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SAMPLE COLLECTION & TRANSPORT MANUAL

AS PER ISO 20658:2023

Guidelines for the collection, handling, labelling, transport and receipt of samples in clinical laboratories.



✓ PATIENT SAFETY | ◉ SAMPLE INTEGRITY | ▣ SAFE TRANSPORT | ☑ QUALITY ASSURANCE | ★ ISO 20658 COMPLIANCE

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Approved by	Medical Director, JDWNRH

JIGME DORJI WANGCHUCK NATIONAL REFERRAL HOSPITAL

Thimphu, Bhutan

11001

SAMPLE COLLECTION AND TRANSPORT MANUAL

Department of Pathology and Laboratory Services

Based on **ISO 20658:2023** and **ISO 15189:2022**

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Version 1

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Foreword

This manual has been developed by the Department of Pathology and Laboratory Services (DPLS) of Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Thimphu, Bhutan, in alignment with the International Standard ISO 20658:2023 - Requirements for the Collection and Transport of Samples for Medical Laboratory Examinations and its normative reference, ISO 15189:2022 - Medical Laboratories: Requirements for Quality and Competence. Aligning this manual with the international standards provides a solid foundation in preparation for standardization and certification of laboratory processes in the near future.

The pre-examination phase encompassing the examination request, patient preparation, patient identification, sample collection, and transport to the laboratory is the single largest contributor to diagnostic error in clinical laboratory diagnostics. Studies consistently demonstrate that 46–68% of all laboratory errors originate in the pre-analytical phase. This manual is designed to substantially reduce these errors through standardised, evidence-based procedures applicable to all clinical areas of JDWNRH.

This manual applies to all personnel involved in sample collection and transport at JDWNRH, including phlebotomists, nurses, doctors, ward assistants, and external transport staff. Compliance is mandatory. Personnel must not deviate from the procedures described herein without written authorisation from the Head of DPLS.

This document is subject to periodic review (every two years, or following significant change) and is maintained under the DPLS's document control system. Users are responsible for ensuring they are working from the current approved version.



Medical Director

Jigme Dorji Wangchuck National Referral Hospital,

Thimphu, Bhutan

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1. Scope and Applicability

1.1 Purpose

This manual specifies the requirements and recommended practices for the identification, collection, handling, storage and transport of patient samples intended for medical laboratory examinations at Jigme Dorji Wangchuck National Referral Hospital (JDWNRH). It provides procedures, criteria, and guidance sufficient for clinical staff to perform these activities consistently, safely, and in a manner that preserves sample integrity.

1.2 Applicability

This manual applies to:

- All clinical departments and wards of JDWNRH including outpatient departments (OPD), and any clinical units that source samples to DPLS JDWNRH for diagnostic testing.
- The JDWNRH sample collection unit
- All JDWNRH employees or contracted sample collection providers, performing sample collection or transport to or on behalf of JDWNRH.

1.3 Related Documents

Document	Reference Number	Relationship
ISO 20658:2023	ISO 20658:2023	Normative - this manual implements ISO 20658:2023
ISO 15189:2022	ISO 15189:2022	Normative reference - laboratory QMS framework
JDWNRH Laboratory Quality Manual	JDWNRH-LAB-QM-001	Parent quality management system document

2. Normative References

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this manual. For dated references, only the edition cited applies.

- ISO 20658:2023 -Medical laboratories -Requirements for the collection and transport of samples for medical laboratory examinations (First Edition, May 2023)
    - ISO 15189:2022-Medical laboratories-Requirements for quality and competence (Fourth Edition, 2022)
- ISO 22870:2016-Point-of-care testing (POCT)-Requirements for quality and competence
- WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy (WHO/EHT/10.10, 2010)
- IATA Dangerous Goods Regulations-Category B Biological Substance, UN 3373 (current edition)
- UN Model Regulations-UN P650 packaging requirements for biological substances
- CLSI H3-A6- Procedures for the Collection of Diagnostic Blood samples by Venipuncture
- CLSI GP41-A6-Collection, Transport, and Processing of Blood samples for Testing Plasma-Based Coagulation Assays

