Joshua Hellmann Foundation Newborn Metabolic Screening Program

Clinical Protocol

The CUHK – BCM Joint Centre for Medical Genetics

The Chinese University of Hong Kong

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Introduction

The Joshua Hellmann Foundation - Newborn Metabolic Screening Program is a newborn screening program which aims to offer an expanded screening test for newborns in Hong Kong. The programme started in 2013, initially targeting at a panel of inborn errors of metabolism (IEM) disorders.

In February of the following year, screening for cystic fibrosis (CF) was added to the programme and was recommended to infants whose parents are both Caucasians. Samples were sent to an overseas laboratory for analysis. The CF screen result was reported separately and the turnaround time was longer (please refer to the section "Screening for cystic fibrosis" for details). Later, the screening panel was further expanded to include congenital adrenal hyperplasia (CAH) in February 2016.

In September 2021, screening for X-linked adrenoleukodystrophy (X-ALD) has been added to the IEM panel, therefore the panel is screening for a total of 31 IEM disorders. The aim is to identify affected infants at the earliest instance, often before they develop any signs or symptoms of the disease and treat them as early as possible so as to achieve a better treatment outcome.

In addition, screening for spinal muscular atrophy (SMA) and severe combined immunodeficiency (SCID) have also been included in the program in September 2021 to identify the affected patients soon after birth and offer treatment earlier to achieve a better outcome in infants with SMA and SCID. For more details, please refer to the section "Screening for spinal muscular atrophy" and "Screening for severe combined immunodeficiency".

The current screening program is complementary to the conventional cord blood screening for congenital hypothyroidism and glucose-6-phosphate dehydrogenase (G6PD) deficiency provided by the Department of Health in Hong Kong.

Referring doctors may choose either options:

- Option 1: IEM + CAH + SMA + SCID screening
- Option 2: IEM + CAH + SMA + SCID + Cystic Fibrosis screening

Workflow of the screening program is shown in Appendix 1.

Newborn Screening for Inborn Errors of Metabolism (IEM)

Background

Inborn Errors of Metabolism (IEM) is a large group of genetic disorders with a collective incidence of 1 in 4000. Infants affected by IEM may appear normal at birth. Some disorders may present with acute metabolic decompensation or organ failure, while some may present with developmental delay or neurological deficit. If not identified and treated early, these IEMs may result in permanent neurological damages or even mortality.

Screening method

We use tandem mass spectrometry (MS/MS) to measure a number of amino acids, free carnitine and acylcarnitines on the dried blood spot cards. Quantities of these analytes and their relationship with one another are used to screen for 31 IEM of amino acids, organic acids and fatty acid oxidation metabolisms. A list of each of the IEMs targeted by the screening test is set out in Appendix 2. The newly added item is Adrenoleukodystrophy (ALD), it is included under the collective term of IEM, as it causes accumulation of very long chain fatty acids (VLCFAs) in the brain. Please refer the section "Screening for ALD" for detail.

Screening result interpretation and reporting

The IEM newborn screening test by MS/MS is a screening but not a diagnostic test. Clinicians must not diagnose or prescribe treatment based solely on the screening test results.

As with any laboratory test, both false positive and false negative results may occur. This means unaffected infants may be falsely identified by the screening test. Thus, it is extremely important that all abnormal screening results should be followed by proper diagnostic tests.

After analysis by MS/MS, three types of results are possible:

1. **Normal** – All metabolites measured are within the pre-defined cut-offs. Normal reports will be sent to the referring doctor.

2. **Positive** – Some of the metabolites measured deviate significantly from the pre-defined cut-offs and the pattern of abnormalities suggests an underlying IEM. Clinical assessment and follow-up diagnostic tests (Appendix 3) are necessary. The referring doctor will be informed of the abnormal results by phone in the earliest instance. Reports will be faxed and mailed to the referring doctor. The metabolic paediatrician at the Centre of Inborn Errors of Metabolism will also be informed. ***All positive screening results must be dealt***

with immediately and without delay.

3. **Uncertain** –Some of the metabolites measured fall slightly outside of the pre-defined cut-offs. Repeat analysis using a second DBS sample is needed. For some infants, additional blood and/or urine tests are also required. The referring doctor will be informed of the abnormal results by phone in the earliest instance. Reports will be faxed and mailed to the referring doctor. It is the responsibility of the referring doctor to contact the parents for a repeat DBS sample as soon as possible. It is estimated that around 6 in 1000 infants screened may require a repeat DBS sample. The majority of repeat DBS samples will have normal analysis results. Infants with persistent abnormal results will require clinical assessment and immediate follow-up diagnostic testing.

