

Specimen Collection Manual  
For  
Laboratory Services

---

Lady Ridgeway Hospital for Children  
2017

First Edition - 2017

ISBN 978-955-7967-00-4

Specimen Collection Manual for Laboratory Services, 2017  
Lady Ridgeway Hospital for Children

<b>Contents</b>	<b>Page</b>
<b>A. General information about the laboratory</b>	
1. Operative laboratories	1
2. Service hours	1
3. Sample acceptance times	2
<b>B. Common to Chemical Pathology and Haematology</b>	
1. Laboratory Requisition	4
2. Tests carried out in the chemical pathology/haematology laboratory	6
3. Sample rejection criteria for chemical pathology /haematology laboratory	7
4. Venepuncture	9
5. Capillary blood sampling	17
6. Special situations	21
<b>C. Chemical Pathology</b>	
1. Introduction to chemical pathology	22
2. List of biochemical tests carried out in the chemical pathology laboratory : routine and on-call	22
3. Types of laboratory request forms	28
4. Collection of venous blood samples, patient preparation and types of containers	29
5. Collection of urine samples	31
6. Sample collection for glucose, protein, lactate in cerebrospinal fluid (CSF)	36
7. Turnaround time	37
8. Sweat test	37
9. Dynamic function tests	38

	<b>Contents</b>	<b>Page</b>
<b>D.</b>	<b>Haematology</b>	
	1. Introduction to Haematology	39
	2. List of tests performed in the Haematology laboratory	39
	3. Routine and special tests, on call lab (night lab) and samples sent outside	39
	4. Types of laboratory request forms	44
	5. Types of containers	44
	6. Haematology referrals and clinics	45
	7. Details of routine tests, special tests and other haematological tests	46
	8. Haematological reference values	54
<b>E.</b>	<b>Histopathology</b>	
	1. Tests performed & Request forms needed	60
	2. General instructions for filling in request forms	61
	3. Special instructions for filling request forms	62
	4. Container labelling	63
	5. Histopathology examination of solid tissue	65
	6. Fine needle aspiration cytology examination- FNAC	65
	7. Cytology examination	66
	8. Examinations of Buccal smear for Barr bodies	67
	9. Frozen sections	67
	10. Post mortem	67

## Contents

## Page

### F. Microbiology

1. General guidelines – Microbiology	68
2. Test turnaround time	70
3. Rejection criteria	71
4. Type of containers	72
5. Anaerobic culture	74
6. Blood culture	75
7. Bone marrow culture	77
8. CSF	78
9. Ear swabs	79
10. Eye	81
11. Faeces	82
12. Fluids from sterile sites	83
13. Intravascular catheter associated infections	84
14. MRSA screening swabs	86
15. Pus and wound swabs	87
16. Respiratory tract secretions	88
17. Throat swabs	89
18. Urine	90
19. Serology tests	91
20. TB culture and PCR (Xpert MTB/RIF)	92

## Contributors

*Dr. Kumudu Karunaratne*  
Consultant Microbiologist

*Dr. Mala Jayatilaka*  
Consultant Haematologist

*Dr. Eresha Jasinge*  
Consultant Chemical Pathologist

*Dr. Sandini Gunaratne*  
Consultant Histopathologist

*Dr. Siromi Perera*  
Consultant Histopathologist

*Dr. Nipunika Senadheera*  
Consultant Haematologist

*Dr. H.W. Dilanthi*  
Senior Registrar – Chemical Pathology

*Dr. Rukmali Ratnayake*  
Medical Officer – Microbiology

## **List of Consultants**

Dr. Kumudu Karunaratne

Consultant Microbiologist

Dr. Eresha Jasinge

Consultant Chemical Pathologist

Dr. Sandini Gunaratne

Consultant Histopathologist

Dr. Nipunika Senadheera

Consultant Haematologist

Dr. Chandima Thevarapperuma

Consultant Haematologist





## General information about the laboratory

Following laboratories are operative in the hospital

- Chemical pathology - Located in the 1<sup>st</sup> floor of the old building
- Haematology - Located in the 1<sup>st</sup> floor of the new building
- Histopathology - Located in the ground floor of the old building
- Microbiology - Located in the 1<sup>st</sup> floor of the old building
- OPD laboratory - Located in the OPD
- On call laboratory - Located in the 1<sup>st</sup> floor of the new building

## Service hours

### Week days, Public holidays and Sundays

Day laboratory - from 8.00 am to 4.00 pm

On call laboratory - from 4.00 pm to 8.00 am the following day

### Saturday

Day laboratory - from 8.00 am to 12.00 noon

On call laboratory - from 12.00 noon to 8.00 am the following day

## Sample acceptance times

### Week days, Public holidays and Sundays

- Chemical pathology - 8.00 am to 3.00 pm\*
- Haematology - 8.00 am to 3.00 pm\*
- Histopathology - Refer next page
- Microbiology

#### **Week days**

- Cultures - 8.00 am to 3.30 pm
- Urine FR and Stool FR - 8.00 am to 3.00 pm

#### **PH & Sundays**

- Cultures - 8.00 am to 3.00 pm
- Urine FR and Stool FR - 8.00 am to 2.30 pm
- OPD laboratory - 8.00 am to 3.00 pm
- On call laboratory - 3.00 pm to 8.00 am the following day

\* Samples sent to chemical pathology and haematology after 2.30 pm should be requested on 'on call request forms' – green colour request form (giving allowance for transport time).

### Saturday

- Chemical pathology - 8.00 am to 11.00 am\*\*
- Haematology - 8.00 am to 11.00 am\*\*
- Histopathology - Refer next page
- Microbiology
  - Cultures - 8.00 am to 11.30 am
  - Urine FR and Stool FR - 8.00 am to 11.00 am
- OPD laboratory - 8.00 am to 11.00 am
- On call laboratory - 11.00 am to 8.00 am the following day

\*\* Samples sent to chemical pathology and haematology after 10.30 am should be requested on "on call request forms" -green colour request form (giving allowance for transport time).

## Histopathology - Sample reception/examination times

### 1. Histopathology samples

#### Time of accepting samples

Week days	- 8.00 am – 3.00 pm
Saturdays	- 8.00 am – 11.30 am
Sundays and Public holidays	- 8.00 am – 11.30 am

### 2. Fine needle aspiration examinations

#### Time of Examination

Week days	- 8.00 am – 3.00 pm
Saturdays	- 8.00 am – 11.30 am

### 3. Cytology examinations

Week days	- 8.00 am – 3.00 pm
Saturdays	- 8.00 am – 11.30 am
Sundays and Public holidays	- 8.00 am – 11.30 am

Note ; If urgent cytology test is needed other than above time please contact Histopathologist via exchange

### 4. Buccal smears, frozen sections, postmortems

Call the laboratory for an appointment

## Laboratory specimen requisition for chemical pathology and haematology

- Only the appropriate investigations should be requested according to the history of the patient, examination findings and previous investigations.
- Each specimen must accompany a completed request form which is signed by the ordering clinician / medical officer looking after the patient.
- Inadequately/incorrectly filled request forms will be rejected at the reception counter (Please refer sample acceptance and rejection criteria).
- **The person collecting the specimen must fill the information in the request form regarding sample collection. Eg: date and time of collection and initial it.**
- All specimens must have proper labelling. (Please refer sample acceptance and rejection criteria)
- Do not duplicate the samples and send under different names.
- If there's a delay in sending samples or an urgent test is required, need to communicate with the laboratory.
- Once the sample is collected, it should be delivered to the laboratory with undue delay.

- All the samples sent to the laboratory should be entered in a delivery book which belongs to the ward with name, age, sex, investigations and time of delivery.
- Validated test reports of the day lab will be available at the lab counter and the ward staff should collect them during the day time.
- When sending same test for the second time for haematology tests, make sure to keep a reasonable interval between the tests, unless there is a specific reason to repeat within a short period.

## Tests carried out in the chemical pathology/haematology laboratory

- There are different levels in which to prioritize result reporting. Each report will contain the specific result/results and reference range.
- Urgent : Highest priority. To be used only for life threatening situations.
- Routine : Regular specimen processing and analysis performed on a daily basis.
- Timed : Tests which require collection and testing at specific intervals.

### Urgent samples

Urgent samples should be restricted to occasions where they are essential for the immediate management of the patient. Samples should be sent along with the request form for urgent biochemical/haematology investigations during day time.

## Sample rejection criteria for chemical pathology/haematology laboratory

Following samples will be rejected at the sample reception.

1) Inadequately written labels/stickers –

Labels/stickers those are incomplete without the following information.

- Name of the patient
- Age of the patient
- Ward/Clinic
- BHT number/Clinic number
- Tests requested
- Signature / name of the nurse who is responsible for the specimen

Should be clearly written in the colour coded sticker
---

2) Incomplete request forms - Request forms that are incomplete without the following information.

- Name, age and sex of the patient
- Ward /Clinic
- BHT number/Clinic number
- Type of the sample (Eg: blood, CSF, urine)
- Tests requested
- Name of the doctor requesting the test
- Date of request
- Time of sample collection

- Signature of the sample collecting nursing officer
- Short, relevant clinical history of the patient

**The details on the label of the sample and the request form should be identical.**

- 3) Unlabeled specimens or specimens without request forms
- 4) Illegible specimen labels/request forms
- 5) Specimens showing gross evidence of decomposition
- 6) Inadequate/over collected volume of the specimen for the tests ordered
- 7) Samples in inappropriate containers
- 8) Specimens which are not transported properly  
Eg: Samples for plasma ammonia and lactate should be transported in ice
- 9) Clotted/partially clotted specimens  
Eg: For FBC, ESR and coagulation tests, fasting plasma glucose, plasma lactate
- 10) Unsatisfactory patient preparation  
Eg: Non-fasting samples for serum phosphate, fasting plasma glucose, serum triglycerides, plasma lactate, plasma ammonia
- 11) Specimens that have leaked or have specimen material on the outside of the container



## Venepuncture

- Staff drawing blood from children and neonates must be well trained and practiced in venepuncture techniques.
- Verify patient preparation. Eg: fasting for serum phosphate, fasting plasma glucose.

