

Document Name: Primary Specimen Manual



PRIMARY SPECIMEN MANUAL
(KEM/ LS/Patho/TP-1/PSM)

Department of Pathology
Seth G.S. Medical College & K.E.M. Hospital

Acharya Donde Marg, Parel (East), Mumbai-400012.

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Issue Date: 10.10.2023

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Professor and Head

Department of Pathology

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*HOD–Head of Department

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AMENDMENT RECORD

Sr No	PgNo	Section/ Clause / Para / Line	Date of Amendment	Amendment made	Reason for amendment	Signature of person Authorizing Amendment
01	15	6.3.5b	27.04.24	Ensure patient privacy with the use of curtains / screens	Provision of patient privacy was not documented	
02	01	--	07.04.26	- Dr Pradeep Vaideeswar as Head of Lab - New 2 tests viz. ESR & RDW added	- Previous Head of lab. Dr Asha Shenoy Superannuated - Expansion of scope of Clinical Pathology & Hematology Division	

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LIST OF ABBREVIATIONS

<u>Abbreviations</u>	<u>Full form</u>
CBC	Complete blood count
CVTS	Cardiovascular and Thoracic Surgery
ENT	Ear, Nose and Throat
ESR	Erythrocyte Sedimentation Rate
FNAC	Fine needle aspiration cytology
HOD	Head of Department
hrs	Hours
I/C	In charge
IPD	In patient Department
MSB	Multi-storeyed Building
OBGY	Obstetrics and Gynaecology
OC	Orthopedic Center
OPD	Outpatient Department
PPE	Personal Protective Equipment
Retic count	Reticulocyte count
TAT	Turnaround time

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1. FOREWORD

This Primary Specimen Manual has been prepared to provide an overview of the tests offered, their indications and limitations and also to facilitate the process of aseptic and standardized collection and transportation of clinical specimens for pathological investigations. Recipients of this manual are requested to share this manual with all members of the department which includes interns, residents, registrars, nursing staff and teaching faculty.

2. INTRODUCTION

‘The result of lab test is only as good as the quality of the specimen.’

A good quality specimen is an important pre- analytic criterion for the accuracy of a test result. This manual is intended to provide the clinicians and the laboratory personnel alike, the instructions on what constitutes appropriate specimens, and where and how they need to be sent/ transported.

The Department of Pathology offers diagnostic services for various diseases through its different divisions viz. Clinical Pathology & Hematology, Cytopathology, and Surgical Pathology, including Neuropathology and Cardiovascular Pathology. Apart from these divisions, the department also offers OPD services for processing samples of OPD patients, and emergency laboratory services (EMS) for specimens of emergency nature or from seriously ill patients, after routine hours. The records of specimens processed are maintained without affecting patient confidentiality by restricting access of these records to only laboratory staff.

QUALITY ASSURANCE

Services are provided using approved reagents and kits, calibrated equipment and controls, and trained and proficient manpower authorized by qualified pathologists. External Quality Assessment and continual improvement programs are in place to assure the quality of the results generated.

SCOPE

This manual is meant for all those health care workers who are involved with specimen collection, labeling, transport, storage, handling and disposal.

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PURPOSE

The purpose of this manual is to facilitate collection and transport of appropriate specimens in a manner that reduces the risk of exposure to blood and body fluids, maintains confidentiality as required and complies with standard collection protocols.

RESPONSIBILITY

a) Healthcare workers

- Should follow the recommendations / procedures described in this manual
- In case a clarification is required, should contact the division- in- charge (Clinical Pathology & Hematology, Cytopathology) or head of the department.
- Should follow standard precautions while collecting, handling, and transporting specimens.
- Ensure that appropriate specimen is collected in adequate quantity in appropriate containers which are labelled and transported along with an appropriately filled requisition form immediately to the laboratory.
- Biohazard spill should be attended to immediately.
- In the event of a needle stick injury, immediate action as per the protocol is indicated.