Newborn Screening for Congenital Adrenal Hyperplasia (CAH)

Background

Congenital adrenal hyperplasia (CAH) is a group of genetic disorders, in which the body cannot produce enough cortisol. Classic CAH describes patients with no or minimal residual enzyme activity presented at birth or soon after birth. There are two different forms of classic CAH – salt-wasting form and simple virilization form. The incidence of classic CAH is around 1 in 10,000 to 1 in 20,000. The most common (>90%) cause of CAH is 21-hydroxylase deficiency. In this form of CAH, 75% of affected patients cannot produce adequate cortisol and aldosterone. Low levels of these hormones may cause nausea and vomiting, tiredness, dehydration, and weight loss. In the most severe case, CAH can lead to "salt-losing crisis" with low blood pressure, shock or even death. Low levels of cortisol also stimulate the production of ACTH from the pituitary gland. This can lead to hyperpigmentation of the skin and overproduction of adrenal androgens. In female infants, exposure to excessive androgens in utero may result in abnormal genital development. In the remaining 25% of affected patients, production of aldosterone is adequate and affected infants are present with virilization at birth or precocious pubertal development in childhood.

Affected female infants are much more readily detected at birth than affected male infants because of abnormal genitalia. However, both genders are at the same risk of developing salt-losing crisis. The aim of newborn screening for CAH is to detect infants affected by salt-losing form of CAH early so that prompt treatment can prevent the development of salt-losing crisis. The milder forms of CAH is not the target of this screening test.

Screening method

The CAH screening test measures the level of 17-hydroxyprogesterone (17-OHP) on the dried blood spots by time-resolved fluoroimmunoassay. All newborns have high levels of 17-OHP at birth. In healthy infants, the levels of 17-OHP decrease with time but for infants affected by CAH 17-OHP, concentrations remain high. Premature and stressed infants (otherwise not affected CAH) have higher 17-OHP levels than full-term healthy infants.

Screening result interpretation and reporting

The CAH newborn screening test is a screening but not a diagnostic test. Clinicians must not diagnose or prescribe treatment based solely on the screening test results.

As with any laboratory test, both false positive and false negative results may occur. This means

unaffected infants may be falsely identified by the screening test. Thus, it is extremely important that all abnormal screening results should be followed by proper diagnostic tests.

Infants with 17-OHP results which fall outside the pre-defined cut-off are at risk of CAH and require further investigation. For most infants, a repeat analysis using a second DBS sample should be sufficient. Further investigations (e.g. electrolytes) may be required depending on the actual 17-OHP results and the clinical condition of each infant. The referring doctor will be informed of the abnormal results by phone as soon as possible. Reports will be faxed and mailed to the referring doctor. It is the responsibility of the referring doctor to contact the parents for a repeat DBS sample as early as possible.

The false positive rate of CAH screening is around 0.5% in general but is higher in premature and stressed infants. The majority of repeat DBS will show normal 17-OHP results. Infants with persistent elevated 17-OHP levels will require clinical assessment and immediate follow-up diagnostic testing.

Newborn Screening for X-linked adrenoleukodystrophy (X-ALD)

Background

X-linked adrenoleukodystrophy (X-ALD), is the most common peroxisomal disorder, caused by mutations in the ABCD1 gene located on the X chromosome, with an estimated incidence of ~1 in 21,000 males in the United States. ABCD1 gene codes for the very long chain of fatty acids (VLCFA) transporter in peroxisomes which results in the accumulation of VLCFA in tissues throughout the body. The most severely affected tissues are the myelin in the central nervous system, the adrenal cortex, and the Leydig cells in the testes. X-ALD patients present with several distinct phenotypes but genotype-phenotype correlation is not clear. About two-thirds of X-ALD patients will present with the most severe childhood cerebral form of the disease. It is characterized by normal development in early childhood, followed by rapid degeneration to a vegetative state.

X-ALD patients can only be diagnosed early because of a known family history or because of etiologic work-up of clinical primary adrenal insufficiency with brain MRI for cerebral disease. As white matter abnormalities on brain imaging can be presented before neurological manifestations, it provides an intervention window for individuals with early, clinically pre-symptomatic, cerebral disease. Hence, newborn screening for X-ALD is recommended and has been implemented in many newborn screening program worldwide.

Screening method

We use tandem mass spectrometry (MS/MS) to measure C26:0 lysophosphatidylcholine (C26:0-LPC) in dried blood spots. C26:0-LPC level is increased in patients with X-ALD. Patients with uncertain and abnormal results will have follow up confirmation tests to confirm the diagnosis at our center.

Newborn Screening for Severe Combined Immunodeficiency (SCID)

Background

Severe combined immunodeficiency (SCID) is a group of rare genetic disorders caused by a profound impairment in T-cell development and function without specific antibody production. SCID patients present with recurrent infections including opportunistic bacterial, viral and fungal infections, chronic diarrhoea and failure to thrive. The incidence of SCID is around 1 in 45,000 to 1 in 65,000. Infants with SCID have poor prognosis due to severe recurrent infections. The affected infant is well at birth and usually presents after the first month of life, while the protection from maternal IgG level is dropped.

Infants with typical SCID have fewer than 300 autologous T cells/uL. Patients with low levels of T cells are susceptible to infection and other complications leading to failure to thrive, pneumonia and other life-threatening infections. X-linked SCID is caused by mutations in the IL2RG gene on the X chromosome, and generally only occur in boys. All other types of SCID are autosomal recessive. Affected newborns generally appear healthy and less readily detected at birth. The aim of newborn screening for SCID is to idenitfy infants with SCID to avoid infections early so that prompt treatment can prevent the development of complications.