## Drawing blood

- Collect all the supplies and equipments needed for the procedure and place them within safe and easy reach on a tray or trolley.
- Select recommended needle gauge and device for routine venepuncture procedures according to age groups. (table 1)
- **Avoid collecting blood using only the needle.**

Patient group	Needle gauge	Device
Neonates	23	Winged set (butterfly cannula)
Children with small veins	23	Winged set (butterfly cannula)
Children with large veins	22/23	Needle fitted to the syringe/ Winged set (butterfly syringe)

Table 1. Recommended needle gauge and device for routine injection and venepuncture procedures according to age groups

- Perform hand hygiene.
- Check that the request form matches the patient’s identity.  
(i.e. match the patient’s details with the laboratory request form to ensure accurate identification - Name, BHT and ward)
- Obtain verbal consent from the parent/ guardian
- Immobilize the child as below. (figure 1, 2)



Figure 1. Immobilization of the child



Figure 2. Immobilization of the child

- Extend the patient's arm and inspect the ante cubital fossa or forearm.
- Locate a vein of a good size that is visible, straight and clear. Do not insert the needle where veins are diverting, because this increases the chance of a haematoma.
- Apply the tourniquet on the patient about two finger breadth above the venepuncture site. **Avoid prolonged application of tourniquet.** (> 1 minute) Prolonged application can lead to haemoconcentration. (figure 3)
- To retie a tourniquet need to wait 2 minutes.

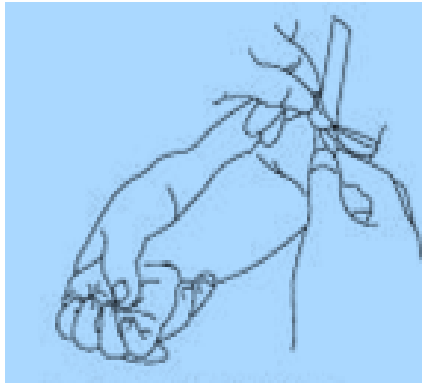


Figure 3. Application of a tourniquet

- Put on well-fitting gloves.
- Disinfect the collection site with a 70% alcohol swab for 30 seconds and allow to dry completely. Apply firm but gentle pressure. Start from the centre of the venepuncture site and work downward and outwards to cover an area of 2 cm or more. Do not allow alcohol to remain in the puncture site as it may cause haemolysis.
- DO NOT touch the cleaned site; in particular, DO NOT place a finger over the vein to guide the shaft of the exposed needle. If the site is touched, repeat the disinfection.

- Once the infant or child is immobilized, puncture the skin 3–5 mm distal to (i.e. away from) the vein at 30° angle; this allows good access without pushing the vein away.
- If the needle enters alongside the vein rather than into it, withdraw the needle slightly without removing it completely, and angle it into the vessel.
- Draw blood slowly and steadily.
- After the required amount of blood has been collected, release the tourniquet **BEFORE** withdrawing the needle.
- Place dry gauze over the venepuncture site and slowly withdraw the needle. Put an adhesive plaster on the patient if necessary.
- Ask the parent to continue applying mild pressure while the arm is extended and raised.
- Inject the blood sample extremely slowly into the tube minimizing the pressure and velocity. **DO NOT** recap and remove the needle.

Vigorous suction on the syringe during collection or forceful transfer of blood from the syringe to the tube may cause haemolysis.

- Before dispatch, invert the tubes containing additives for the required number of times as mentioned in table 2.
- Properly dispose all contaminated supplies.
- Remove gloves, dispose them appropriately and perform hand hygiene.

## Order of draw for vacuum tubes and needles






Order of draw	Colour of the stopper	Type of tube	No. of inversions recommended
1		Blood culture bottle	Not required
2		Citrate tube (Coagulation and ESR)	3-4 times
3		Non-additive tube (serum/glass tube)	Glass - none
4		Serum separator tube (gel tube)	5 times
5		Sodium Heparin tube (microtainer)	8-10 times
6		EDTA tube	8-10 times
7		Oxalate/fluoride (glucose tube)	8-10 times

Table 2. Order of draw for vacuum tubes and needles

- Label the sample correctly and transport to laboratory as soon as possible.
- For instructions in collecting blood for specific investigations please refer the relevant section.  
Eg: Sample collection for plasma lactate and ammonia - chemical pathology section

## Complications of venepuncture

<b>Adverse event</b>	<b>Cause</b>	<b>Prevention/Management</b>
Bruising/ haematomas	Leakage of a small or large amount of blood	Keep patient's arm raised and apply pressure to venepuncture site for 3-5 minutes to allow a platelet plug to form.
Fainting (syncope)/ fits	Patient's condition or a reaction to pain or fright	Remove the needle and protect the patient hitting his head or hurting himself. Activate specific medical emergency plan if needed.
Arterial puncture	Accidental puncture (Blood will be bright red)	Apply direct pressure to the puncture site for a minimum of 5 minutes.
Needle stick /blood borne infections	Inadequate precautions to prevent infections	Inform the infection control unit. Hepatitis/HIV post exposure prophylaxis if needed.
Allergies to antiseptics and / or adhesive tapes		Ask for allergies before venepuncture. Avoid use of particular allergic material.
Collapsed veins	Forceful drawing of blood	Use a butterfly needle. Use appropriate needle and syringe.
Nerve damage	Excessive or blind probing for a vein	Begin procedure from the beginning with a new needle. Ask for assistance if still unable to find a vein.

Table 3. Complications of venepuncture



## Capillary blood sampling

Capillary sampling from a finger, heel or (rarely) an ear lobe may be performed on patients of any age, for specific tests that require small quantities of blood.

Condition	Heel-prick	Finger-prick
Age	Birth to 6 months	Over 6 months
Weight	Approximately 3-10 kg	Greater than 10 kg
Placement of lancet	On the medial or lateral plantar surface (figure 4)	On the side of the ball of the finger perpendicularly to the lines of the fingerprint
Recommended finger	Not applicable	Third and fourth finger (i.e. middle and ring finger) of the non-dominant hand; avoid the thumb and index finger because of calluses and avoid the little finger because the tissue is thin
Depth of puncture	Not beyond 2.4 mm In neonates not beyond 1.0 mm	6 months- 8 years -: 1.5 mm Over 8 years -: <2.4mm

Table 4. Site and indications for capillary blood sampling

## Preparation for capillary blood sampling

The appropriate sized, automated lancet devices for use in infants should be selected. Manual lancets should not be used.

A clinical hand wash should be performed prior to starting the procedure.

**Select an appropriate site for blood sampling.** (figure 4)

The site chosen for sampling should be free from previous injury.

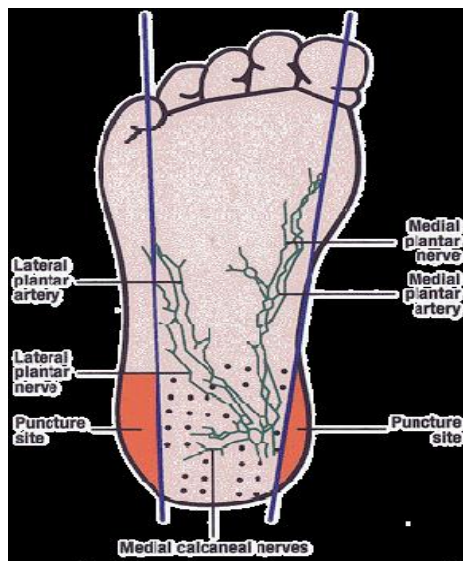


Figure 4. Appropriate sites for capillary blood sampling

## **Obtaining the sample**

- Gloves should be worn.
- Ensure baby is lying in a safe and secure position.
- Clean the proposed puncture site with 70% alcohol. In neonates use warm water and gauze. Alcohol impregnated wipes should not be used.
- Allow the area to dry. Gently compress the heel and hold the skin under tension.
- Puncture the skin with one quick, continuous and deliberate stroke. DO NOT puncture the skin more than once with the same lancet.
- Relax tension and wipe away initial blood flow with cotton wool or gauze.
- Do not squeeze the heel or finger.
- Hold the capillary tube or blood tube to the blood droplet and touch.
- Momentarily release pressure to collect subsequent blood then reapply pressure, allowing the blood to flow.
- Continue until sufficient blood has been obtained.
- Once the sample has been obtained apply pressure to the site with gauze. Maintain pressure until bleeding has stopped.

**Order of draw**

With skin punctures, collect the specimens in the order below,

- haematology specimens
- chemistry specimens

This order of drawing is essential to minimize the effects of platelet clumping. If more than two specimens are needed, venepuncture may provide more accurate laboratory results.

**Complications that can arise in capillary sampling include:**

- Collapse of veins if the tibial artery is lacerated from puncturing the medial aspect of the heel
- Osteomyelitis of the heel bone (calcaneus)
- Nerve damage if the fingers of neonates are punctured
- Haematoma and loss of access to the venous branch used
- Scarring
- Localized or generalized necrosis (a long-term effect)
- Skin breakdown from repeated use of adhesive strips – this can be avoided if sufficient pressure is applied and the puncture site is observed after the procedure.

## Special situations

Situation	Action
IV or blood transfusion running	As far as possible avoid drip arm and use the opposite arm. If not, turn off the IV drip for at least 3 minutes before the venepuncture and select a vein other than the one with IV drip. In the request form note that the blood was taken from a drip arm and IV line was turned off for 3 minutes.
Indwelling lines	The first 5 mL of blood should be discarded.
Sclerosed veins, scars, oedema	Select another site.
Haematoma	Draw from a vein below the site.

Table 5. Special situations

## Chemical Pathology

### Introduction to chemical pathology

The area of clinical pathology that is concerned with analysis of body fluids

### List of biochemical tests carried out in the chemical pathology laboratory: routine and on-call

#### Blood - Routine

Regular specimen processing and analysis of following analytes is performed on a daily basis.

Calcium	Indirect bilirubin
Phosphate	Gamma Glutamyl Transferase (GGT)
Magnesium	C Reactive Protein (CRP)
Alkaline Phosphatase (ALP)	Creatine Kinase (CK)
Total protein	Amylase
Albumin	Urea
Globulin	Creatinine
Aspartate Transaminase (AST)	Uric acid
Alanine Transaminase (ALT)	Sodium
Total bilirubin	
Direct bilirubin	

Potassium	Post prandial/ post 2 hour plasma glucose
Chloride	
Osmolality	Cholesterol Triglycerides
Random plasma glucose (RPG)	Lipid profile
Fasting plasma glucose (FPG)	Ceruloplasmin

### **Blood – Special**

Need to obtain a date from the laboratory.