3. Terms and Definitions

For the purposes of this document, the following terms and definitions apply (adapted from ISO 20658:2023 and ISO 15189:2022):

Term	Definition
Examination request	A formal instruction, electronic or paper-based, from an authorised healthcare professional requesting one or more laboratory examinations on a specified patient
Pre-examination processes	Steps that begin with the clinician's examination request and end when analytical examination begins; includes request, patient preparation, collection, transport, and pre-analytical preparation

Term	Definition
Primary sample	The original sample drawn or collected directly from a patient for examination or analysis (e.g. whole blood in a blood tube, urine in a container)
Aliquot	A portion of the primary sample transferred to a secondary container for examination
Sample integrity	The condition of a sample that ensures examination results accurately reflect the analyte concentration or characteristic in the patient at the time of collection
Haemolysis	Disruption of red blood cell membranes releasing intracellular contents into the serum or plasma, resulting in visible reddish discolouration and interference with multiple analytes
Lipaemia	Turbidity in serum or plasma due to excess lipids (triglycerides >3.4 mmol/L), causing interference with spectrophotometric assays
Icteric sample	A serum or plasma sample that is excessively yellow due to elevated bilirubin (>342 µmol/L), causing potential interference in some assays
Order of draw	The recommended sequence for collecting blood into multiple tubes during a single venepuncture to prevent additive carryover between tubes
Chain of custody	A documented, unbroken record of sample possession, transfer, and handling from collection to final disposal
Competency	Demonstrated ability to apply knowledge and skills to perform a task correctly and consistently according to defined standards
Non-conforming sample	A sample that fails to meet one or more acceptance criteria, rendering it unsuitable for analysis as submitted
Rejection criteria	Pre-defined conditions under which a sample will not be accepted for examination, or examination results will not be reported without qualification

Term	Definition
Transport medium	A liquid or gel medium designed to maintain the viability of microorganisms during transport to the laboratory
Point-of-care testing (POCT)	Laboratory examination performed near the patient, outside the central laboratory, by clinical staff using portable or bedside analysers
Minimum sample volume	The least volume of primary sample that must be collected to permit examination of all requested tests with appropriate quality
Turnaround time (TAT)	The elapsed time from sample collection (or receipt) to reporting of results

4. Abbreviations

Abbreviation	Full Form
ABG	Arterial Blood Gas
AFB	Acid-Fast Bacilli
APTT	Activated Partial Thromboplastin Time
BAL	Bronchoalveolar Lavage
BMA	Bone Marrow Aspiration
CBC	Complete Blood Count
C&S	Culture and Sensitivity
CSF	Cerebrospinal Fluid
CSU	Catheter Specimen of Urine
DAT	Direct Antiglobulin Test
DPLS	Department of Pathology and Laboratory Services
EDTA	Ethylenediaminetetraacetic Acid

Abbreviation	Full Form
EMU	Early Morning Urine
ESR	Erythrocyte Sedimentation Rate
FVU	First-Voided Urine
HbA1c	Glycated Haemoglobin
HIV	Human Immunodeficiency Virus
HVS	High Vaginal Swab
ICU	Intensive Care Unit
INR	International Normalised Ratio
ISO	International Organization for Standardization
JDWNRH	Jigme Dorji Wangchuck National Referral Hospital
LDH	Lactate Dehydrogenase
LFT	Liver function test
MRN	Medical Record Number
MRSA	Methicillin-Resistant Staphylococcus aureus
MSU	Midstream Urine
NICU	Neonatal Intensive Care Unit
O&P	Ova and Parasites
OPD	Outpatient Department
OT	Operating Theatre
POCT	Point-of-Care Testing
PPE	Personal Protective Equipment
PT	Prothrombin Time
QMS	Quality Management System