X-linked agammaglobulinemia (XLA) is one type of Primary immunodeficiency disorder (PID) which is caused by a mutation or deletion in the BTK gene. The gene defect prevents the normal development of B lymphocytes and that results in a severe antibody deficiency. Similar to SCID, XLA patients' blood have decreased copy number of Kappa deleting recombination excision circles (KRECs), which are circular DNA fragments produced during the maturation of B lymphocytes and this can be used as a marker for screening of the disease.

Screening method

T cell receptor excision circles (TREC) are present when T cells are produced. Since newborn infants with SCID have few or no T cells, they have low levels of TRECs in blood. We use multiplex real time PCR based assay to measure the copy numbers of TREC, KREC in the dried blood spot. This assay allows the screening of infants with severe forms of PID manifested by T and B cell lymphopenia .

Screening result interpretation and reporting

The SCID newborn screening test is a screening but not a diagnostic test. Clinicians must not diagnose or prescribe treatment based solely on the screening test results.

As with any laboratory test, both false positive and false negative results may occur. This means unaffected infants may be falsely identified by the screening test. Thus, it is extremely important that all abnormal screening results be followed by proper diagnostic tests.

Infants with TREC results fall outside the pre-defined cut-off are at risk of SCID and require further investigation. Low levels of TREC may also result in infants with prematurity, other less severe immune disorders or other syndromes.

For most infants with false positive screening result, a repeat analysis using a second DBS sample should be sufficient. More investigations (e.g. complete blood count, lymphocyte subset) may be required depending on the actual TREC results and the clinical condition of the infants. The referring doctor will be informed of the abnormal results by phone as soon as possible. Reports will be faxed and mailed to the referring doctor. The false positive rate of SCID screening is around 0.5% in general but is higher in premature and stressed infants. The majority of repeat DBS will show normal TREC results. Infants with persistent low TREC levels will require clinical assessment and immediate follow-up diagnostic testing.

Screening for Spinal Muscular Atrophy (SMA)

Spinal Muscular Atrophy (SMA) is a group of hereditary diseases that progressively destroys motor neurons and leads to muscle wasting. SMA is one of the most common lethal recessive genetic disorders and has an incidence of approximately 1/10,000 live births with a carrier frequency of approximately 1 in 57. SMA patients commonly present with motor disability, respiratory and nutritional compromise. The mortality rate of SMA is more than 50% in affected infants or children. SMA is caused by mutations in both copies of the survival motor neuron 1 gene (SMN1) on chromosome 5q. It is classified into four types based on the age of onset, symptoms, and rate of progression. In type I, patients present at birth or before six months of age with significant loss of motor neuron within the first six months of life. They typically have the lowest level of functioning SMN protein and have significant motor neuron loss within the first six months of life. Majority of type I SMA cases are due to homozygous deletion in exon 7 of SMN1 gene. In types 2 and 3, patients usually have a later onset in childhood or teenage. The neighboring SMN2 genes can in part compensate for non-functional SMN1 genes and hence high SMN2 copy numbers often decrease the severity of the phenotype. For patient present in early adulthood, they typically have higher levels of SMN2 copy, and they are classified as type 4. In this screening test, only SMN1 copy will be screened, patient with homozygous deletion of exon 7 of SMN1 gene will be called back follow up investigations including blood test for SMN2 copy numbers.

Screening method

We use multiplex real time PCR based assay to measure the copy numbers of SMN1 in dried blood spot. The assay amplifies the exon 7 of the SMN1/2 genes in a PCR reaction. This screening test identify the absence of exon 7 in the SMN1 gene, which is present in approximately 96% of patients with SMA.

Target Infants

Dried Blood Spots Collection for Well Infants

All healthy newborns born at or after 34 weeks of gestation[1] who have completed oral feeding for 24 hours are suitable for the screening test. Dried blood spots (DBS) should be collected after completion of oral feeding within 24 hours to 7 days after birth. In general, the best time for screening for the majority of IEM and CAH is between 24 and 72 hours of life. This means that the sample collection is best done in hospital before the infants are discharged.

Dried Blood Spot Collection for Premature (<34 weeks of gestation), Low Birth Weight and Sick Newborns

For newborns who fit into one of the following criteria, repeated dried blood spots collection at different time points are required for a better analysis as a newborn screening test for inborn errors of metabolism.

- Premature (< 34 weeks of gestation)
- Low birth weight of < 2,000 g
- Sick newborns who require admission to Neonatal Intensive Care Units
- Newborns who have been kept nil by oral, or have received parenteral nutrition, blood transfusion

Schedule of blood sample collection:

- First blood sample: blood should be drawn at 24 to 72 hours of life at the unit, preferably before transfusion of red cell or whole blood.
- For infants that require immediate whole blood or red cell transfusion soon after admission to NICU, an additional blood sample should be drawn before transfusion of such products is instituted. (Note: Transfusion of platelet and/or plasma does not warrant an additional

[1] During Phase I (15 July 2013 – 31 August 2013), we accepted DBS from healthy term newborns (>/=37 weeks of gestation). Phase II was started from 1 September 2013, we had extended our service to include slightly preterm infant with body weight >2kg.

blood sampling.)