Done only on Tuesdays and Thursdays (Except public holidays)

- Plasma ammonia
- Plasma lactate

### **Urine - Routine (On daily basis) (random)**

Sodium	Clini test for reducing substances
Potassium	Clinistix test for glucose
Chloride	Ketone bodies
Osmolality	Bile
pH	Urobiliogen
Transtubular potassium gradient	

### **Urine (in a spot urine sample)**

Only on Mondays, Wednesdays and Fridays (Except public holidays)

Calcium : creatinine ratio

Phosphate : creatinine ratio

Magnesium : creatinineratio

Uric acid : creatinine ratio

Protein : creatinine ratio

Tubular reabsorption of phosphate

Tubular maximum phosphate reabsorption

Fractional excretion of sodium/phosphate/magnesium/uric acid

### **Urine Special tests**

Only on Mondays (Except public holidays)

Nitrosonaphthol test for tyrosinaemia

Cyanide Nitroprusside test for cystinuria

Silver Nitroprusside test for homocystinuria

Berry spot test for mucopolysaccharidoses

Total porphyrin



Watson-Schwartz test for porphobilinogen

Ammonical silver nitrate test for homogentisic acid in alkaptonuria

Ferric chloride test for phenylpyruvic acid in phenylketonuria

Dinitrophenyl hydrazine test for ketoacids in Maple syrup urine disease (MSUD)

**Timed urine (24-hour urine collection)**

Only on Mondays, Wednesdays and Fridays (Except public holidays)

Protein

Calcium

Phosphate

Magnesium

Uric acid

Creatinine clearance

Electrolytes

### **Cerebrospinal fluid (CSF)**

- Lactate - Need to obtain a date. Done only on Tuesdays and Thursdays (Except public holidays)
  - Sugar
  - Protein
- } Routine

### **Analysis of other body fluids**

- Pleural fluid
  - Peritoneal fluid
- } Routine

**Tests available in night laboratory/on-call laboratory****Blood**

Calcium	Amylase
Magnesium	Urea
Total protein	Creatinine
Albumin	Sodium
AST	Potassium
ALT	Chloride
Bilirubin (total/ direct/ indirect)	
CRP	
Cholesterol	
Random plasma glucose (RPG)	

**Urine**

Ketone bodies
Clinitest and clinistix test
Urine full report (UFR)

**CSF**

Protien
Glucose
CSF full report

**Serum samples sent to other laboratories**

25 hydroxy vitamin D }  
- Colombo North Teaching Hospital  
- Medical Research Institute (MRI)  
- Apeksha Hospital, Maharagama

T<sub>4</sub>, TSH }  
LDH }  
Tumour markers }  
- Apeksha Hospital, Maharagama  
- MRI

**Types of laboratory request forms**

1. For routine blood investigations
2. For urgent biochemical investigations during day time
3. For special biochemical investigations and other body fluids
4. For investigations of urine samples
5. For on-call laboratory
6. For sweat test
7. Samples sent to Apeksha Hospital, Maharagama and MRI should be accompanied by a special request form.

**Collection of venous blood samples (Table 1)**

Investigation	Container with colour of the stopper	Preparation of the patient and special requirements	Sample volume
Calcium	Gel <input type="checkbox"/>		} 2mL up to the mark
Phosphate	Gel <input type="checkbox"/>	Need fasting Newborn : Before the next feed Child : 4 hours Adult : 6 hours	
Magnesium	Gel <input type="checkbox"/>		
Alkaline Phosphatase	Gel <input type="checkbox"/>		
Total protein	Gel <input type="checkbox"/>		
Albumin	Gel <input type="checkbox"/>		
Globulin	Gel <input type="checkbox"/>		
AST	Gel <input type="checkbox"/>		
ALT	Gel <input type="checkbox"/>		
GGT	Gel <input type="checkbox"/>		
Total bilirubin	Gel <input type="checkbox"/>	} Protect from light	
Direct bilirubin	Gel <input type="checkbox"/>		
Indirect bilirubin	Gel <input type="checkbox"/>		
Sodium	Gel <input type="checkbox"/>		
Potassium	Gel <input type="checkbox"/>	Do not refrigerate the sample	
Chloride	Gel <input type="checkbox"/>		
Creatinine	Gel <input type="checkbox"/>		
Urea	Gel <input type="checkbox"/>		
Uric acid	Gel <input type="checkbox"/>		
C Reactive Protein	Gel <input type="checkbox"/>		
Creatine Kinase	Gel <input type="checkbox"/>		
Amylase	Gel <input type="checkbox"/>		
Ceruloplasmin	Gel <input type="checkbox"/>		
Triglycerides Lipid profile	Gel <input type="checkbox"/>	-Need 10-12 hours fasting -Patient can be on an intravenous drip (5% dextrose) in the fasting state	
Cholesterol	Gel <input type="checkbox"/>		
Osmolality	Gel <input type="checkbox"/>		

Investigation	Container with colour of the stopper	Preparation of the patient and special requirements	Sample volume
RPG  FPG  Post prandial/post 2 hour plasma glucose	Sodium fluoride/ oxalate ■	<p>Need fasting Newborn : Before the next feed Child : 6 hours Adult : 8-10 hours</p> <p>2 hours after a meal/glucose load</p>	1 mL (Strictly adhere to this volume as the ratio between the sample and the preservative should be maintained)
Plasma lactate	Sodium fluoride/ oxalate ■	<p>4-6 hours fasting/just before the next meal</p> <p>Avoid exercise of the hand or arm immediately before and during the procedure.</p> <p>Sample should be taken <b><u>without a tourniquet by using a needle and a syringe.</u></b> Once the sample is transferred to the tube, <b><u>mix it well</u></b> and <b><u>transport it in ice to the laboratory immediately.</u></b></p>	1mL (Strictly adhere to this volume as the ratio between the sample and the preservative should be maintained)
Plasma ammonia	EDTA ■	<p>4-6 hours fasting /just before the next meal</p> <p>Sample should be taken <b><u>without a tourniquet using a needle and a syringe.</u></b> Once the sample is transferred to the tube, <b><u>mix it well</u></b> and <b><u>transport it in the ice</u></b> to the <b><u>laboratory immediately.</u></b></p>	2mL up to the mark

Table1. Patient preparation and collection of venous blood samples

## Collection of urine samples

### Spot urine samples (Table 2)

Investigations	Preparation of the patient	Sample volume	Container	Remarks
<b><u>Routine Tests</u></b>				
Electrolytes	Early morning fresh sample preferred	5 mL	Plain bottle	For interpretation of results a blood sample should be obtained for serum electrolytes at the time of urine collection
Fractional excretion of sodium (FE <sub>Na</sub> )	Early morning fresh sample preferred	5 mL	Plain bottle	A urine sample for sodium and creatinine and a blood sample for sodium and creatinine are needed
Trans tubular potassium gradient (TTKG)	Early morning fresh sample preferred	5 mL	Plain bottle	A urine sample for potassium and osmolality and a blood sample for potassium and osmolality are needed
Osmolality	Early morning fresh sample	5 mL	Plain bottle	For interpretation of results a blood sample should be obtained for serum osmolality at the time of urine collection
pH	Early morning fresh sample	5-10 mL	Plain bottle	Fasting preferred
Ketone bodies Urobilinogen Bile Clinitest Clinistix test		5 mL	Plain bottle	

(Table 2 contd.)

Investigations	Preparation of the patient	Sample volume	Container	Remarks
<u>Spot urine samples</u> Ca : creatinine ratio	Fasting - Newborn: Before the next feed  Child:4 hrs  Adult:6hrs	5 mL	Bottle containing 1mL of 0.6N HCl as a preservative	
*PO <sub>4</sub> : creatinine ratio * Fractoinal excretion of PO <sub>4</sub> (FE <sub>PO4</sub> ) * Tubular reabsorption of phosphate (TRP) * Tubular maximum reabsorption of phosphate (Tm PO <sub>4</sub> /GFR)	Fasting - Newborn: Before the next feed  Child : 4 hrs  Adult:6hrs	5 mL	Bottle containing 1mL of 0.6N HCl as a preservative	For FE PO <sub>4</sub> , TRP, Tm PO <sub>4</sub> /GFR : At the time of urine collection obtain a blood sample for serum creatinine and serum phosphate in the fasting state.
Mg : creatinine ratio		5 mL	Bottle containing 1mL of 0.6N HCl as a preservative	
Protein : creatinine ratio	Early morning fresh sample	5 mL	Plain bottle	



**(Table 2 contd.)**

Investigations	Preparation of the patient	Sample volume	Container	Remarks
Uric acid : creatinine ratio	Early morning fresh sample	5 mL	Plain bottle	
Fractional excretion of uric acid (FE <sub>UA</sub> )	Early morning fresh sample	5 mL	Plain bottle	A blood sample for uric acid and creatinine should be obtained at the same time

Table 2. Patient preparation and collection of spot urine samples

**Special tests (Table 3)**

Investigations	Sample volume	Container	Remarks
<b>Special tests</b>			
Total porphyrin		Plain bottle covered with an aluminium foil	
Watson-Schwartz test for porphobilinogen			
Nitrosonaphthol test for tyrosinaemia	Early morning fresh urine sample 5 – 10 mL	Plain bottle	
Cyanide Nitroprusside test for cystinuria			
Silver Nitroprusside test for homocystinuria			
Ammonical silver nitrate test for homogentisic acid in Alkaptonuria			
Ferric chloride test for phenylketonuria			
2,4-Dinitrophenyl hydrazine test for keto acids in MSUD	Second voided urine sample 5 mL		Need 3 samples on 3 consecutive Mondays
Berry spot test			

Table 3. Patient preparation and collection of urine samples for special tests

## Timed urine – Sample collection

### Steps

- Obtain a container from the laboratory to collect urine.
- Empty the bladder fully into the toilet soon after rising and record time (eg 5.30 am). This is the **START** time of the collection. The urine is discarded as it is formed before the beginning of the collection period.
- Collect all the urine passed in the next 24-hours into the bottle. Empty the bladder on rising next morning at the same time (5.30 am) into the bottles and record the **FINISH** time.(If child passes urine before the finished time and if there is no urine to pass at finish time, mention the time urine was added into the bottle for the last time as the finish time.)
- The patient should be instructed to pass urine into a separate wide mouth clean container and transfer it to the bottle provided by the laboratory using a funnel.
- Label the container properly.
- Hand over the sample to the laboratory without delay. The **START** and **FINISH** time should be clearly mentioned in the request form.
- If a clearance test has been requested (creatinine clearance), a blood sample too should be collected at any time during the urine collection period (not after the urine collection period.)

**For timed urine, spot urine samples and special tests for urine;**

- To obtain a date a completely filled request form should be sent to the laboratory.
- After collection samples should be sent as soon as possible to the chemical pathology laboratory.

**Sample collection for CSF glucose, protein and lactate****(Table 4)**

Investigations	Patient preparation	Remarks	Container
Protein			Plain bottle in the lumbar puncture set
Glucose		For an accurate evaluation, a blood sample should be obtained for plasma glucose 1-2 hours before the lumbar puncture. If the patient is fasting for 4-6 hours, blood for glucose can be obtained immediately before the lumbar puncture.	
Lactate	Need fasting for 4-6 hours	Transport it to the laboratory immediately.	