Abbreviation	Full Form
RCOG	Royal College of Obstetricians and Gynaecologists
RFT	Renal Function test
SAF	Sodium Acetate-Acetic Acid-Formalin (fixative)
SOP	Standard Operating Procedure
SPA	Suprapubic Aspirate
SST	Serum Separator Tube
STAT	Statim - immediately/urgent
STI	Sexually Transmitted Infection
TAT	Turnaround Time
TFT	Thyroid Function Tests
VBD	Vector-Borne Diseases
SPS	Sodium polyanethol sulfonate

5. Management Requirements

5.1 Quality Management System

DPLS, JDWNRH maintains a documented quality management system (QMS) covering all pre-examination activities in accordance with ISO 15189:2022 Clause 8 and ISO 20658:2023 Clause 5. The QMS includes:

- Documented policies and procedures for all pre-examination processes
- Mechanisms for identification, documentation, and correction of non-conformities
- Processes for continued improvement of pre-examination quality
- Management review of pre-examination performance indicators at defined intervals (at least annually)

5.2 Responsibility and Authority

The Head of Department of DPLS holds overall responsibility for this manual and for the quality of all pre-examination processes at JDWNRH. Delegated responsibility is as follows:

Role	Responsibility
Head of DPLS	Overall accountability; approval of SOPs; management review
Laboratory Quality Manager	Document control; audit programme; non-conformity management; training oversight
Unit Incharges	Monitors samples collected safely, correctly, and efficiently, with proper documentation, staff supervision, and compliance with procedures.
Phlebotomist In-charge	Day-to-day supervision of the phlebotomy unit; competency assessment of phlebotomy staff.
Ward Nurse In-Charge	Ensuring ward nursing staff are trained and competent for IPD ward sample collection
Emergency Department Nurse Incharge	Ensuring ED staff follow STAT collection protocols
ICU Nurse Incharge	Critical care sample collection protocols; ABG handling
Infection Control focal	PPE, hand hygiene, and biohazard compliance in sample collection
All Staff collecting samples	Compliance with this manual; immediate reporting of non-conformities and incidents

5.3 Personnel Competency and Training

All personnel performing sample collection or transport at JDWNRH:

- Receive documented training on this manual before performing independent sample collection

- Demonstrate initial competency through supervised practice and competency assessment before working independently
- Undergo annual competency reassessment covering venepuncture technique, patient identification, order of draw, labelling, and sample transport
- Receive documented training on relevant safety procedures including sharps safety, PPE use, and management of blood/body fluid exposure

Competency records maintained in the personnel file and in the laboratory training register. The competency assessment tools are held in QSP-15 of QMS (F-PER-03).

WARNING: Competency reassessment shall be triggered immediately following any significant pre-analytical error, near-miss, or patient safety incident attributable to sample collection or transport.

5.4 Document Control

All documents within the scope of this manual are controlled in accordance with the JDWNRH Document Control Procedure (QSP-2 of QMS). Specifically:

- Only current, approved versions shall be used in clinical practice
- Superseded versions shall be removed from circulation and archived
- Proposed amendments shall be reviewed by the Laboratory Quality Manager and approved by the Head of DPLS before implementation
- A master copy list shall be maintained by the Laboratory Quality Manager

5.5 Equipment for Sample Collection

All equipment and other articles used for sample collection (tourniquets, needles, syringes, tubes, lancets, containers cotton balls or antiseptic swab, PPE, vacutainer holders, Barcode labels and Disposal container) are:

- Sourced from approved suppliers on the JDWNRH Approved Supplier List
- Stored in designated, clean areas away from clinical waste and contaminants
- Used within their stated expiry date - expired consumables must not be used under any circumstances

- Inspected visually before use - damaged, deformed, or compromised items must be discarded
- Disposed of safely in designated sharps or clinical waste containers immediately after use.

Examination Request

6.1 General Requirements

A valid examination request must accompany every sample to be collected or submitted to the laboratory for testing. The request may be electronic (through the JDWNRH electronic Patient Information System, ePIS) or in paper format prescription with sign and seal of the prescriber. No sample shall be collected, received or processed without a valid, identifiable examination request.