- Second blood sample: blood should be drawn at day 28 of life or before discharge, whichever comes first.

Recommendation:

We recommend proceeding directly to diagnostic testing for the following cases:

- If there is clinical suspicion for IEM or metabolic derangements such as recurrent hypoglycaemia, hyperammonaemia, or ketonuria;
- If there is clinical suspicion for CAH;
- Infants with a family history of IEM (e.g. affected older siblings) or CAH.

Referral sites:

1. Hospital-based Obstetrics or Paediatrics ward or clinic

   To ensure the accuracy in timing of blood sampling and adequate follow-up of results, we accept samples from all obstetric or paediatric departments in local Hong Kong hospitals.

2. Private clinic

   We also accept samples from private obstetric or paediatric clinics with their own logistic arrangements. Please contact our laboratory at 6806 4590 for further details.

Collection Card

A special filter paper (referred to as "collection card") is used to collect a few drops of blood obtained by pricking the heel of the infant. This kind of sample is commonly referred to as Dried Blood Spot (DBS).

Collection cards can be obtained from the Prenatal Genetics Diagnosis Laboratories at Prince of Wales Hospital. (Address: 4/F, Block K, DTB, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong)

Unused collection cards should be stored properly. Do not place heavy objects on top of unused collection cards as this will cause compression on the cards.

Request Form

(Appendices 4 and 5)

Please make sure that all necessary information on the request form is accurate and complete. ***It is vital to provide the name and contact of referring doctor who will be contacted directly by phone should there be any positive or uncertain results.***

It is the responsibility of the referring doctors to explain the screening test in details to parents before blood collection. Parents who agree to the screening should give their consent in writing by signing at the end of the request form.

The request form can be obtained from the **Prenatal Genetics Diagnosis Laboratories;** the address is set out at the end of this document.

Dried Blood Spot (DBS) Collection and Handling Guide

1. Collect the sample after completion of oral feeding within 24 hrs to 7 days after birth. The best time for collection is between 48 and 72 hours after birth. DO NOT collect samples before 24 hours of age. (For preterm, low birth weight, sick infants, please refer to the specific blood taking schedule on page 11.)

2. The shaded areas (see picture) indicate the puncture sites on the heel where blood is collected from.

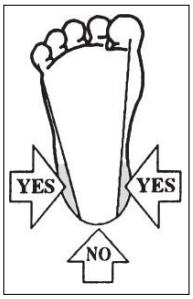
3. To prevent specimen contamination, DO NOT touch any part of the circled areas on the filter paper before, during or after blood collection.

4. Complete the infant's demographic data (name, date of birth and ID or hospital reference number) or affix infant's GUM label on the collection card before proceeding to collection.

5. Cleanse site with alcohol swap and allow to dry.

6. Puncture heel with sterile lancet designed for heel prick for infants. Blade-type lancet with incision depth of 2 mm is recommended.

7. Wipe away the first drop of blood with sterile gauze and allow the next large drop of blood to form.



8. Place collection card over this large blood drop and allow it to soak through and completely fill the circular area in one single application. DO NOT apply blood to both sides of the collection card. DO NOT layer several blood drops on top of each other.

9. Fill the remaining circles on the collection card with successive blood drops. A minimum of four circles is necessary for each collection card. *(A minimum of 5 completely filled circles are required if Cystic Fibrosis Screening is requested.)*

10. Allow blood spots to dry thoroughly in a flat position on a non-absorbent surface for at least 3 to 4 hours at room temperature. DO NOT leave wet collection cards in a hanging position as this will cause the heavier red cells to migrate to the dependent end of the circle resulting in uneven saturation. (See picture)

11. Keep the collection card away from direct sunlight and heat. DO NOT dry blood spots on a heater, in a microwave, with a hair dryer or under sunlight. DO NOT stack collection cards on top of each other before thorough drying.

Accuracy of the screening test results will be compromised if the specimen is improperly collected or handled.

Delivery of the Collection Card to the Laboratory

1. Transport each collection card in separate envelopes. DO NOT use plastic bags.

2. Deliver collection cards and completed request forms to the **Prenatal Genetics Diagnosis Laboratories**; the address is set out at the end of this document.

3. DBS sample reception time:

   Monday to Friday (except public holidays) 9:00am – 3:00pm (closed at 1:00 – 2:00 pm)

4. DBS received before 10:00am on Friday (or the day before public holiday) will be analyzed on the same day. Reports with normal results will be sent out on the following Monday (or the next working day). If results are positive or uncertain, referring doctors will be contacted in the earliest instance.

5. For DBS collected after 10:00am on Friday, Saturday, Sunday and any public holiday, please send them to the laboratory on the following Monday (or the next working day). For DBS samples that cannot reach the laboratory on the day of collection, store them in a cool dry place at room temperature for no more than four days before sending them to the laboratory.