Table 4. Patient preparation and collection of cerebrospinal fluid (CSF) samples

## Turnaround time

Routine tests	-	varies depending on the number of tests requested
Urgent tests	-	one hour
Plasma lactate	-	two hours
Plasma ammonia	-	two hours
Spot urine tests	-	two days
Timed urine tests	-	three days
Special urine tests	-	one week
Berry spot test	-	four weeks
Sweat test	-	three hours

## Sweat test

Performed only on Tuesdays and Thursdays.

Contact the laboratory to obtain a date and for further details.

## Dynamic Function tests

Following tests are performed in the department of chemical pathology.

1. Water deprivation test
  2. Acid loading test
  3. Ischaemic forearm test
- A referral should be sent to the chemical pathologist in order to assess the patient and get a date for the test. Instructions on procedures and containers for sample collection should be collected from the department of chemical pathology prior to the date of the test.

## References

1. United States National Committee Clinical Laboratory Standards consensus in 2003
2. GOSH (2014) [On - line] Blood Sampling, Neonatal Capillary. [http://www.gosh.nhs.uk/health\\_professionals/clinical\\_guidelines/blood-sampling-neonatal-capillary/](http://www.gosh.nhs.uk/health_professionals/clinical_guidelines/blood-sampling-neonatal-capillary/) (Accessed 16/11/14)
3. WHO guideline on drawing blood, Best Practices in Phlebotomy, 2010
4. Dean AS, MARK MR, Demitri AF. Cerebrospinal Fluid Analysis. *American Family Physician* 2003; 68: 1103-08
5. Geetha A, Sandeep J, Tony M, Michael P. It is all in the history, or is it? *Arch Dis Child EducPract Ed* 2012; 97: 17-22

## Haematology

### Introduction to Haematology

Haematology includes the analysis of diseases that affect the production of blood and its components such as blood cells, haemoglobin, bone marrow, plasma proteins, blood vessels, spleen and the mechanism of coagulation.

### List of tests performed in the Haematology laboratory

#### Routine tests:

Regular specimen processing and analysis of following tests will be done on daily basis.

- FBC
- Blood picture
- Reticulocyte count
- ESR
- Malarial parasites
- Prothrombin time (PT)
- Activated Partial Thromboplastin Time (APTT)
- Thrombin Time (TT)

**Special tests:**

Need to inform Consultant Haematologist/MO Haematology and obtain a date. Preferably patient should be sent to haematology laboratory for collection of the blood sample on the given date.

- Sickling test
- Brewer's test -Screening for G6PD deficiency
- Cryohaemolysis test for hereditary spherocytosis
- Red cell inclusion bodies (Heinz bodies, Hb H inclusions)
- Heat precipitation test for unstable haemoglobin
- HAM test - screening for Paroxysmal Nocturnal Haemoglobinuria(PNH)
- Hand spectroscope for urine haemoglobin
- Urine Haemosiderin to detect chronic intravascular haemolysis
  
- Bleeding Time - As a special investigation in bleeder screening, not as a routine pre-operative investigation.
- Clotting factor levels ( Factor VIII, IX and XI)
- Inhibitor screening
- Clot solubility test for factor XIII deficiency
- von Willebrand's investigations ( vWF Ricof activity, vWF antigen)
- Plasma fibrinogen (Clauss method)–to assess the fibrinogen level. Prior appointment not required.
- ROTEM as a special investigation when there is acute haemostatic failure –prior date not required ,however it is essential to inform the Consultant Haematologist, MO or MLT before sending the specimen.



- Rheumatoid factor
- LE cell test
- Acid elution test (Kleihauer test)
- Bone marrow aspiration with special stains (pearl stain, Sudan Black , PAS)
- Trepine biopsy

**Other tests :**

Other reference laboratories that the of specimens are referred; MRI/ Haematology laboratory and RIA laboratory of NHSL/ Faculty of Medicine Colombo and Ragama, private sector

- Serum Ferritin – MRI,RIA – NHSL
- Serum iron studies ( Serum Iron, TIBC, transferrin saturation) - MRI
- Serum Vit B<sub>12</sub>, Serum Folate, Red cell Folate – MRI
- HPLC for Hb A2 quantification, Hb F estimation and Hb sub typing – MRI, specimen should be sent through the haematologist.
- $\alpha$  Thalassaemia gene studies – Human Genetic Unit- Faculty of Medicine Colombo,Thalassaemia unit –NCTH /Ragama
- $\beta$  Thalassaemia gene studies - Not available at state sector
- Platelet aggregation studies - MRI
- vWF investigations – LRH & MRI
  
- ANA - MRI
- Anti DS- DNA - MRI
  
- D – dimer - MRI (Depending on availability)

- Serum LDH – MRI
  - Serum Methaemoglobin– MRI
  - Serum Haptoglobin
  - Protein C
  - Protein S
  - Anti Thrombin
  - Activated Protein C Resistance (APC) for factor V leiden
- } Available at NHSL  
(Dates will be given)
- Genetic studies for ,  
Factor V leiden gene mutation,  
Prothrombin gene mutation,  
MTHFR mutation,  
JAK2V617F mutation
- } Faculty of Medicine  
Colombo – Human  
Genetic Unit
- Screening for lupus antibodies - DRVVT - Haematology lab NHSL
  - Anticardiolopin antibodies (IgM, IgG)
  - Serum erythropoietin
- } Not available at  
state sector  
hospitals
- Flow cytometry –1) Cancer Hospital – Patient should have a registered number from CIM.  
2) MRI – Haematology without acute leukemia panel

- PCR
- Cytogenetic studies, chromosomal fragility test
- FISH technique



Faculty of Medicine  
Colombo – Human  
Genetic Unit

**Test done at night on call laboratory:**

From 3.00 pm to 8.00 am next day

- FBC/Blood picture /Retic count
- PT
- APTT
- ROTEM

**Tests done at OPD laboratory:**

- FBC
- Blood picture
- ESR
- Retic count

## Types of laboratory request forms

1. For routine blood investigations - Colour coded request forms  
EDTA specimen – Lavender  
Coagulation – Blue  
ESR – Black & white
2. For very urgent FBC during day time and night – Pink form.  
A preliminary report will be sent to ward immediately through the same staff member who brought the specimen to the lab.
3. For on-call laboratory -Green
4. For special haematological investigations – MRI forms, HPLC requests forms, and RIA lab forms are available in the laboratory.

## Types of containers

### Colour code for tubes used in haematology laboratory:

- Lavender  EDTA
- Blue  Citrate /coagulation
- Yellow  Plain/Gel tube
- Black  Citrate/ESR

## Haematology Referrals & Clinics

### Referrals:

Monday to Saturday.

Sunday morning - very urgent referrals only. (On-call medical officer will be available on Sunday morning)

### Clinics:

**Tuesday:** Anaemia clinic, Thalassaemia screening clinic  
Bone marrows under GA in the theatre (ASOT).

**Wednesday:** Haemophilia Clinic and clinic to investigate bleeding disorders. *(If a patient suspected of bleeding disorder needs urgent treatment, investigations will be done urgently on any day)*

**Thursday:** ITP clinic  
Bone marrows under GA in the theatre (ASOT).

**Friday:** Warfarin Clinic (Anticoagulation)





**Saturday:** -Haemophilia Clinic,

Haematology clinics are flexible for children who are following another clinic in this hospital. They can come to the haematology clinic in the morning on the same day of the other clinic and get their investigations done from LRH HAEMATOLOGY/BIOCHEMISTRY LAB.

If any urgent haematological information / advice are required you may contact the Consultant Haematologist **SOS** at any time.





## Details of routine tests, special tests and other haematological tests

### Routine Tests:








Test	Container	Volume	Patient preparation and special requirements
<b>1. Full Blood Count (FBC)</b> Hb, HCT, Red Cell indices (MCV,MCH,MCHC) WBC/DC, Platelet Count	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Mix well by 8- 10 inversion</li> <li>• Fresh sample (within six hours).</li> <li>• Do not keep in the refrigerator.</li> </ul>
<b>2. Blood picture</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• For finger prick sample patient need to be sent to the laboratory whenever possible.</li> </ul>
<b>3. Reticulocyte count</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Fresh sample</li> <li>• Do not keep in the refrigerator.</li> </ul>
<b>4. Malarial parasites (Thick &amp; thin films)</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• For finger prick sample patient should be send to the laboratory.</li> </ul>
<p><b>If FBC, Blood picture, Retic count and Malarial parasites are necessary for your patient please note that it can be done from one single EDTA specimen.</b></p>			




<b>5. ESR</b>	Citrate ■ (3.8%)	1.6mL	<ul style="list-style-type: none"> <li>• Blood and Citrate volume should be correct.</li> <li>• Mix well by 8 -10 inversions.</li> <li>• Fresh sample (within 2hrs).</li> <li>• Do not keep in the refrigerator.</li> </ul>
<b>Coagulation Profile:</b>  <b>6. Prothrombin Time (PT)</b> <b>7. Activated Partial thromboplastin Time (APTT)</b> <b>8. Thrombin Time(TT)</b> <b>9. Plasma Fibrinogen</b>	Citrate ■ (3.2%)	1.8mL	<ul style="list-style-type: none"> <li>• Mix well with 4-5 gentle inversions.</li> <li>• Send to the laboratory without undue delay.</li> </ul>
<b>10. ROTEM</b>	Citrate ■ (3.2%)	1.8mL X 2	<ul style="list-style-type: none"> <li>• Two specimens required as above.</li> <li>• Please write it clearly that it is for ROTEM.</li> <li>• Need to discuss with Consultant haematologist before sending.</li> </ul>

**Special Tests:** Prior appointment is necessary and the patient needed to be sent to the lab.



Test	Container	Volume	Patient preparation and special requirements
<b>1. Sickling test</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary and send the patient to lab on due date.</li> </ul>
<b>2. Screening for G6PD deficiency (Brewer's test)</b>	EDTA 	2mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> <li>• Control: 2ml EDTA blood from a non related preferably age and sex matched person.</li> <li>• Mix well.</li> <li>• Test to be done once reticulocyte count is normal.</li> </ul>
<b>3. Osmotic fragility test</b>	EDTA 	2mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> <li>• Mix well</li> <li>• 1ml blood + 15 -20IU of Heparin can also be used.</li> <li>• Control from a non related person.</li> </ul>
<b>4. Cryohaemolysis test</b>	EDTA 	2mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> <li>• Mix well.</li> </ul>