All healthcare workers (HCWs) should complete the full course of Hepatitis B vaccination and receive tetanus toxoid (TT).

b) Hospital administration

- Provide the containers and PPE as required for collection and transport
- Facilitate immunization of healthcare workers

c) Head of Laboratory

- Sensitize health care workers on procedures described in the manual through designated staff.
- Make a copy of the manual available to all the departments.

d) Supervisory Staff and Division – in- charge

- Periodically audit compliance and suitability of the procedures.
- Take corrective action in case non-compliance is detected.

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3. STANDARD PRECAUTIONS (Collection, handling, transport of Sample at Clinical areas)

These precautions should be followed by all health care workers to prevent the transmission of infectious agents while providing healthcare, which also includes specimen collection, handling, and transport.

- All clinical specimens should be considered as potentially infectious.
- All cuts and dressings should be completely covered with impervious dressing.
- Appropriate personal protective equipment should be worn while performing collection as per expected exposure risk (e.g. a pair of clean gloves, apron, mask).
- Hands should be washed before and after a procedure irrespective of glove use
- Where there is a risk of splash occurring, face shield and gown should be worn in addition.
- All spills of blood and body fluids should be decontaminated with an absorbent containing 0.5-1% sodium hypochlorite (freshly prepared) immediately
- Used items must be discarded as per KEMH waste disposal policy.

4. LABORATORY WORKING HOURS

The working hours, for the various divisions and specimen acceptance timings are provided in the tables below.

Routine working hours	Weekdays	9.00 am to 5.00 pm (Clinical Pathology Hematology & Division) 9.00 am to 4.00 pm (Cytopathology Division)
	Saturdays & Bank Holidays	9.00 am to 2.00 pm (Clinical Pathology Hematology & Division) 9.00 am to 1.00 pm (Cytopathology Division)
	Sundays & OPD holidays	Closed

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SPECIMEN ACCEPTANCE TIMINGS:

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	Division	Timing
ONLY Indoor patients (Hematology investigations)	Clinical Pathology & Hematology	9.30 am –10.30 am
OPD & IPD patients for FNAC	Cytopathology	9.00 am- 4.00 pm weekdays 9.00 am to 1.00 pm Saturday

5. TESTS/ SERVICES OFFERED:

Division /Location	Tests offered	Specimen type Where applicable	Contact Person with intercom number
Clinical Pathology & Hematology, 6th floor, Multistoreyed building	CBC with RBC indices	Whole Blood EDTA	Dr. Tejaswini Waghmare 022-24107556 / 7526
	Peripheral Smear study (PS) for RBC morphology, Differential WBC & morphology, Platelet morphology	Whole Blood EDTA	
	PS for Malarial Parasite	Whole Blood EDTA	
	PS for Filarial Parasite	Whole Blood EDTA	
	Reticulocyte count	Whole Blood EDTA	
	Sickling test	Whole Blood EDTA	
	Erythrocyte Sedimentation Rate (ESR)	Whole Blood EDTA	
Cytopathology Division, 2nd floor, Library Building	Fine needle aspiration cytology (FNAC)	--	Dr Kanchan Kothari/ Dr Mona Agnihotri 022-24107566

*Details about the specimen collection will be provided in the sections below.

- All sample containers should be adequately labeled/ bar-coded.
- All samples should be accompanied by adequately filled requisition form/ made entry in HMIS system.

6. TEST INDICATIONS AND LIMITATIONS

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Sr.no.	Specimen/test performed	Indications (major)	Limitations

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Clinical Pathology & Hematology Division			

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1	Blood For CBC	Monitor overall health	Cryoprotein/ cryoglobulins.

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	With RBCIndices	and diagnose wide	Erythrocyte agglutination

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	rangeof hematological	(cold agglutinin)	

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	conditions, including	Clotted Samples	

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	anemia, infection and	Hemolysed or Lipaemic	

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	leukemia	Samples	

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		Pseudo thrombocytopenia.	