6. Special arrangement for long public holidays (> 3 days) will be announced if necessary.

Unsatisfactory Dried Blood Spot Samples

The following DBS samples are unsatisfactory for the screening test:

1. Incomplete information on request form or collection card, rendering it impossible to determine the infant's identity or age at the time of collection;
2. Insufficient quantity of blood on the card to perform the analysis;
3. Damaged or contaminated collection card.

When unsatisfactory DBS samples are received, the laboratory will not proceed to the analysis. The referring hospital or clinic will be informed in the earliest instance for recollection of samples.

Out-patient Dried Blood Spot Collection

If DBS cannot be collected before a newborn is discharged from the hospital, parents may bring their infant to the Newborn Screening Clinic of the CUHK–BCM Joint Centre for Medical Genetics, The Chinese University of Hong Kong, at the Prince of Wales Hospital for blood collection.

Turnaround Time

A report will be issued at around three working days after the collection card is delivered to the laboratory.

Testing Laboratory

Xcelom Ltd., Hong Kong

Storage and Disposal of Dried Blood Spots

All dried blood spot cards will be stored in the laboratory for a minimum of two years. The laboratory will ensure the appropriate and proper protection of sensitive personal information. With informed consent from parents, the laboratory may store residual DBS cards for more than two years and use them for internal studies or research after all identifying information has been removed. Amino acids and acylcarnitines in DBS cards will deteriorate after prolonged storage which will render retrospective diagnosis of IEM impossible.

Screening for Cystic Fibrosis

Background

Cystic fibrosis (CF) is the most common autosomal recessive disease in white populations. CF is caused by mutations in the *CFTR* gene, which encodes a chloride channel called cystic fibrosis transmembrane conductance regulator (CFTR) protein. Affected patients develop various gastrointestinal, pulmonary and endocrine problems from the neonatal period to adulthood. (3) Newborn screening and early treatment can improve the nutritional, growth and intellectual outcomes in CF patients.(4)

The incidence of CF varies among different ethnic groups (5-8):

- Non-Hispanic Caucasians 1:2,500
- Ashkenazi Jews 1:2,270
- Hispanics 1:13,500
- African Americans 1:15,100
- Asians 1:35,100 – 350,000

A commonly adopted CF screening strategy is the IRT/DNA approach. Immunoreactive trypsinogen (IRT) is first measured in dried blood spot samples (Tier 1 test). IRT is a marker of pancreatic injury and is not specific to CF. If IRT concentration is elevated, a panel of *CFTR* mutations are then tested on the same dried blood spot (Tier 2 test). Infants with elevated IRT concentrations and one or two *CFTR* mutations are reported to have positive screening results.

Further laboratory testing and clinical assessment are necessary to confirm the diagnosis of CF.

The sensitivity of newborn screening for CF is around 95% in developed countries such as Australia, the United Kingdom and the United States. (9-11) Approximately 15% of infants with CF are born with meconium ileus. These patients may have normal IRT concentrations and thus be overlooked by newborn screening. Therefore, neonates with meconium ileus or a history of CF in siblings should always be followed up regardless of the screening result. The same principle applies to patients who develop signs and symptoms suggestive of CF. Nonetheless, there is evidence demonstrating that false negative newborn screening results do not result in delayed diagnosis or poorer outcomes in affected patients. (11,12)

Sample requirement

Two dried blood spots of 12 mm in diameter (i.e. two completely filled circles).

Newborn Metabolic plus Cystic Fibrosis Screening: a minimum of 5 completely filled circles are required.

Testing laboratory

Newborn Screening Laboratory, Wisconsin State Laboratory of Hygiene,
Madison, Wisconsin, USA

Testing algorithms

Tier 1 test: immunoreactive trypsinogen (IRT)

Tier 2 test: mutation analysis of 23 *CFTR* mutations
(http://www.slh.wisc.edu/clinical/newborn/health-care-professionals-guide/nbs-test-panel-of- diseases/#cf).

Tier 2 test is performed on the highest 4% of the daily IRT results.

Interpretation

Infants with elevated IRT concentrations and one or two CFTR mutations are reported to have positive screening results. Further laboratory testing and clinical assessment are

necessary to confirm the diagnosis of CF.

Provision of accurate ethnicities of parents to the laboratory aids interpretation of Tier 2 test (CFTR gene analysis) results.

- Potential false negative IRT results
  - Affected infants with meconium ileus;
  - Affected infants with pancreatic sufficiency;
  - IRT levels in affected infants remain elevated for 2 to 4 weeks and may decline in some patients at 1 month old. (13) Thus, this newborn screening test is not suitable for older infants or children suspected to have CF.
- Potential false positive IRT results
  - IRT may be falsely elevated in premature or sick infants.
- Mutation detection rate of the 23-CFTR-mutation panel (CFTR gene analysis)
  - Ashkenazi Jewish 94%
  - Non-Hispanic white 88%
  - Hispanic white 72%
  - African American 64%
  - Asian American 49%