<b>Red cell inclusion bodies:</b>			
<b>5. Heinz bodies</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Patient should be sent to the lab.</li> </ul>
<b>6. Hb H inclusions</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Fresh sample</li> </ul>
<b>7. Heat precipitation test for unstable haemoglobin</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> </ul>
<b>8. Screening for PNH – Ham test</b>	Patient: EDTA  Clotted blood   Control EDTA  Clotted blood 	2mL 10mL  2mL 12mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> <li>• Control from a non related person with same ABO blood group.</li> <li>• Patient and donor should be sent to lab.</li> <li>• Test is not reliable if blood transfusion was done during last 3 months.</li> </ul>
<b>9. Urine Haemoglobin by hand spectrometry</b>	Plain bottle		
<b>10. Urine haemosiderin</b>	Iron free container	5mL	<ul style="list-style-type: none"> <li>• Early morning first voided urine samples from 3 consecutive days.</li> </ul>
<b>11. Bleeding Time (BT)</b>			<ul style="list-style-type: none"> <li>• Need to contact MO/ haematology.</li> <li>• Patient should be sent to the laboratory whenever possible with a recent platelet count.</li> </ul>

<b>12. Clotting factor levels</b>  <b>13. Inhibitor screening</b>  <b>14. Clot solubility test for factor XIII</b>  <b>15. Von Willebrand Antigen &amp; Ricof activity</b>	Citrate  (3.2%)	1.8mL	<ul style="list-style-type: none"> <li>• Prior appointment is necessary.</li> <li>• Send the patient to the haematology lab for blood sampling.</li> <li>• Preferably need two samples.</li> <li>• Need a control from a nonrelated person.</li> </ul>
<b>16. Rheumatoid factor</b>	Gel 	1mL	<ul style="list-style-type: none"> <li>• Sample can be kept for few days in the refrigerator.</li> </ul>
<b>17. LE cell test</b>	Defibrinatingflask	5mL	<ul style="list-style-type: none"> <li>• Mix well by rotation.</li> <li>• Patient should be sent to the lab.</li> </ul>
<b>18. Acid elution test (Kleihauer test)</b>	EDTA 	1mL	<ul style="list-style-type: none"> <li>• Communicate with the lab MO.</li> <li>• Finger prick can also be used.</li> </ul>
<b>19. Bone marrow aspiration</b>  <b>20. Trepine biopsy</b>			<ul style="list-style-type: none"> <li>• Need a haematology referral.</li> <li>• Two days (Tuesday &amp; Thursday ) under general anaesthesia at AS/OT</li> <li>• Urgent bone marrow done on other days, ward has to arrange the theatre list.</li> </ul>

**Samples collected and sent outside:**

Test	Container	Volume	Patient preparation and special requirements
<b>1. Serum Ferritin</b>	Plain tube	2mL	<ul style="list-style-type: none"> <li>• MRI /RIA lab</li> </ul>
<b>2. Serum iron studies (Serum Iron, TIBC, Transferrin saturation)</b>	Acid wash tubes	2mL	<ul style="list-style-type: none"> <li>• MRI</li> </ul>
<b>3. HPLC for , Hb identification Hb A2 estimation Hb F estimation</b>	EDTA 	2mL	<ul style="list-style-type: none"> <li>• MRI through consultant haematologist</li> <li>• HPLC specimens should only be sent from haematology lab</li> </ul>
<b>4. ANA 5. Ds DNA</b>	Plain tube	2mL	<ul style="list-style-type: none"> <li>• MRI</li> </ul>
<b>6. Bone marrow for Flow cytometry</b>	EDTA 	2mL	<ul style="list-style-type: none"> <li>• CIM, private sector MRI – limited panel</li> <li>• Should be sent through the haematologist</li> </ul>
<b>7. Bone marrow for TB PCR</b>			<ul style="list-style-type: none"> <li>• Faculty of Medicine Colombo, Chest Hospital Welisara.</li> <li>• Need the container from the relevant laboratory</li> </ul>

**Other tests:**

<b>Test</b>	<b>Sample and required volume</b>	<b>Patient preparation and special requirements</b>
<b>1. Serum Vit B12</b> <b>2. Serum Folate</b> <b>3. Red cell Folate</b>		<ul style="list-style-type: none"> <li>• Done at private sector/ MRI</li> <li>• Need to collect the special container from the relevant laboratory.</li> </ul>
<b>4. Platelet aggregation studies</b> <b>5. von willebrand investigations</b>		<ul style="list-style-type: none"> <li>• MRI</li> <li>• Age &gt;1year</li> <li>• Patient to be sent to the MRI with a referral letter from Consultant Haematologist.</li> </ul>
<b>6. D - dimer</b>	1.8mL blood + 0.2mL Sodium Citrate (3.2%)	<ul style="list-style-type: none"> <li>• Private sector</li> </ul>
<b>7. Serum LDH</b>	2mL clotted blood	<ul style="list-style-type: none"> <li>• MRI</li> </ul>
<b>8. Serum Methaemoglobin</b>		<ul style="list-style-type: none"> <li>• MRI</li> <li>• Need to inform prior to sending the sample.</li> </ul>
<b>9. Serum Haptoglobin</b>	2mL clotted blood	<ul style="list-style-type: none"> <li>• Private sector</li> </ul>
<b>10. Serum Erythropoietin</b>	2mL clotted blood	<ul style="list-style-type: none"> <li>• Private sector</li> </ul>
<b>11. Protein C</b> <b>12. Protein S</b> <b>13. Anti thrombin</b> <b>14. Activated protein C (APC) resistance</b>	2mL citrated blood	<ul style="list-style-type: none"> <li>• NHSL (Dates need to be taken)</li> </ul>

Test	Sample and required volume	Patient preparation and special requirements
<b>15. Genetic tests for: Factor V Leiden mutation, Prothrombin gene mutation, MTHFR mutation, JAK2V617F mutation</b>		<ul style="list-style-type: none"> <li>• Faculty of Medicine – Colombo.</li> <li>• With a referral letter from the Consultant Hematologist.</li> </ul>
<b>16. DRVVT/KCT</b>		<ul style="list-style-type: none"> <li>• Patient to be sent to NHSL with a referral letter from the Consultant Haematologist.</li> </ul>
<b>17. Anti Cardiolipin antibodies IgM&amp;IgG</b>		<ul style="list-style-type: none"> <li>• Private sector</li> </ul>
<b>18. Cytogenetic studies &amp; chromosomal fragility tests</b>	3mL blood in to a special container containing Lithium heparin	<ul style="list-style-type: none"> <li>• Need to collect the special container from the relevant laboratory.</li> </ul>
<b>19. FISH analysis</b>	3mL blood in to a special container containing Lithium heparin	<ul style="list-style-type: none"> <li>• Need to collect the special container from the relevant laboratory.</li> </ul>

## Simplified chart of Haematology reference values

Red cell count: <ul style="list-style-type: none"> <li>• Men</li> <li>• Women</li> <li>• Infants (Cord blood)</li> <li>• Children</li> </ul>	4.5 – 6.5 x 10 <sup>12</sup> /l 3.6 – 5.6 x 10 <sup>12</sup> /l 7.0 – 8.0 x 10 <sup>12</sup> /l 4.1 – 5.5 x 10 <sup>12</sup> /l
Haemoglobin: <ul style="list-style-type: none"> <li>• Men</li> <li>• Women</li> <li>• Infants (Cord blood)</li> <li>• Children 3 months</li> <li>• Children 1yr</li> <li>• Children 3 – 6 yrs</li> </ul>	13.5 – 17.5 g/dl 11.5 – 15.5 g/dl 13.5 – 19.5 g/dl 9.5 – 13.5 g/dl 10.0 – 13.5 g/dl 11.0 – 14.0 g/dl
Packed Cell Volume (PCV): <ul style="list-style-type: none"> <li>• Men</li> <li>• Women</li> <li>• Infants (Cord blood)</li> <li>• Children 3 months</li> <li>• Children 3 – 6 yrs</li> </ul>	40 – 52 % 36 – 48 % 44 – 64 % 32 - 44 % 36 – 44 %
Mean Cell Volume (MCV): <ul style="list-style-type: none"> <li>• Adults</li> <li>• Neonates</li> <li>• Children 3 months</li> <li>• Children 3 -6 yrs</li> </ul>	80 – 90 fl 120 fl 95 fl 70 – 86 fl
Mean Cell Haemoglobin (MCH): <ul style="list-style-type: none"> <li>• Adults</li> <li>• Children 3 months</li> <li>• Children 3 -6 yrs</li> </ul>	27 – 32 pg 24 – 34 pg 24 – 30 pg

Mean Cell Haemoglobin Concentration (MCHC):	
<ul style="list-style-type: none"> <li>• Adults &amp; Children</li> <li>• Neonates</li> </ul>	<p>30 – 35 g/dl</p> <p>27.5 g/dl – 32.5 g/dl</p>
Leucocyte count:	
<ul style="list-style-type: none"> <li>• Adults</li> <li>• Neonate (Day 1)</li> <li>• Infant (1year)</li> <li>• Children (4 -7 year)</li> </ul>	<p>4 – 11 x10<sup>9</sup>/l</p> <p>10 – 26 x10<sup>9</sup>/l</p> <p>6 – 18 x10<sup>9</sup>/l</p> <p>5 – 15 x10<sup>9</sup>/l</p>
Neutrophil Count:	
<ul style="list-style-type: none"> <li>• Adults</li> <li>• At birth</li> <li>• Neonate</li> <li>• Infant (1 year)</li> <li>• Children (2 -6 year)</li> <li>• Children ( 6 – 12 year)</li> </ul>	<p>2 – 7 x10<sup>9</sup>/l</p> <p>4 – 14 x10<sup>9</sup>/l</p> <p>3 – 9 x10<sup>9</sup>/l</p> <p>1 – 7 x10<sup>9</sup>/l</p> <p>1.5 – 8 x10<sup>9</sup>/l</p> <p>2 – 8 x10<sup>9</sup>/l</p>
Lymphocyte Count:	
<ul style="list-style-type: none"> <li>• Adults</li> <li>• At birth</li> <li>• Neonate</li> <li>• Infant (1 year)</li> <li>• Children (2 -6 year)</li> <li>• Children ( 6 – 12 year)</li> </ul>	<p>1.5 – 3.5 x10<sup>9</sup>/l</p> <p>3 – 8 x10<sup>9</sup>/l</p> <p>3 – 16 x10<sup>9</sup>/l</p> <p>3.3 – 11 x10<sup>9</sup>/l</p> <p>6 – 9 x10<sup>9</sup>/l</p> <p>1 – 5 x10<sup>9</sup>/l</p>
Eosinophil Count:	
<ul style="list-style-type: none"> <li>• 2 months – 12yrs</li> <li>• Adults</li> </ul>	<p>0.05 – 1.1 x10<sup>9</sup>/l</p> <p>0.04 – 0.44 x10<sup>9</sup>/l</p>
Platelet count:	150 – 400 x10 <sup>9</sup> /l
Reticulocyte count:	
<ul style="list-style-type: none"> <li>• Adults &amp; Children</li> <li>• Neonates</li> </ul>	<p>0.5 – 2.5 %</p> <p>2 – 6 %</p>
Bleeding Time (BT)	2 – 7 minutes