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2	Peripheral Smear study (PS) for RBC morphology, Differential WBC count & morphology, Platelet morphology	Diagnose & monitor numerous hematological diseases (anemias, leukemias, acute and chronic infection, allergies, cancer care, autoimmune disorders)	Requires a skilled systematic approach artefacts may interfere results
3	Peripheral Smear Study For Malarial Parasite	Malaria	Low sensitivity in low parasitemia Laborious Cannot distinguish between past and present infections
4	Peripheral Smear Study For Filarial Parasite	Filariasis	Laborious, tedious Time of sample collection
5	Blood For Reticulocyte Count	Hemolytic anemias, Erythroblastosis fetalis, Response to iron or Vitamin B12 therapy	Confusion of reticulocytes with red cell inclusions like basophilic stippling, Recent blood transfusion. Mishandling, contamination of the sample.
6	Blood For Sickling Test	Sickle cell anemia	Erythrocytosis, hyperglobulinemia, extreme leukocytosis, hyperlipidemia can cause false positive results. Anemia, high levels of Hb F, and recent transfusions can cause false negative results.
7	Erythrocyte Sedimentation Rate (ESR)	Detecting and monitoring non-specific systemic inflammation, chronic infections, and certain cancers like plasma cell neoplasms	Long 60-minute testing time, high sample volume requirements, and susceptibility to manual error.

CYTOPATHOLOGY DIVISION

1	FNAC	Pathologic diagnosis of superficial or deep-seated mass lesions e.g. Thyroid, Lymphnode, Breast, Salivary gland, intra-abdominal lesions, Soft tissue, etc.	Architecture cannot be studied. Intra-lesional heterogeneity may lead to sampling errors and affect diagnosis
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7. SPECIMEN COLLECTION (General Instructions)

A) General instructions and Pre-collection activities

1. Confirm the identity of the patient
2. Explain the procedure to the patient and obtain consent (as appropriate)
3. Wear appropriate PPE.
4. Prepare patient as required for the collection
5. Ensure patient privacy with the use of curtains/screens.
6. Collect the specimen aseptically
7. Collect at the appropriate time (wherever recommended) and inadequate quantity (Appendix1, page 23)
8. Label the specimen container with date, name, registration number, ward, unit, specimen, and test required.
9. Fill the requisition form completely, legibly and sign before transporting to the laboratory/ Print the bar code by entering details and paste that bar-code to test- specific vacutainers.
10. The minimum details required in the requisition form/ HMIS data entry in computer would include:

Name, age, gender, registration number, ward, unit, specimen, date of collection, time of collection where applicable, site from where specimen was collected (where applicable), presumptive diagnosis, nature of investigation required. Any other relevant clinical information if provided will be of assistance.

11. Remove PPE and discard in the red bag. Masks are discarded in yellow bag
12. After collection, close the container and keep in upright position
13. If the outside of the container is contaminated while collection, decontaminate with 70%alcohol or 0.5% sodium hypochlorite (1:10dilution) wipe.
14. Wash hands and dry with a clean towel or use an alcoholic hand rub.
15. If during collection / handling / transport the specimen container breaks, evacuate areaadjacent, inform sister-in-charge / place a large absorbent immediately, and instructlabor staff to immediately follow spill control protocol.
16. Specimens which do not satisfy acceptance criteria will be rejected (page 20)

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a. **Note**

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- The type of specimen required, their quantity for the various investigations carried out in the different divisions and their turnaround time are mentioned at the end of this manual. (Appendix 1, page 24)
- No emergency testing is done at the Clinical Pathology & Hematology Division and reports are issued as per the turnaround time mentioned in the appendix.
- Specimens will not be stored for any other investigation.
- No verbal requests will be entertained for testing.

8. SPECIMEN COLLECTION- BLOOD FOR HEMATOLOGICAL INVESTIGATIONS

[In Clinical Area ONLY]

The resident doctors/ the nursing staff of particular ward is responsible for specimen collection of indoor patients

- Blood collection is performed only by well-trained experienced phlebotomists (Laboratory technicians/Doctors).
- Ensure that the patient is at least 2 hours fasting before specimen collection.
- Requirements – Gather material required for collection and biomedical waste disposal.
 - This includes- Tourniquet, Alcohol wipes, Sterile syringe and needle (21G preferably) or appropriate evacuated tube sets, cotton ball, gloves, alcoholic hand rub solution, collection container - preferably pre-labelled/ pre- bar coded [clean / sterile , dry test tube or evacuated tubes - purple
 -
 - cap for EDTA], sharps can, requisition form, red bag, and yellow bag.
- If multiple collections are done using the same gloves, and if the gloves are visibly clean, the same pair of gloves can be used provided the gloves are disinfected after every collection using 70% alcohol/ alcoholic hand rub.
- In case there is contamination with blood, the gloves should be removed immediately and discarded in the red bag and replaced with new pair of plastic and latex gloves.