<u>Turnaround time</u>

Samples with normal IRT results: approximately three to four weeks

Enquiries

Office hours: Monday to Friday 9:00am – 5:00pm
CUHK – BCM Joint Centre for Medical Genetics, The Chinese University of Hong Kong
Enquiry hotline for general public (during office hours): 6806 4590
General Enquiries (e.g. request form, collection card and DBS reception)
Prenatal Genetics Diagnosis Laboratories 4/F, Block K, DTB, Prince of Wales Hospital 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong Tel: 6806 4590 Fax: 3505 4810
Obstetrician
Prof. LEUNG Tak Yeung Professor and Chairman, Department of Obstetrics and Gynaecology, CUHK Tel: 3505 2806 Email: tyleung@cuhk.edu.hk
Newborn Screening Laboratory
Dr. LAW Lap Kay Eric Honorary Scientific Officer, Department of Obstetrics and Gynaecology, CUHK Tel: 6806 4590 Email: ericlaw@cuhk.edu.hk
Paediatricians
Dr. CHONG Shuk Ching Associate Professor of Medical Genetics Deputy director, Joint Centre of Medical Genetics Department of Paediatrics, CUHK

Tel: 3505 2982

Email: chongsc@cuhk.edu.hk

Prof. Fernando SCAGLIA

Honorary Professor, Department of Obstetrics and Gynaecology, CUHK

Director, Joint BCM-CUHK Centre of Medical Genetics, CUHK

Tel: 3505 2982

Email: fscaglia@bcm.edu

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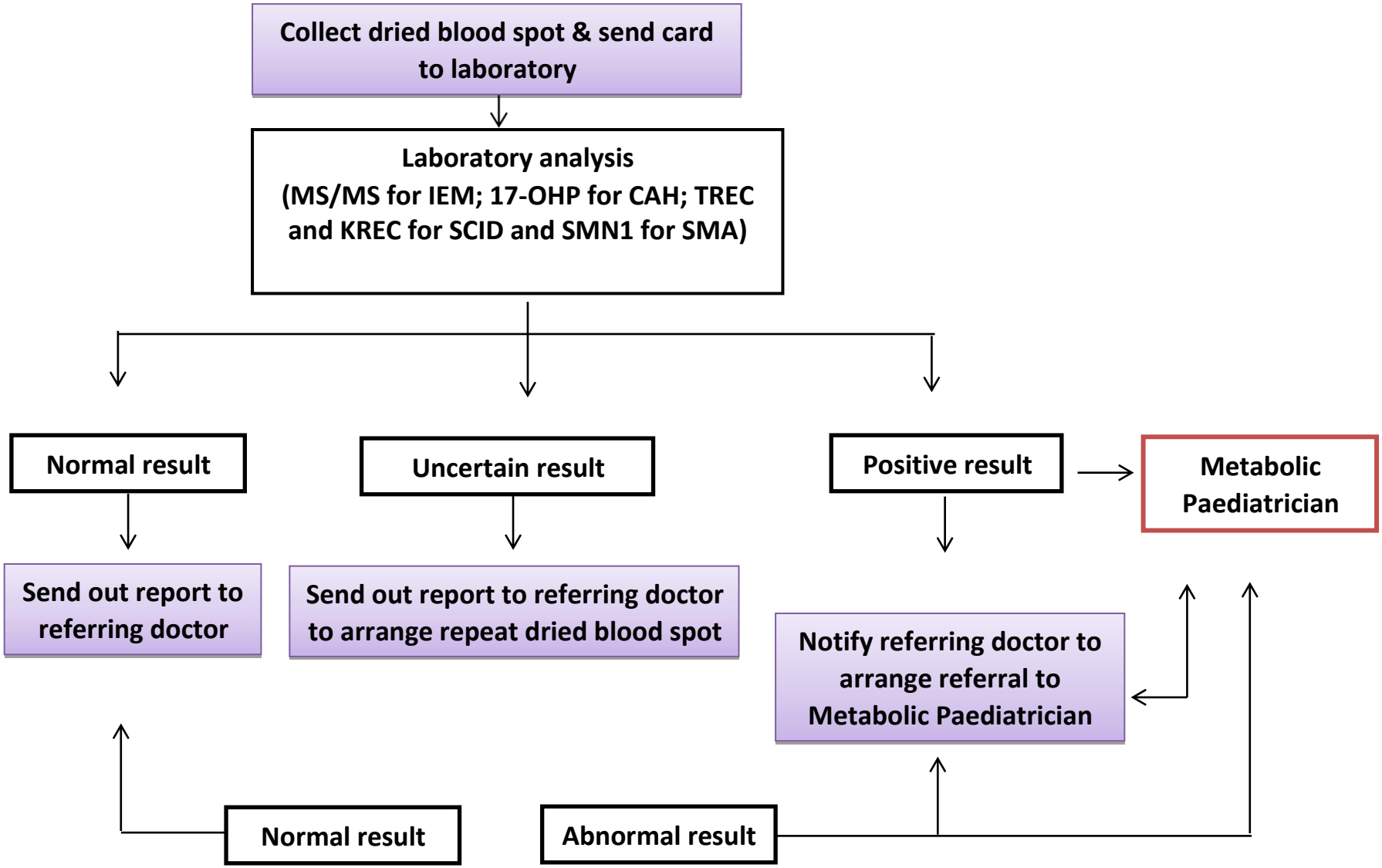
Appendix 1. Workflow of the Newborn Metabolic Screening Program