PT APTT TT <ul style="list-style-type: none"> <li>• &lt; 6 months please refer the chart for coagulation reference values.</li> <li>• &gt;6 months - Adults             <ul style="list-style-type: none"> <li>PT 11.4 -14 (s)</li> <li>APTT 25 – 39 (s)</li> <li>TT 12 -16 (s)</li> </ul> </li> </ul>	
Plasma Fibrinogen	1.5 – 4.0g/l
<u>Serum Ferritin</u>	
New born	110 - 500 µg/l
2 month -1year	4 - 400µg/l
1 -5 years	6 - 24µg/l
5 -9 years	10 - 550 µg/l
<i>Adult</i>	
Male	40- 340µg/l
Female	14-150µg/l
Osmotic Fragility	3- 5g/l NaCl
Cyrohaemolysis	3 – 15 % lysis – Normal >20% lysis - Positive



**Haematological reference values for normal infants & children:**

	Birth	Day 3	Day 7	1 month	2 months	3 months - 1year	1 – 6 year	6 – 12 year
Red cell count (x10 <sup>12</sup> /l)	5 - 7	4 – 6.6	4.9 - 6.1	3 – 5.4	3.1 -4.3	3.9 - 5.3	4–5.2	4 – 5.2
Hb (g/dl)	14-22	15 -21	17.9 – 17.1	11.5 – 16.5	9.4 – 13.0	11.1 – 14.1	11 - 14	11.5 – 15.5
PCV l/l	0.45 - 0.75	0.45 – 0.67	0.42 – 0.66	0.33 – 0.53	0.28 – 0.42	0.3 - 0.4	0.34- 0.4	0.35- 0.45
MCV (fl)	100-120	92- 118	88- 126	92- 116	87-103	68 - 84	75 -87	77 -95
MCH (pg)	31-37	31-37	31-37	30-36	27-33	24-30	24-30	25-33
MCHC (g/l)	30-36	29-37	28-38	29-37	28.5- 35.5	30-36	31-37	31-37
RDW CV %					11 -17	11 - 17	11 - 16	11- 16
Reticulocyte x10 <sup>9</sup> /l	120-400	50- 350	50- 100	20-60	30-50	30-100	30- 100	30- 100
WBC x10 <sup>9</sup> /l	10-26	7-23	6-22	5-19	5-15	6-18	5-15	5-13
Neutrophils x10 <sup>9</sup> /l	4-14	3-5	3-6	3-9	1-5	1-7	1.5-8	2-8
Lymphocytes x10 <sup>9</sup> /l	3-8	2-8	3-9	3-16	4-10	3.5 - 12	6-9	1-5
Monocytes x10 <sup>9</sup> /l	0.5-2	0.5-1	0.1- 1.7	0.3-1	0.4-1.2	0.2-1.2	0.2-1	0.2-1
Eosinophils x10 <sup>9</sup> /l	0.1-1	0.1-2	0.1- 0.8	0.2-1	0,1-1	0.1-1	0.1-1	0.1-1
Platelets x10 <sup>9</sup> /l	150- 450	150- 450	150- 450	150- 450	150- 450	150- 450	150- 450	150- 450

**Reference values for coagulation tests in healthy full term infants during first 6 months of life:**

	D1	D5	D30	D90	D180
PT (s)	11.5 – 14.5	11 – 13.8	10.6 – 13	10.8 – 13	11.5 – 13
APTT (s)	37.1 – 48.7	34 – 51	33 – 47	31 – 43	31 – 39
TT (s)	21 - 26	20 - 26	22 - 27	23 - 27	23 -28

After 6 months of age, adult ranges can be applied.

PT (s) – 11.4 -14

PT ratio – 0.8 – 1.2

APTT (s) – 25 – 39

TT (s) – 12-16

## References

1. Practical Haematology ( 10<sup>th</sup> edition), Lewis S.M, Bain B.J, Bates I. 11-24
2. Manual on Laboratory Services – Ministry of health 2007
3. Paediatric Haematology. (2<sup>nd</sup> edition).LilleymanJ.S., Ian M. Hann, BlanchetteV. S,1-8, 2001.

## Histopathology

### Instructions for collection and transport of specimens to the histopathology laboratory

[Contact No –Histopathologist 319, Lab /MLT 261]

### Tests performed in the histopathology section & Request forms

1. Histopathology examination	General Histology request form (white form) Renal request form.( green form) Liver request form.(blue form)
2. Fine needle aspiration examination (FNAC)	FNAC request form (white form)
3. Examination of fluids for cytology - Eg. Pleural fluid	Cytology request form (white form)
4. Examination of Buccal smears	Health 359 Form
5. Frozen sections	Frozen section request form and General histopathology request form when relevant (pink form)
6. Postmortems	Postmortem request form

If any other special tests are needed please call the consultant Histopathologist /MO/lab at any time.

## **A) General instructions**

### **Filling in request forms**

All the forms should be filled in the following manner

- A.1) Name of patient -At least 2 names should be written in Block Letters (CAPITALS) or one name and date of birth  
Eg :*HASINI SUMUDU or SUMUDU-08/06/2010* .
- A.2) Age - Please state years months or days.
- A.3) Gender-F or M.
- A.4) Clinical details - relevant clinical details and relevant investigations including previous history and reports and lab reference number.
- A.5) Details of surgery- relevant to diagnosis/endoscopic diagnosis.
- A.6) Many samples from the same patient should be filled in the same request form.
- A.7) Name of the doctor filling in the request form should be clearly written.
- A.8) THE REGISTRAR OR CONSULTANT SHOULD OVERSEE THE REQUEST FORMS IN MAJOR SURGERY/LIVER /MUSCLE BIOPSIES AND ASSURE THAT IMPORTANT RELEVANT INFORMATION IS MENTIONED.
- A.9) The reports are handed over to the ward mentioned in the request form. (Therefore Please avoid writing the BHT number, Ward number of previous admissions or the theatre name in the Ward column as these cause unnecessary difficulties to the patients in obtaining the report)

## B) Special instructions for filling request forms.

### B.1) Tests which are requested by one unit for a procedure performed by another unit

*Eg. Paediatric ward requests US guided liver /lymph node/ trucut biopsy/pericardial biopsy/colonoscopy biopsies or peritoneal aspiration to be performed by the Radiologist or surgeon.*

- Contact the lab for the special request forms if relevant and sample collection bottles and instructions.
- A filter paper will be given for small trucut / colonic biopsies and it should be folded with the specimen inside and put into the container.
- When placing mucosal biopsies on the filter paper, Mark a cross “X” with a pencil on the paper to identify the superficial surface before folding the biopsy.
- If the differential diagnosis requires any special frozen sections for fat etc. the case should be discussed with the Pathologist or MO at the time of ordering the test.
- The request form should be filled by the unit **requesting the test.**
- A medical officer from the unit requesting the test should be present at the procedure and complete the **relevant cage on the reverse of the form.**
- If a medical officer is not present it is the responsibility of the unit requesting the test to ensure that procedure details are supplied to the pathologist.

## B.2 ) Liver and Renal biopsy

- Liver and Renal biopsies which are not suspicious for a malignancy have a special request form. A book must be maintained in the ward with the clinical details and investigations since a multidisciplinary meeting will be held prior to issuing of the report. The patient's details must be available in the ward at all times.

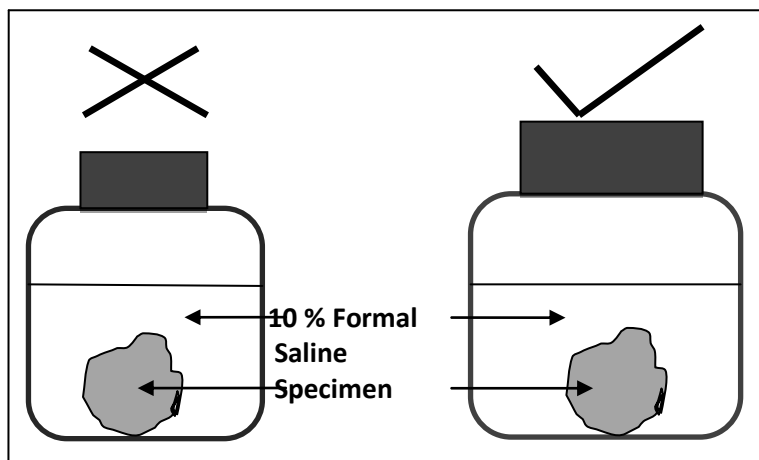
## B.3) Hirschsprungs sample

- Large specimens for Hirschsprungs disease should be orientated and accompanied by a diagram
- The above details in B.1 apply to these biopsies.

## C) Container labelling

### Containers and fixatives

- C.1) Wide mouth, leak proof, plastic, clean container with a tight fitting lid should be used.



- C.2) The lab provides bottles with the letter H on the lid for use of small specimens.
- C.3) 10 % Formal Saline ten times the volume of the tissue should be in the bottle.
- C.4) A firmly attached label containing the following information should be on the bottle. Type of Specimen/ biopsy, Name of patient, Age , BHT No ,Ward / Clinic
- C.5) If in one surgery the colon and lymph nodes were removed and put into 2 bottles, the sample with colon should be labeled as bottle A- Colon and the sample with lymph nodes should be labeled as bottle B- lymph nodes
- C.6) Store at room temperature until dispatch to the laboratory.(Do not refrigerate formalin fixed specimens)
- C.7) Send the specimen & request form with the properly filled register.Eg:

Date	Pt Name	BHT No.	Ward	Specimen	Officer sending	Officer receiving
2015/08/04	1.xxxxxxx	222201	15	Vvvvvv	ssssss	rrrrrrr

- C.8) Check once again whether all the above requirements have been filled in the register before sending the specimens to the lab.
- C.9) Do not send specimen taken in OT back to wards.
- C.10) If any of the above requirements are not fulfilled the specimens will not be accepted. For further clarification contact **Ext. no. 261**



## Tests performed in the histopathology section

### **1. Histopathology examination of solid tissue**

- Fill in the relevant form.
- General histology form- for all specimens other than those stated below
- Renal biopsy form – for renal biopsies which are not suspicious for malignancy
- Liver biopsy form for liver biopsies which are not suspicious for malignancy.
- For Liver, muscle and renal biopsy contact the lab for further information and sample containers
- Label the specimen according to “C”

### **2. Fine needle aspiration cytology examination- FNAC**

#### **2.1) Unguided FNA examination.**

- Please send the patient with duly filled request form with relevant clinical history during the times previously specified.