Procedure

- Help the patient sit comfortably on a chair with an arm rest / or lie down on a bed/couch.
- Use alcoholic hand rubs to disinfect your hands.
- Wear plastic and clean latex gloves. Also wear a plastic apron if required.

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- Place absorbent material (cotton/gauze piece) below the patient's elbow to avoid soiling due to any

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leakage.

- Inform patient about the collection and the discomfort that is likely to be felt [a small prick like an insect bite].
- Pre label with the name, registration number, unit, specimen, type of investigation requested and the date and time of specimen collection / pre - bar code the collection vacutainer.
- Tie a tourniquet above the site of blood collection to make the vein prominent. [This is usually above the patient's anterior cubital fossa on the forearm].
- Instruct the patient to clench his/her fist while collection is on.
- Disinfect the site of collection [patient's] with an alcohol swab [clinical spirit, 70% ethyl or isopropyl alcohol].
- After use, discard the alcohol swab in the yellow bag.
- Take a new sterile needle [preferably 21 G for an adult and 22 G for a child] and syringe /sterile evacuated tube set in front of the patient. The needle is attached to the syringe.
- Discard the paper/plastic cover of the syringe and needle in the blue bag.
- Insert the needle aseptically into the vein at an angle of 45 degrees.
- Allow blood to flow and collect 3-6ml/as per evacuated tube capacity.
- Release the tourniquet.
- Tell the patient to release the clenched fist.

- Withdraw the needle slowly and place a dry cotton swab at the puncture site.
- Ask patient to keep the elbow flexed until blood flow stops. [Usually 2-5 minutes]
- If syringe has been used, transfer the blood gently along the wall without squirting into appropriate pre-labelled/ pre bar coded collection vacutainer.
- Discard in the designated sharp can.
- Where collection is done at the laboratory, ask patient to leave after checking that there is no bleeding from the puncture site and to discard the used cotton swab in the yellow bag.
- Any used cotton/ gauze should be discarded in yellow bag.

9. SPECIMEN COLLECTION – FNAC (For Cytopathology Investigations)

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FNAC should preferably be performed by Pathologist and patient should be referred to

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Cytopathology laboratory for the procedure.

In special circumstances (e.g. Endoscopy/ USG guided) if FNAC is done by clinicians/ radiologists and slides are sent to laboratory, procedure for FNAC is outlined below

- Explain the procedure to the patient clearly.
- Consent for FNAC to be taken
- Identify the lesion
- Clean the skin over site of aspiration with a spirit swab.
- Take a 23-26 gauge sterile needle and attach a 10 cc -sterile syringe to it.
- Fix the lump with one hand for superficial swellings.
- Insert the needle in the target tissue.
- Now withdraw the plunger (piston), to apply negative pressure.
- Move the needle back and forth rapidly within the target tissue.
- Release the negative pressure before withdrawing the needle from the tissue.
- Detach the needle from the syringe.
- Draw air into the syringe.
- Attach syringe back again to the needle.
- Expel out the aspirate onto the microscopy slides with the bevel of the needle facing the slide and close to it.
- Spread the aspirate with the flat of another glass slide applying a light pressure to achieve an evenly thin smear.
- Immerse some slides in an alcohol fixative kept in a Coplin jar immediately after preparation for PAP staining.
- Rapidly air dry few slides to prepare air-dried smears for Giemsa staining
- After withdrawing needle from the patient, apply a spirit swab to the site with pressure to prevent bleeding.
- Label the Coplin's jar and Air dried slides with patient details (Name, Unit, OPD/IPD number, Test name) and send to Cytopathology laboratory

10. DISPOSAL OF WASTE GENERATED (as applicable)

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- Segregate waste into appropriate color coded bags/containers.