Appendix 2. Target Inborn Errors of Metabolism

Inborn Errors of Metabolism	ACMG# classification	Key metabolites
Amino Acid Disorders		
Phenylketonuria (PKU)	Core	↑ Phe
Maple syrup urine disease (MSUD)	Core	↑ Leu/Ile
Citrullinaemia type 1	Core	↑ Cit
Argininosuccinic aciduria	Core	↑ Cit
Homocystinuria	Core	↑ Met
Tyrosinaemia type 1	Core	↑ Tyr, SA*
Arginase deficiency	2°	↑ Arg
Defects of biopterin cofactor biosynthesis or regeneration	2°	↑ Phe
Citrullinaemia type 2 (Citrin deficiency)	2°	↑ Cit
Organic Acid Disorders		
Propionic acidaemia (PA)	Core	↑ C3
Methylmalonic aciduria (MUT, cbl A/B)	Core	↑ C3
Isovaleric acidaemia (IVA)	Core	↑ C5
ß-ketothiolase deficiency (BKT)	Core	↑ C5OH, C5:1
Glutaric acidaemia type 1 (GA1)	Core	↑ C5DC
3-Hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG)	Core	↑ C5OH
Multiple carboxylase deficiency (MCD)	Core	↑ C5OH
3-Methylcrotonyl-CoA carboxylase deficiency (3MCC)¶	Core	↑ C5OH
Malonic aciduria (Malonyl-CoA decarboxylase deficiency)	2°	↑ C3DC
3-Methylglutaconic aciduria type I (3MGA)	2°	↑ C5OH
Cbl C/D	2°	↑ C3

Fatty Acid Oxidation Disorders		
Primary carnitine deficiency (Carnitine update defect, CUD)¶	Core	↓ C0
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	Core	↑ C8
Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	Core	↑ C14:1
Long-chain hydroxyl-acyl-CoA dehydrogenase (LCHAD)	Core	↑ C16OH
Trifunctional protein deficiency (TFP)	Core	↑C16OH
Carnitine palmitoyltransferase I deficiency (CPT1)	2°	↑ C0
Carnitine palmitoyltransferase II deficiency (CPT2)	2°	↑ C16
Carnitine-acylcarnitine translocase deficiency (CACT)	2°	↑ C16
Multiple acyl-CoA dehydrogenase deficiency (Glutaric aciduria type 2, GA 2)	2°	↑ C4, C5
Medium/short-chain hydroxyl-acyl-CoA dehydrogenase deficiency (M/SCHAD) (3-Hydroxyacyl-CoA dehydrogenase deficiency)	2°	↑ C4OH
Peroxisomal Disorders		
X-linked adrenoleukodystrophy	Core	↑ C26:0-lysoPC

#ACMG: American College of Medical Genetics (Ref 13, 14). Core conditions: newborn screening for these disorders are mandated in the United States. 2° conditions: they are part of the differential diagnosis of a core condition, they are clinically significant and revealed with the screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance.

*SA: Succinylacetone. A second tier test for dried blood spots with tyrosine concentration above the pre-defined cut-off.

¶ A positive screening test result may suggest inborn errors of metabolism in the mother or infant.

Appendix 3. First line diagnostic tests for Inborn Errors of Metabolism

Amino acid profile	
Details:	Diagnostic test for amino acid disorders Abnormal in some organic acid disorders
Sample requirement:	Heparin blood 3 mL (minimum 1 mL) or Plasma 1 mL (minimum 0.2 mL)
Carnitine and acylcarnitine profile	
Details:	Diagnostic test for fatty acid oxidation disorders Abnormal in some organic acid disorders
Sample requirement:	Clotted blood in plain bottle 1 mL or Serum 0.5 mL
Urine metabolic screen	
Details:	Diagnostic test for organic acid disorders Abnormal in some amino acid and fatty acid oxidation disorders
Sample requirement:	Fresh spot urine in plain bottle 20 mL

Appendix 4. Request form (consent in Chinese)

THE CHINESE UNIVERSITY OF HONG KONG

The CUHK – BCM Joint Centre for Medical Genetics

Joshua Hellmann Foundation Newborn Metabolic Screening Program

Tel: 6806-4590 Fax: 3505-4810

JOSHUA HELLMANN FOUNDATION FOR ORPHAN DISEASE

Affix Baby's GUM label here or fill in the following Name: DOB: ID / Hospital no.:	Affix Mother's GUM label here or fill in the following Name: ID / Hospital no.:

Gestation age at delivery :_____week_____day Birth weight :_____grams Hospital / Place of birth: _____

Date and time of birth :_____/_____/_____:_____am/pm Parental Consanguinity: ☐ Yes ☐ No

Ethnicity: ☐ Chinese ☐ Caucasian ☐ Indian ☐ Pakistani ☐ Other (please specify:_____)