#### **2.2) Ultrasound guided FNA examination.**

- The lab should be contacted to obtain the request form slides and alcohol jar.
- The FNA request form should be completed by the unit that requests the test and the unit performing the test should fill in the relevant section. See special instructions.

- If slides are made after spreading the material on the glass slide it should be immersed immediately in alcohol while the smear is wet. The alcohol Jar & slides are provided by the laboratory. Alcohol fixed samples for cytology can be kept at room temperature for any period till it is sent to the laboratory
- The alcohol jar with slides with a duly completed FNA request form should be sent to the lab.

### 3. Cytology examination-

#### Specimens for Cytology / Malignant Cells.

- Aspirated fluids suspected of having malignant cells should be sent for cytology.  
(*Ex. CSF, Pleural fluid, Peritoneal, any other fluid*)
- Cytology request form must be filled according to general instructions and the unit performing the test must fill in the appropriate manner.(See special instructions )
- Collection should be into a clean leak proof container without preservatives.
- Label should contain type of specimen, Name, Age, Sex, Ward, Date and time of collection.
- After collection the sample should be sent to the histopathology lab immediately.
- If the specimen is taken after 4.00pm – keep refrigerated at 4<sup>0</sup>c and send to the lab next morning with the duly completed request form.

**[ Pls note: Samples delayed more than 24 hrs. and kept at room temperature for longer periods are not suitable for Cytological Studies.]**

#### 4. Examinations of Buccal smear for Barr bodies

- Please send the patient with mother with a referral letter and the duly filled request form (H359) to the Consultant Histopathologist for an appointment.

#### 5. Frozen sections

- Contact the lab for an appointment and to obtain the Request forms and special instructions.

#### 6. Post mortem

- Contact the histopathology lab to obtain the request form and to arrange for an autopsy examination.

**IMPORTANT: Duplicates of Histopathology or Cytology reports will not be issued as the Original reports are dispatched by hand to the Wards /Units mentioned. Only the Clinic & OPD reports are being filed in the department and these reports can be collected personally from the Histopathology Lab.**

If any other information or further clarification is required please contact:

Department of Histopathology / L R H

Extensions - M L T and MO PATH -261

Consultant Histopathologist and MO PATH -319

Location- Ground Floor of Old Building adjoining Kitchen

## Microbiology

### General guidelines for Microbiology specimens

The goal of microbiologic evaluation is to provide accurate, clinically pertinent results in a timely manner. The quality of the specimens submitted to the microbiology laboratory is critical for optimal specimen evaluation.

- Make every effort to obtain specimens prior to the initiation of antimicrobial therapy.
- Take measures to minimize contamination by the normal colonizing flora of the skin or mucous membranes.
- Collect an adequate volume of specimen.
- Send tissue or fluid whenever possible rather than submitting a specimen collected on a swab.
- Label all specimen containers with identifying information about the patient (name, BHT, ward/clinic and date of collection).
- If several specimens are collected at the same time from different sites, indicate the site also on the bottle label in addition to the request form.

- Similarly if several samples are taken from a same type of specimen on the same day (e.g. Blood cultures in suspected endocarditis) indicate the time of collection both on the request form as well as on the label of the container.
- Request forms
  - Use green colour microbiology request forms (H 1222) to send specimens for culture to LRH lab.
  - Use MRI request forms to send specimen to MRI. (H 275a)
  - For TB – use TB request form. (TB-06)
  - Obtain MRI request form from general stores and other forms from microbiology lab.
- Complete the request form in clear handwriting, including requested details on probable clinical diagnosis, antimicrobial therapy etc. Time of collection is important in CSF and blood cultures.
- Notify the laboratory in advance if special tests are requested.
- Place warning indication on specimens from patients suspected of having highly contagious diseases as a safety measure.
- If there is any question about the optimal sample to collect, call the microbiology laboratory before obtaining specimens.
- Reports of specimens ordered from OPD/clinic are available in microbiology lab. Others will be issued to wards.
- All microbiology investigations to be sent to MRI should be sent to microbiology laboratory.







## Microbiology Test Results - Turnaround Time

Test	Turnaround time
Blood culture & ABST	1 – 7 days
CSF antigen	Same day
CSF culture & ABST	2 days
CSF full report	1 hour
Dengue antibody	Same day
Dengue antigen	Same day
Fluid full report	Same day
IV cannulae tip culture	1 –2 days
MRSA screening swabs	1 –3 days
Pus culture & ABST	1 - 3 days
Respiratory sample culture & ABST	1 – 3 days
Sterile fluid culture & ABST	1 – 5 days
Stool culture & ABST	2 – 5 days
Stool full report	Same day
Swabs – wound/Ear /Eye/Throat	1 – 3 days
Urine culture & ABST	1 – 3 days
Urine deposits	Same day

## **Rejection criteria followed by the Microbiology laboratory on specimen acceptance**





1. Broken or cracked bottles
2. Patient details on request form does not match with the container label
3. Specimen leaking from container (likely contamination of sample)
4. Specimen sent in non-sterile container
5. Swabs of endotracheal (ET) secretion
6. Tips of endotracheal tubes
7. Unlabelled specimen
8. Urinary catheter tips
9. Urine specimens - refrigerated for more than 24 hours
10. Urine specimens taken from a receptacle (catheter bag or bed pan)

## Types of containers issued from Microbiology Laboratory

<p style="text-align: center;">Blood and Bone marrow cultures</p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p style="border: 1px solid black; padding: 5px; width: 80px; margin: 0 auto;">Bactec Bottle</p> </div> <div style="text-align: center;">  <p style="border: 1px solid black; padding: 5px; width: 80px; margin: 0 auto;">In- house bottle</p> </div> </div>
<p style="text-align: center;">CSF culture</p>	<div style="text-align: center;">  </div>
<p style="text-align: center;">Urine culture</p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  </div> <div style="text-align: center;">  </div> </div>
<p style="text-align: center;">Pus and fluids</p>	<div style="text-align: center;">  </div>



## Types of containers issued from Microbiology Laboratory

<p>TB culture bottle</p> <p>(Obtain from microbiology laboratory and send back to microbiology laboratory)</p>	
<p>Swabs</p>	
<p>Fungal blood cultures</p> <p>(Obtain from microbiology laboratory and send back to same).</p>	
<p>Virology transport media</p> <p>(Obtain from microbiology laboratory and send back to same).</p>	

## Specimens for anaerobic cultures

1. Optimal time of specimen collection is before starting antibiotic treatment(local/systemic)
2. Specimen should be collected using aseptic techniques, and taking precautions to minimize contamination with endogenous microflora.
3. The best specimens would be those collected with a needle and syringe and biopsies taken during surgery.

### Transport

- All specimens must be transported as rapidly as possible with minimum exposure to oxygen. Therefore Samples for anaerobic culture should reach the laboratory as early as possible.
- Use anaerobic transport containers if available.
- If they are not available, transport pus in a sterile screw capped container or in a sealed syringe itself.
- Biopsies - sterile saline in a screw capped container.
- Stools for *Clostridium difficile* toxin and culture - in a wide mouthed container with a lid. Transport specimen in ice.
- Specimen should be sent to microbiology laboratory, LRH with accompanying MRI request form giving patient identification details and clinical details.
- Specimen will be sent to MRI from LRH microbiology laboratory.

## Blood culture

### Procedure for collection of peripheral blood for culture

1. Select the vein to be used for the venipuncture.
2. Clean with 70% ethyl alcohol and allow drying. It takes about 30 – 60 seconds for antiseptic action to occur.
3. Wipe concentrically starting at the center with 7.5% povidone iodine.
4. Allow iodine to dry for 2 minutes and avoid touching the site.
5. Label the bottle with the patient identification details, date, time and site of collection.
6. Wipe top of the blood culture bottle with 70% alcohol after removing the cap or the tape covering the lid. Allow to dry completely which usually takes about 30 - 60 seconds. Do not open the lid of the in-house blood culture bottles.
7. Wash hands with soap and water and wear sterile gloves before collecting blood.
8. Use a disposable sterile needle and syringe and draw blood taking aseptic precautions. Generally it is recommended to use a new sterile needle if the first attempt is not successful. Take precautions for the prevention of sharp injuries.
9. Volume of blood to be collected is as follows:

	<b>Bactec bottle</b>	<b>In-house bottle</b>
<b>Neonate</b>	Minimum of 1 ml	Minimum of 1 ml
<b>2-5 yrs</b>	Minimum of 2 ml	2-3 ml
<b>&gt;5 yrs</b>	2-3 ml	3-5 ml

10. Inoculate into the blood culture bottles carefully.
11. Thoroughly mix bottle to avoid clotting.
12. After phlebotomy, dispose needles and syringes in to a sharp bin.
13. Remove iodine from the skin with a 70% alcohol swab.
14. Specimen should be sent to laboratory immediately with accompanying properly filled Microbiology request form.
15. If there is a delay in transport store the bottles at room temperature. Do not refrigerate blood culture collected.
16. For infective endocarditis - Obtain 3 blood cultures depending on the urgency to start antibiotics. Preferably these samples should be collected 12 hours apart. If the patient needs antibiotics urgently all samples can be collected within 1 hour (with first and last samples drawn at least 1 hour apart) from different venepuncture sites.

## Bone marrow culture

1. Collect the specimen aseptically.
2. Label the bottle with the patient identification details, date, time and site of collection.
3. Wipe top of the blood culture bottle with 70% alcohol after removing the tape or the cap covering the lid. Allow to dry completely which usually takes about 30 - 60 seconds. Do not open the lid of the in-house blood culture bottles.
4. Inoculate into the blood culture bottle carefully.
5. Thoroughly mix bottle to avoid clotting.
6. Dispose needles and syringes in to a sharp bin.
7. Specimen should be sent to laboratory immediately with accompanying properly filled Microbiology request form.
8. If there is a delay in transport store the bottles at room temperature. Do not refrigerate.
9. For TB culture – use the appropriate bottle and the request form provided by the microbiology laboratory.

## **CSF culture, Cell count and Antigen testing**

Whenever feasible collect specimen before starting antibiotic treatment. Clean skin with 70% alcohol. Allow to dry for 30 seconds. Clean with povidone-iodine and wait for 1-2 minutes before inserting the needle.

1. Containers for collection:
  - a. Sample 1 – to Biochemistry for Sugar and Protein
  - b. Sample 2 – 1 ml into a sterile cryo-vial provided by microbiology laboratory both for culture and antigen detection
  - c. Sample 3 – 0.3 -0.5 ml for cell count
2. Transport immediately to the laboratory with appropriately filled request form. Document date and time of collection on the request form.

### **CSF obtained from EVD or shunt**

1. Clean site of puncture with 70% alcohol.
2. Wait for about 1 minute until it is dried.
3. Aspirate CSF Using sterile needle and syringe.
4. Containers for collection and transport as indicated above.