6.2 Minimum Information Requirements

Every examination request shall contain, at minimum, the following information:

Data Element	Required?	Notes
Patient full name	Mandatory	As per official medical record - no abbreviations
CID	Mandatory	Primary unique identifier at JDWNRH
UHID	Mandatory	Secondary unique identifier at JDWNRH
Date of birth	Mandatory	For age-specific reference intervals
Gender / sex	Mandatory	Affects reference intervals and test interpretation
Ward / OPD / department	Mandatory	For sample return and clinical communication
Date and time of collection	Mandatory	Critical for result interpretation and sample stability
Name/MHPC No of requesting clinician	Mandatory	Accountability and result communication
Specific examinations requested	Mandatory	Unambiguous test names or code
Urgency (STAT / routine)	Mandatory	Determines processing priority and TAT
Sample type if non-standard; If it's not given	Conditional	e.g. CSF, BAL, joint fluid must be specified

Data Element	Required?	Notes
in the dropdown lists (Epis).		

6.3 Incomplete Requests

If a request is received with insufficient patient identification details or missing mandatory information, the laboratory:

1. Contacts the requesting clinician or ward immediately and document the communication
    a. Holds the sample pending clarification - do not process unless the incompleteness to be resolved
2. Finds if the sample is irreplaceable (e.g. CSF, bone marrow, intraoperative tissue) or urgent, proceed with testing and document the deficiency; notify the clinician and request corrected information
3. Records the non-conformity in the laboratory non-conformity log using QSP-08, F-NCR-01 of QMS

CRITICAL: Samples with no patient identification whatsoever shall not be processed. A new, correctly labelled sample must be collected.

6.4 Urgent / STAT Requests

STAT requests require immediate communication with the laboratory. The requesting clinician or ward nurse:

- Telephones the laboratory before or at the time of sample collection to alert laboratory staff
- Clearly marks the request form and sample label with 'STAT' or 'URGENT' and mention sample source (ward, unit, department)
- Ensures the sample reaches the laboratory within 15 minutes of collection for STAT analytes (glucose, electrolytes, ABG, troponin, blood gases)

7. Patient Preparation

7.1 General Principles

Inadequate patient preparation is a leading cause of pre-analytical variation. The collecting staff is responsible for verifying that appropriate patient preparation has been followed before proceeding with collection. If preparation is inadequate, the collection is deferred and rescheduled, unless clinical urgency overrides this.

7.2 Fasting Requirements

Test	Fasting Requirement	Notes
Fasting glucose	8-12 hours (water permitted)	Morning collection preferred
Fasting lipid profile (cholesterol, triglycerides, LDL, HDL)	8-12 hours	Avoid alcohol 24 hrs prior
Fasting insulin / C-peptide	8-12 hours	-
Oral glucose tolerance test (OGTT)	8-12 hours; no exercise	Patient must remain seated for entire test Dissolve 75 grams of glucose in 250-300 ml of water and consume within 5 minutes. SOP ??
Random glucose	Not required	Document time of last meal on request
Routine biochemistry (RFT LFT, TFT, CRP)	Not required	Though fasting preferred for lipid-linked panels
Haematology (CBC, ESR, coagulation)	Not required	-
Iron studies,(TIBC)	Morning fasting preferred	Iron shows diurnal variation - morning collection recommended
Cortisol	Not required	Document exact collection time, Avoid heavy exercise and physical stress

Test	Fasting Requirement	Notes
	Morning 7:00-9:00 AM collection(basal) Evening PM collection(3.00-5.00 pm) 8 hours gap	
Testosterone, LH, FSH	Morning preferred;	Document time

7.3 Other Pre-Collection Requirements

- Posture: Patient is seated and relaxed for at least 15 minutes before blood collection. Patients who were lying (inpatient) are kept in the same (seated) position unless clinically inappropriate.
- Exercise: Strenuous exercise within 24 hours of collection is documented; it significantly affects CK, LDH, potassium, and lactate.
- Medications: Some medications require dose-specific timing particularly TDM SOP (JDW/PHARM/TDM/02), (e.g. trough/peak levels for aminoglycosides, vancomycin). Document the time on the request form. Do not withhold medications without clinical authorisation.
- Alcohol: Patients are refrained or advised to avoid alcohol for at least 24 hours before lipid profiles and liver function tests.
- Smoking: Note smoking status; nicotine affects catecholamines, cortisol, and certain tumour markers.
- Hydration: Dehydrated patients may have falsely concentrated analytes; document clinical status.