Feeding type: ☐ Breast ☐ Formula ☐ Mixed *(please wait until feeding for > 24 hrs before blood collection)*

Baby / Mother: ☐ Antibiotics (_____) ☐ Immunosuppressant (_____) ☐ Steroids

Family history: ☐ IEM ☐ Congenital adrenal hyperplasia ☐ Immunodeficiency disease ☐ Cystic fibrosis

Screening Test:	☐ **IEM, CAH, SMA & SCID** Minimum 4 blood spot circles	☐ **IEM, CAH, SMA, SCID & Cystic Fibrosis** Minimum 5 blood spot circles

Referring doctor information (contact person for positive results)

Name: Dr_____ Signature: _____

Phone: _____ Fax:_____

Hospital or Clinic: _____

Dried Blood Spot (DBS) Information

See back of collection card for DBS collection and handling guide.
Provide baby's name, ID / hospital reference no, and date of birth on the collection card.

DBS collection date:_____ DBS collection time:_____

Test information: ☐ 1st sample ☐ 2nd sample ☐ Pre-transfusion sample ☐ Others:_____)

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家長同意書:

- 我明白這個新生兒代謝病篩查計劃的目的和可能出現的結果。
- 我明白如果寶寶的第一個血樣顯示不確定或陽性結果，我們將會被安排重新抽取血樣本再作篩查。
- 我明白在極少數情況下，患病的寶寶有可能未被檢出。
- 我同意樣本會被送往其他化驗所 (本地或海外; 服務或研究實驗室) 以針對以上及其他相關代謝病。
- 我同意，剩餘的樣本經刪除所有的身份信息後，可能會被保留並用於實驗室內部試驗，醫學研究以及刊登醫學文獻。拒絕允許使用寶寶的剩餘血樣將不會影響寶寶的篩查結果。

如果您不希望寶寶的剩餘血樣被保留作以上用途，請在這裡劃上✓號：☐

母／父親姓名:_____ 電話號碼:_____

母／父親簽署:_____ 簽署日期:_____

Version 9 (effective date: 1 September 2021)

c/o Prenatal Genetics Diagnosis Laboratories, 4/F, Block K, DTB, Prince of Wales Hospital, Shatin, N.T., Hong Kong
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Appendix 5. Request Form (consent in English)

THE CHINESE UNIVERSITY OF HONG KONG
The CUHK – BCM Joint Centre for Medical Genetics
Joshua Hellmann Foundation Newborn Metabolic Screening Program
Tel: 6806-4590 Fax: 3505-4810

Affix Baby's GUM label here or fill in the following Name: DOB: ID / Hospital no.:	Affix Mother's GUM label here or fill in the following Name: ID / Hospital no.:

Gestation age at delivery :____week____day Birth weight :_____grams Hospital / Place of birth: _____

Date and time of birth :_____/_____/_____:_____am/pm Parental Consanguinity: ☐ Yes ☐ No

Ethnicity: ☐ Chinese ☐ Caucasian ☐ Indian ☐ Pakistani ☐ Other (please specify:_____)

Feeding type: ☐ Breast ☐ Formula ☐ Mixed *(please wait until feeding for > 24 hrs before blood collection)*

Baby / Mother: ☐ Antibiotics (_____) ☐ Immunosuppressant (_____) ☐ Steroids

Family history: ☐ IEM ☐ Congenital adrenal hyperplasia ☐ Immunodeficiency disease ☐ Cystic fibrosis

Screening Test:	☐ **IEM, CAH, SMA & SCID** Minimum 4 blood spot circles	☐ **IEM, CAH, SMA, SCID & Cystic Fibrosis** Minimum 5 blood spot circles

Referring doctor information (contact person for positive results)

Name: Dr_____ Signature: _____

Phone: _____ Fax:_____

Hospital or Clinic: _____

Dried Blood Spot (DBS) Information
See back of collection card for DBS collection and handling guide.
Provide baby's name, ID / hospital reference no, and date of birth on the collection card.

DBS collection date:_____ DBS collection time:_____

Test information: ☐ 1st sample ☐ 2nd sample ☐ Pre-transfusion sample ☐ Others:_____)

~~~~~~

Parent consent:

1. I understand the purpose and possible results of the newborn metabolic screening test.
2. I understand that my baby will be called back for a second heel prick if the first sample showed uncertain or positive results.
3. I understand that, in rare circumstances, variant forms of target diseases may escape screening.
4. I agree that my child's sample can be sent to other laboratories (local or overseas, service or research laboratory) for further testing of the aforementioned disease and related disorders.
5. I agree that leftover samples may be retained for laboratory internal use, medical research and publication after all identifying information has been removed. Refusal to permit the use of my baby's sample will not affect the screening test result.
   ***If you don't want your baby's sample to be kept for these purposes, please tick here*: ☐**

Name of mother / father:_____ Tel no.:_____

Signature of mother / father:_____ Date of signature:_____

Version 9 (effective date: 1 September 2021)

c/o Prenatal Genetics Diagnosis Laboratories, 4/F, Block K, DTB, Prince of Wales Hospital, Shatin, N.T., Hong Kong
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