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- Discard all blood soaked non plastic items in yellow bags, all used plastics in red bag, and all sharps in sharp waste disposal container.
- Do not disassemble needle and syringe assembly. Discard the assembly in sharp waste disposal can.
- Fill the bags/containers only to 3/4th of its capacity.
- Untreated waste should not be stored beyond 48 hrs.
- The red and yellow bags and the sharp cans should be tied, labeled, entered in log book and sent to temporary biomedical waste storage room near gate number

11. NEEDLE STICK INJURY PROTOCOL

Needle- stick injury, while collecting/ transporting/ handling/ disposing specimens/ collection devices, is an indication for post exposure prophylaxis (PEP).

Procedure to be followed when exposure has occurred

- Wash the area with soap and water
- Avoid squeezing or milking the wound
- Do not use caustic agents, such as bleach
- Inform your superior and consult ART (anti-retroviral therapy) center, Ground floor, MSB, during routine hours for PEP drugs.
- After routine hours, consult MICU (2nd floor, main hospital building) for PEP drugs
- The medical officer at each of these places will determine risk i.e. Type of exposure and Infection status of source and decide on treatment
- Get Lab tests done and follow up in 3-6 months
- Follow medical officer's advice for duration of PEP.
- It is important to initiate PEP as early as possible and within 2 hours.

12. SPILL PROTOCOL

For spills with blood and body fluids

- Clear the area of spill and start spill containment

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Instruct the housekeeping staff on the protocol which is as follows:

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- Don appropriate personal protective equipment (impervious gown, gloves, face shield or goggles as appropriate and boots if spill is large.).
- Wear heavy duty gloves and then pick up any broken glass with the help of forceps and discard into a sharps container.
- Cover spill with paper towels /absorbent (gauze) and allow soaking.
- Discard in yellow bag.
- Cover spill again with paper towels/absorbent (gauze).
- Squirt disinfectant (1% Na hypochlorite; 1:5 dilution) on to absorbent with circular motion, from the outside towards the center.
- Allow to stand for at least 10 minutes.
- Discard used paper towels/absorbent (gauze) in the yellow biohazard bag.
- Mop the area with 1% Na hypochlorite.
- Disinfect the heavy duty gloves and forceps with 1% Na hypochlorite before storage, wash well in running water and store dry.

13. SPECIMEN TRANSPORT

- The transport of specimens should be done as soon as possible to the respective divisions, preferably within 2 hours of collection (particularly for Hematological investigations) along with the completely filled and signed requisition form. Check specimen acceptance timings.
- Place the specimen container in a tray / container in such a manner that it remains upright and does not spill/ fall. Do not transport specimens in apron or shirt pockets.
- The person transporting the specimen should be instructed as to the location for the test and provided with gloves by the clinician and sister-in-charge respectively.
- If specimens are not transported as per requirement particularly for Hematological investigations, they may be rejected. (see rejection criteria below)
- The sample entries are made in HMIS and are bar-coded/ if not then requisition forms should accompany the specimen and should not be placed in the same tray as the specimen. Do not wrap the requisition form around the specimen container.
- The specimens and forms should be transported in a separate container/tray.

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REQUISITION FORMS SOILED WITH SPECIMEN WILL NOT BE ACCEPTED.

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14. SPECIMEN RECEPTION AND ACCEPTANCE

- The specimens are accepted at the reception counter for each division
- This section is manned by a trained laboratory technician and assistant /laboratory attendant or resident who also guides the patients for other investigations if required.
- The designated person checks transport conditions and instruct for corrections if deviations found.
- Validate the details by checking entries of samples through HMIS system/ or on the requisition form with the specimen and the label on the container.
- If appropriate, the dispatch is signed
- Acceptance is based on the following criteria being satisfied.

SPECIMEN ACCEPTANCE CRITERIA:

General :

- Entry in HMIS system/ Completely filled and signed requisition form
- Details on bar code/ label of specimen container and requisition form should match

a) For Clinical Pathology & Hematology Division:

- Appropriate specimen: Whole blood EDTA
- Appropriately bar coded / labelled container: Patient's Name, ward number, Indoor registration number
- Appropriate volume: minimum quantity 2 ml
- Appropriate transport: Section 13
- No breakage/ leakage/ soiling of container/requisition form

b) for Cytopathology Division:

- Smears to be sent in Coplin's Jar with Absolute alcohol and/ or Air Dried

15. CRITERIA FOR SPECIMEN REJECTION

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General :

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- No entry in HMIS & no requisition/ Incomplete requisition
- Mismatch between details on HMIS entry/ requisition form and specimen container
- No signature of clinician on requisition form

A) For samples in Clinical Pathology & Hematology Division:

- Soiled/ blood-stained requisition form (specimen is accepted; new form is asked)
- Specimen transport time has exceeded two hours
- Leaking or broken specimen container
- Specimen not barcoded/ labelled
- Specimen in wrong container
- Insufficient specimen quantity
- Hemolysed /Clotted blood specimen for hematological investigations
- Lipemic blood specimen for hematological investigations

B) For samples of Cytopathology Division:

- Broken slides
- Sample sent in Formalin

16. REPORT DISPATCH

The reports are delivered through various modes:

Clinical Pathology & Hematology division:

- If specimens are received through HMIS entries, then reports are approved online, through HMIS
- If specimens are sent without HMIS entries (offline), reports of the IPD patients are handed over to the nursing staff on duty/ the resident doctor in respective wards. Record of the same is maintained in the report dispatch book.

Cytopathology division:

- If patients' entries are done through HMIS system, then reports are given on line through HMIS system.
- If not, then FNAC reports are handed over to patient/ authorized representative at the Cytopathology laboratory reception.

17. COMPLAINTS

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For any complaints pertaining to any of the services offered, a note may be sent any time to the

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HOD to facilitate correction as required and improvement of services. Clinicians are also requested to fill the annual feedback forms with relevant suggestions for improvement.

18. REFERENCES

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- 4) Ruilan Du, et al . A low-cost, accurate method for detecting reticulocytes at different maturation stages based on changes in the mitochondrial membrane potential. Journal of Pharmacological and Toxicological Methods.2020, ISSN 1056-8719,
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APPENDIX 1

Tests offered and their TAT

Division	Test Offered	Volume	TAT After specimen receipt
Clinical Pathology & Hematology Division	CBC with RBC indices	2 ml blood in an EDTA test tube or purple cap evacuated tube	Within 10 hours
	PS study for RBC morphology, Differential WBC count & morphology and platelet morphology	2 ml blood in an EDTA test tube or purple cap evacuated tube	

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Laboratory Services
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Seth GS Medical College and KEM Hospital

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	Reticulocyte count	1-2 ml blood in an EDTA test tube or purple cap evacuated tube	
	Sickling test	1-2 ml blood in an EDTA test tube or purple cap evacuated tube	
	PS for Malarial Parasite	1-2 ml blood in an EDTA test tube or purple cap evacuated tube	
	PS for Filarial Parasite	1-2 ml blood in an EDTA test tube or purple cap evacuated tube	
	Erythrocyte Sedimentation Rate	2 ml blood in an EDTA test tube or purple cap evacuated tube	
Cytopathology Division	FNAC	Material is aspirated Air dried and alcohol fixed smears to be made	3 working days

APPENDIX 2

(Requisition form): If samples received offline

LABORATORY SERVICES
Dept. of Pathology
Seth GSMC and KEM HOSPITAL

बृहन्मुंबई महानगरपालिका
विकृतिशास्त्र आणि अणुजीव शास्त्र विभाग
विकृतिचिकित्सा

रुग्णालय

दिनांक २० आंतर/बाह्य रुग्ण नोंद क्रमांक

वक्ष/विभाग खाट क्र. डॉ.

नांव

पुरुष/स्त्री वय व्यवसाय

चिकित्सालयीन रोग निदान रोगाचा कालावधी

पाठविलेली सामुग्री

आवश्यक तपास

व्याधि विवरण

पूर्वीची प्रयोगशालेय तपासणी

अन्य संबंधित माहिती

विकृति चिकित्सेचे निष्कर्ष

दिनांक रोजी सकाळी/दुपारी वाजता बोलाविले.

Sample	Date	Time
Collected		
Received		

वैद्यकीय अधिकारी

HP1-BMPP-65284-2023-24-5,000 Pads (200 Lvs)(M-2)

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Kindly send your suggestions if any

to

The office of

Professor and Head,

Department of Pathology

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