1. General Procedure For Submission Of Specimen

Blood specimen meant for *Parseh Pathobiology & Genetics Laboratory* should be processed and delivered immediately after collection. Apart from tests which are performed on whole blood, only serum or plasma should be sent. If specimen cannot be delivered on the same day, freeze the specimen and transport frozen in dry ice to prevent denaturation, decomposition or bacterial growth.

Timed urine specimens should be collected with great care and preserved with the appropriate preservatives. The total volume of urine voided should be measured and recorded clearly on the label stuck to the container.

Every specimen must be accompanied by a duly completed IMR request form. For tests such as Inborn error of metabolism and Multiple Myeloma, special request forms has to be filled up. (Forms could be obtained from the Biochemistry Unit, Specialised Diagnostic Centre). Each specimen must be accompanied by a completed request form containing the following:

a. Patient's identification data, especially I/C number, and/ or R/N, age, and sex

b. Relevant clinical summary

c. Relevant routine laboratory results

d. Provisional Diagnosis

e. Doctor's name and signature

f. Ward/clinic and hospital's official rubber stamp.

g. Date of specimen collected, and

h. Date of specimen sent

i. Test requested

j. Type of specimens

Container for specimen should be labelled with patient's name, I/C or R/N, and name of the test requested.

2. Specimen Collection and Preparation

i. Blood

Collect blood into a plain container or one with the appropriate anticoagulant/preservative in it. Separate the plasma or serum (after the blood has been allowed to clot) as appropriate and transfer the specimen into a clean container. Freeze the specimen and transport frozen in dry ice to the Biochemistry laboratory. In the case of tests requiring whole blood, send the blood specimens immediately after collection.

Note: If possible, all specimens must arrive at the laboratory on the same day of collection.

ii. Urine

A timed urine specimen is required for most tests performed in this laboratory. The specimen should be collected with great care and preserved with the appropriate preservative. Send an aliquot of about 20ml to the laboratory. Note: The total volume of urine voided should be measured and recorded, both on the label stuck onto the specimen bottle and on the request form.

Procedure for collection of a 24-h specimen is given below:

a. At the start of the collection period (note the time) ask the patient to empty his/her bladder completely. Discard this urine specimen.

b. Collect all urine specimens passed during the next 24h in the container provided.

c. If a preservative is used, mix the contents thoroughly after each addition of urine.

d. At the end of the collection period (at approximately the same time the following day), ask the patient to empty his/her bladder completely. Include this last specimen in the total collection.

e. Measure the urine volume and written it down on the request form. Send an aliquot of about 100ml immediately to our laboratory, otherwise store it at 4°C and deliver to the laboratory as soon as possible.

3. Rejection of Request for test.

Request form and specimen will be reviewed in this laboratory to see whether they are conforming to specification. Request for test will be rejected if:

a. The test requested is not offered by the laboratory

b. Name of test is not written in the request form

c. Name of patient and identification is not filled up

d. Request form is not accompanied with specimen (no specimen received)

e. Information about patient identification does not match with the label at the specimen's container

f. Name and Address of client is not stated.

g. Broken or empty specimen container

h. Insufficient volume of specimen for the test requested.

i. Grossly heamolysed specimen.

j. Specimen sent is not suitable for the test requested

4. Special Tests

4.1. Metabolic Diseases/Inborn Error of Metabolism (IEM)

Inborn Error of Metabolic Diseases (IEMD) is single gene defects that cause abnormalities in the synthesis or catabolism of proteins, carbohydrates, fats or their derivatives. Both urine and plasma/serum is needed for laboratory diagnosis of these groups of diseases.

Simple urine tests can be done at peripheral laboratories, such as urinary colour and odour, simple chemical tests such as ferric chloride, DNPH, urine reducing sugars and ketones. Although some are non-specific, positive test may direct the physician to more specific tests indicating the need for further evaluations.

Routine chemistry tests in blood serum or plasma can also give further diagnostic clue to the type of metabolic disorder and help narrow down the diagnostic possibilities. Blood glucose, blood gases, blood ammonia, liver function tests, serum pyruvate and lactate are the most important routine chemistry tests and they should be done to any patient suspected of having IEM before sending the samples to this laboratory for further confirmatory tests.

Please use special form (IEM form) when requesting tests for IEMD. The form can be obtained from the laboratory. This form has been specially designed to help in the correct interpretation of the test result. Please fill up the important clinical signs and results of the routine chemistry tests done at the site laboratory.

Metabolic screening will be done to urine specimen by Thin Layer Chromatography. This procedure will be replaced by analysing 3mm blood spot using tandem mass spectrometry when it is available. If results are positive, plasma/serum amino acids and urine organic acids will be done. For patient with positive clinical signs and routine chemistry tests, plasma amino acids and urine organic acids will be done as soon as possible.

For the diagnosis of most amino acids disorder, morning fasting blood specimens are preferred to avoid post-prandial increase of most of the amino acids. Samples from young infants who are fed at frequent intervals, should be collected immediately before the next scheduled feeding. For hyperammonemia screening, postprandial blood is more suitable since an elevation of blood ammonia may be intermittent and present only in the fed state.

Certain amino acids are maintained at higher concentration in blood cells compared to plasma. Thus haemolysis often increases levels of certain amino acids in plasma or serum.

Improper handling of specimens (such as leaving unspun blood left at room temperature, prolonged storage and unclean specimen tubes) can result in artefactual changes in the amino acids contents.

Please follow carefully the instructions for collection of specimen.

i. Plasma (Amino acids, carnitine, homocysteine)

Plasma obtained from whole blood anticoagulated with heparin has been reported to be the best material for investigation of the steady state of amino acid concentration.

Precautions:

The presence of heparin in excess will lead to haemolysis. The use of EDTA as an anticoagulant can lead to artefacts caused by the presence of ninhydrin positive contaminants in the anticoagulant, and contaminants which also react with reagents in HPLC methods. Insufficient anticoagulation leads to partial clotting of the sample and a mixture of plasma and serum.

a. Collect whole blood directly into heparinised tube.

b. Centrifuge and separate from cells within 4 hours after collection, ensuring that the cellular buffy-coat is completely avoided.

c. Freeze the sample (-20C) if it could not be send to the laboratory within 4 hours.

d. Transport cold or frozen in dry ice to the laboratory.

ii. Serum: (Amino acids, carnitine, homocysteine)

a. Collect 20 ml blood into a clean, plain, unused tube without preservative.

b. Allow the blood to clot, and centrifuge to separate the red blood cells and serum before transportation.

c. Collect at least 2 ml of serum in a clean, unused, screw-capped tube.

c. Freeze the sample (-20C) if it could not be send to the laboratory within 4 hours.

d. Transport cold or frozen in dry ice to the laboratory.

iii. Urine (Organic acids, succinylacetone, orotic acids and urine for metabolic screening)

Generally performed on untimed random urine samples preferably early morning samples. Please state accurate and complete information on the clinical status, dietary manipulations and full drugs history of the patient because all these factors can also change the normal pattern of organic acids profile.

a. Collect a 10- 20 ml random urine specimen in a clean unused tube or bottle without preservative (except when specified that a 24 h urine collection is required).

b. Freeze the specimen (-20C) if it could not be transported within 4 hours to the laboratory.

c. Transport cold or frozen in dry ice to prevent bacterial overgrowth and lose of volatile substances. Some of the compounds of interest are ‘photo sensitive', so please protect the specimen from light by wrapping it with dark plastic bag.

d. A few drops of HCL may be added if specimen cannot be frozen or transported in ice.

For specimen collection and transport of other metabolic tests, please refer to the individual test.