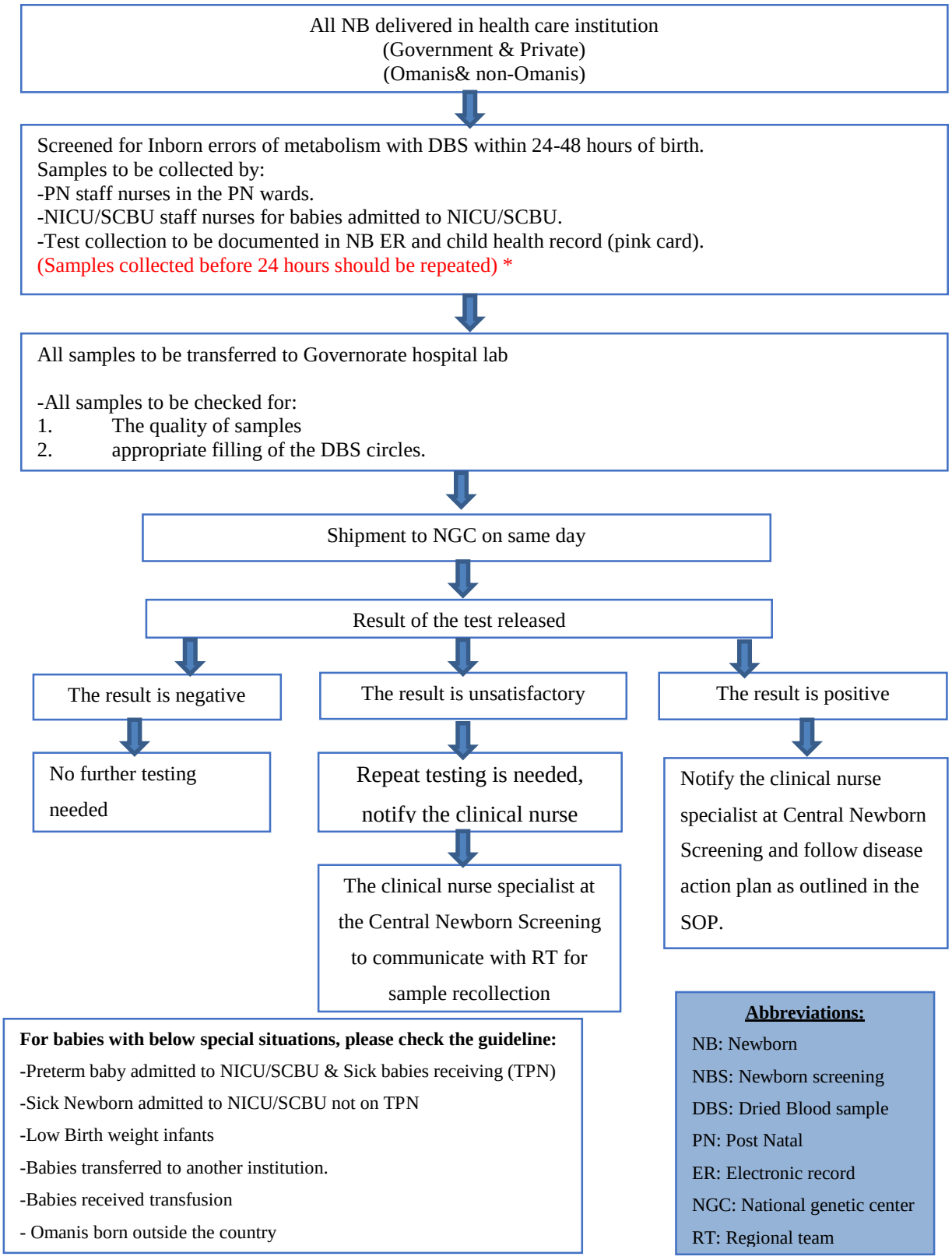
Disorders included in the expanded newborn screening program in Oman and the proposed primary and secondary analytes/ biomarkers:

Disorders intended for Screening	**Primary analyte/biomarker**	**Secondary analyte/ratio**
Phenylketonuria (PKU)	Phenylalanine	Phe/Tyr
Maple syrup urine disease (MSUD)	xLeusine (Leucine + Isoleucine)	
Tyrosinemisa Type I	Succinylacetone	
	Tyrosine	
Homocystinuria	Methionine	
Citrulinemia type I	Citrulline	
Argininosuccinic acidemia (ASA)	Citrulline	
	ASA	
Propionic acidemia (PA)	C3	C3/C2
Methylmalonic acidemia (MMA)	C3	C3/C2
Isovaleric acidemia (IVA)	C5	
Glutaric aciduria type I (GA-I)	C5DC	
3-Hydroxy-3-methylglutaryl CoA lyase Defeciency (HMG)	C5OH	
	C6-DC	
Beta-Ketothiolase deficiency	C5OH	
	C5:1	
Carnitine transporter deficincy	C0	
Carnitine palmitoyl transferase I (CPT-I) deficiency	C0/C16+C18	
Carnitine palmitoyl transferase II (CPT-II) deficiency	C16	
	C18:1	
Carnitine-acylcarnitine translocase deficiency	C16	
	C18:1	
Medium-chain acyl CoA dehydrogenase deficiemcy (MCAD)	C8	
Long-chain 3-hydroxyacyl CoA dehydrogenase deficiency (LCHAD)/ TFP	C16-OH	
	C18:1-OH	
Very long-chain acyl CoA dehydrogenase deficiency (VLCAD)	C14:1	

Disorders intended for Screening	**Primary analyte/biomarker**	**Secondary analyte/ratio**
Congenital hypothyroidism	TSH	
Biotinidase deficiency	Biotinidase	
Congenital adrenal hyperplasia	17-OHP	
Galactosemia	GALT	GAL-1-P

Annex 3: An Overview of the Newborn Screening Program:

Algorithm of NBS Program



Annex 4: Medication

The emergency medications and special metabolic medical formula that MUST be available in all regional hospitals as follow:

Medications:

- L-Carnitine injection
- Sodium Benzoate injection
- Sodium Phenylbutyrate injection
- L-Arginine injection
- Carglumic acid powder/capsules
- Biotin
- Hydroxocobalamin injection

Formula:

- MSUD formula e.g MSUD Anamix
- MCT based medical formula e.g Monogen
- Glucose polymer powder e.g. Maxijul
- Galactose/lactose free formula e.g. Similac Isomil

Medications and special metabolic medical formula that MUST be available in metabolic centers (Royal Hospital and SQUH):

Medications:

- L-Carnitine injection
- L-Carnitine oral solution
- L-Carnitine 500mg capsules
- Sodium Benzoate injection
- Sodium Phenylbutyrate injection
- Sodium Benzoate solution/capsules
- Sodium Phenylbutyrate solution/capsules
- L-Arginine injection
- L-Citrulline powder and/or capsules

- Carglumic acid powder/capsules
- Carbidopa/Levodopa 25/100mg tablets
- L-5-hydroxy-tryptophan 50mg capsules/tablets
- Sapropterin dihydrochloride powder/capsules
- Nitisinone solution/tablets
- Biotin
- Hydroxocobalamin injection
- Coenzyme Q 10 (Ubiquinone or preferably Ubiquinol) oral solution and capsules

Formula:

- Phenylketonuria e.g. PKU Anamix
- Tyrosinemia e.g. TYR Anamix
- Homocystinuria e.g. HCU Anamix
- Urea Cycle Disorders e.g. UCD Anamix
- MSUD e.g. MSUD Anamix
- Propionic acidemia/Methylmalonic acidemia e.g. PA Anamix
- Isovaleric acidemia and HMG CoA Lyase deficiency e.g. IVA Anamix
- Glutaric Aciduria e.g. GA Anamix
- Galactose/lactose free formula e.g. Similac Isomil
- MCT based medical formula e.g. Monogen
- Glucose polymer powder e.g. Maxijul
- Essential Amino Acid Mix