## Ear swabs/aspirates for culture

- Optimal time of specimen collection is before starting antibiotic/ antifungal treatment (local/systemic).

### Otitis media

1. Best specimen for otitis media would be the middle ear fluid aspirated by tympanocentesis.
  - Normally reserved for complicated, recurrent or chronic persistent otitis media.
  - Clean the external canal with mild detergent & aspirate fluid using a sterile syringe and a needle.
2. For perforated ear drum, collect the fluid using a sterile swab on flexible shaft inserted via an auditory speculum. Rotate swab & allow fluid to collect on swab. Ideally obtain 2 swabs (one for Gram stain and the other for culture).

### Otitis externa

1. Initially remove any debris using a moist swab.
2. Obtain sample by firmly rotating a fresh sterile swab in the outer canal.
3. Ideally obtain 2 swabs (one for Gram stain and the other for culture).

Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## Eye swabs and other specimens for culture

- Optimal time of specimen collection is before starting antibiotic/antifungal treatment (local/systemic).

### Conjunctival swabs

1. Wash hands with soap and water or clean with alcohol hand rub.
2. Sample both eyes by rolling over each conjunctiva using separate swabs pre-moistened with sterile normal saline.
3. Pass the swab firmly over lower lid fornix from nasal canthus to the lateral canthus.
4. Swab the normal eye with a separate swab. This helps to compare the identified pathogen with the normal flora.
5. Indicate on the request forms clearly.

### Corneal scraping

1. Needs to be done by trained staff in eye clinic.
2. Instill 2 drops of local anaesthetic.
3. Use a sterile spatula to scrape the corneal ulcers or lesions and inoculate scrapings directly on to medium.
4. Apply remaining material to 2 sterile glass slides for further study.

Corneal scrapings are collected (as given above) after anaesthetic drops are instilled. If swabs are collected specimens need to be collected prior to instillation of anaesthetic drops.



### **Intraocular fluids**

1. Needs to be collected by an eye surgeon using a sterile needle & a syringe.
2. Bedside inoculation is ideal. If not can transport the fluid in the syringe itself as the amount of sample is scanty. Make sure that the cap of the needle is in place and secure, to avoid any accidental needle stick injuries.

### **Corneal button**

1. Sample is collected by the attending surgeon at the time of surgery.
2. Send to the lab in a sterile screw capped container or directly inoculate into BHI broth at the bedside.

Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## **Faecal specimens for culture**

1. Optimal time of specimen collection is early in the course of diarrhoea, before starting antibiotic treatment.
2. Faeces specimen - If it is blood and mucous diarrhoea collect a portion of faeces containing blood and mucous. Peanut size portion is adequate. Collect into a clean/sterile, wide mouthed, screw capped container or leak proof container with a tight fitting lid.
3. Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## Fluids from sterile sites other than CSF

**Types of specimens :**

- Ascitic fluid
- Pleural fluid
- Synovial fluid (joint fluid)
- Peritoneal fluid
- Peritoneal dialysis fluid
- Pericardial fluid
- Other fluids obtained by aspiration (cysts etc.)

1. Optimal time of specimen collection is before starting antibiotic treatment.
2. Clean skin with 70% alcohol and povidone iodine.
3. Aspirate with sterile needle and syringe. Transfer into sterile screw capped bottle.
4. Specimen should be sent to lab immediately with accompanying request form giving patient identification and clinical details.
5. Date and time of collection should be written on request form.
6. Specimens collected after routine working hours should be stored at room temperature until transport to the laboratory on the following morning.

## **Investigation of infections associated with intravascular (IV) catheters**

### **While catheter is in-situ**

1. Need 2 specimens of blood collected at the same time into Bactec bottles.
2. One sample through the line (catheter) and the other from a peripheral site.
3. A similar blood volume from both sites is preferable. Therefore first take the specimen from the likely difficult site.

### **Collection of blood for culture from intravascular catheters**

1. Avoid drawing from lines within an hour of completion of administration of an antimicrobial agent.
2. Label bottles with patient identification details. In addition include collection time, whether the collection was from a peripheral vein or through the catheter.
3. Wipe the top of the blood culture bottle with 70% alcohol and allow drying completely usually for 30 to 60 seconds as for a peripheral draw.
4. Clean the catheter hub with alcohol and allow adequate drying (usually for 30 to 60 seconds).
5. Attach the syringe to the hub, draw a small volume of blood (suggestive amount 0.2 ml for paediatric patients) and discard without using it for culture.
6. Then using a new sterile syringe, collect recommended volume of blood for culture through hub taking aseptic precautions. Quickly reconnect tubing.

7. Inoculate into the blood culture bottles carefully, not more than the recommended volumes.
8. Thoroughly mix bottles to avoid clotting.

**Tip of intravenous catheter (when removing catheter on suspected infection)**

1. Clean skin at insertion site using 70% alcohol.
2. Allow surface to dry.
3. Remove catheter aseptically using sterile forceps.
4. Avoid contact of tip of catheter with skin.
5. Using a sterile pair of scissors, cut the distal 5-6 cm (area under skin) of the catheter.
6. Place the cut portion in a dry sterile screw capped bottle.

Please note that routine culture of intravenous catheter tip on removal is not recommended.

Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## Screening swabs for *Staphylococcus aureus* including MRSA

Screening of patients from high risk units on admission (ICU, burns unit and cardiac surgery patients) and others depending on specific clinical situations listed below.

- a) Screening of an individual patient in the event of recurrent staphylococcal sepsis
- b) If MRSA is isolated from one site in in-ward patients, for decolonization purposes
- c) Screening for MRSA carriage in the event of a suspected hospital outbreak (eg.post operative sepsis) which may be performed on patients as well as on health care workers as instructed by the infection control unit
- d) Screening carried out before high risk surgery such as cardiac, orthopaedic and neuro surgery

### Correct specimen type and method of collection

- Sterile cotton swabs are provided by the laboratory. Skin and nasal swabs should be collected with swabs moistened with sterile normal saline.
- Either attending medical officer or nurse should collect the swab.

#### Nasal swab

Swab both nostrils with a single swab in a circular motion.

### Throat swab

A swab of the back of the throat is taken by rotating a swab as it is moved gently across the throat.

### Perineal or groin swab

The swab should be rotated whilst being brushed across the area. Use a single swab for both sides.

Other sites –Send a swab from the site e.g. axilla, skin lesions, drain sites, wound sites, umbilical discharge etc.

Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## **Pus and wound swabs for culture**

1. Optimal time of specimen collection is before starting antibiotic treatment.
2. Tissue or fluid is always superior to swabs. Therefore whenever possible send aspirated pus even if it is a small quantity, rather than a swab.
3. If swabs are used collect two, one for culture and one for Gram stain.
4. Remove surface exudate with sterile saline.
5. Aspirate if possible or pass a swab deep into the lesion.(Aspirate abscess with a sterile needle and syringe).
6. Transfer aspirated material into a sterile container.
7. Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## Respiratory tract secretions for culture

Optimal time of specimen collection is before starting antibiotic treatment.

### Expectorated sputum

1. Ask the patient to gargle throat & rinse mouth with water (without antiseptics) & then to collect at least 1ml of sputum by deep coughing.
2. Postural drainage with help from a physiotherapist would be helpful when expectoration is poor.

### Induced sputum

1. Rinse mouth of the patient with water after brushing gums and tongue.
2. With the aid of a nebuliser, let the patient inhale approximately 25ml of sterile normal saline. (Steam inhalation also can be used for this purpose).
3. Collect sputum in to a sterile container.

Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.



### **Nasopharyngeal aspirate (NPA)**

1. Use a mucous collection device (eg: disposable mucous extractor).
2. Insert the nasogastric tube into the posterior naso-pharynx.
3. Apply suction, using intermittent suction as the tube is withdrawn.
4. Wash aspirate through tubing using 2 ml of sterile saline if sent for bacterial culture. Use viral transport medium (VTM) instead of saline if the specimen is sent for viral studies.

### **Throat swabs for culture**

Optimal time of specimen collection is before starting antibiotic treatment and antiseptic mouth washes

1. Explain the procedure to the patient.
2. Ensure adequate lighting.
3. Ask the patient to extend the neck and open the mouth.
4. Swab tonsils, soft palate, uvula and finally the posterior pharyngeal wall. Use of a tongue depressor may be helpful.
5. Care should be taken to avoid touching the tongue and other parts of the mouth.
6. Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.

## Urine for culture

- Please do not wash the bottle.
- Do not leave the container lid open prior to collection.
- Avoid touching the mouth of the container or inside part of the lid during collection.

### Method of collection

1. Clean the urethral area with soap and water.
2. When the child is passing urine allow the initial part of urine to pass out. Then from the mid part collect few milliliters of urine to the container.
3. Avoid touching the mouth of the bottle in urethral area when collecting.
4. As soon as urine is collected close the lid tightly.
5. Wipe the outer surface of the bottle with a piece of clean paper or tissue if available.
6. Do not hold the bottle under the tap to clean the outer surface.
7. Handover the specimen to ward nurse.
8. Specimen should be sent to lab with accompanying request form giving patient identification details and clinical details.
9. Specimens collected out of routine working hours of the microbiology laboratory should be kept in the refrigerator and send to lab the following morning without delay.

## **Serology tests**

### **Collection of blood samples for serology**

1. Clean skin with 70% alcohol.
2. Draw 2 ml of blood using a sterile, disposable syringe and needle.
3. Collect blood in to a dry sterile screw capped container.
4. Label bottle with patient identification data.
5. Send blood directly to the laboratory along with a properly filled request form.

### **Dengue Antigen and Antibody detection**

1. Collect the blood sample as mentioned above.
2. Document the duration of illness (number of days of fever) on the request form for result interpretation and for appropriate test selection.

## **TB culture**

Obtain container and specific request form from the microbiology laboratory, LRH.

### **Bone marrow for TB culture**

Add 2 ml of sterile normal saline to the sterile screw cap bottle provided and collect the bone marrow specimen into it.

### **Other specimens for TB culture**

Collect specimen to the sterile screw cap bottle provided.

### **TB PCR ( Xpert MTB/RIF)**

This will be performed specially on respiratory samples (sputum and early morning gastric aspirates) and CSF.

Test can be performed on lymph node aspirates and fluid aspirates from sterile sites.

Collect 2 ml volume to the sterile screw capped bottle provided.

(Currently WHO does not recommend Xpert MTB/RIF on blood, bone marrow, urine & faecal specimens).

Hand over the specimen and the specific request form to the microbiology laboratory, LRH.

Reference for Microbiology section:  
Laboratory Manual in Microbiology,  
Sri Lanka College of Microbiologists: 2011