8. Patient Identification

8.1 The Three-Point Identification Check

Patient misidentification is one of the most serious and preventable errors in the pre-examination process. Every sample collection episode shall begin with a three-point identification check, regardless of how well the collector believes they know the patient.

CRITICAL: NEVER identify a patient from a bed number, room number, or verbal confirmation from a third party alone. ALWAYS apply all three identification points directly with the patient.

The three identification points are:

Point	Method	Action
1.CID	Ask for the CID	Verify CID and Patient details
2. Ask the patient to state their full name	Open-ended question: 'Please tell me your full name'	Do not suggest the name the patient must state it unprompted
3. Ask the patient to state their date of birth	Open-ended question: 'Please tell me your date of birth'	Do not suggest the date the patient must state it unprompted

8.2 Situations Requiring Special Identification Procedures

Situation	Procedure
Unconscious / sedated patient	Use medical record ONLY; a second staff member must verify; document double-check
Confused / dementia patient	Two-staff verification mandatory; use medical record; do NOT rely on verbal response alone
Neonates and infants	Parent/guardian states name; check wristband (maternal wristband if baby not yet individually banded); cross-check with medical record
Emergency - unknown patient	Use temporary emergency ID (e.g. 'UHID'); document on both request form and label; update when identity confirmed
Identical name on ward	Flag to ward In-charge; verify by additional identifiers (UHID, DOB,Bed No, diagnosis)

8.3 At Point of Collection

Before proceeding with sample collection confirm the identification of the patient and complete labeling of the tube/ container at the point of care.

Note : never pre-label tubes in advance.

9. Sample Labelling

9.1 Mandatory Label Information

Every primary sample container must bear a label, applied at the point of care immediately after collection, containing the following minimum information:

Element	Required on Label	Notes
Patient full name	Yes	Exactly as on request and wristband
CID & UHID	Yes	Primary unique identifier
Age/ sex	Yes	Verify against request
Date of collection	Yes	DD/MM/YYYY format
Time of collection	Yes	24-hour HH:MM format

9.2 Labelling Rules

- Labels shall be machine-printed (from ePIS barcode system) wherever available.
- Labels must be affixed to the tube/container body and never to the lid/cap
- Labels must not cover any part of the tube that is used to assess sample volume or appearance
- Barcoded labels must be scannable without removing the label from the tube
- Where multiple aliquots are produced from one collection, each aliquot must be individually and completely labelled
- Smear slides prepared at the bedside must be labelled on the frosted end in pencil immediately after preparation

CRITICAL: A sample whose label does not match the request form in any mandatory field (name, UHID, or DOB) is a MISLABELLED sample and must be rejected. Do not attempt to resolve discrepancies by relabelling recollection is required.

10. Safety and Infection Control

10.1 Personal Protective Equipment (PPE)

All personnel performing sample collection shall wear appropriate PPE in accordance with the JDWNRH Infection Control Policy (JDW/AMS/IPC/02). Minimum requirements are:

Procedure	Gloves	Apron/Gown	Mask	Eye Protection
Routine venepuncture	Yes (non-sterile)	If splash risk	Yes	No (routine)
Blood culture collection	Yes (non-sterile)	Yes	Yes	No
Arterial blood gas (ABG)	Yes (non-sterile)	If splash risk	Yes	No
Lumbar puncture / CSF collection	Yes (sterile)	Sterile gown	Surgical mask	No
Microbiology swabs (routine)	Yes (non-sterile)	Yes	Yes	No
Sputum induction	Yes (non-sterile)	Yes	FFP2/N95	Yes (goggles)
Known / suspected TB patient	Yes (non-sterile)	Yes	FFP2/N95	Yes
Known infectious hazard (e.g. VHF)	Double gloves + full PPE per protocol	Yes	FFP3	Full face shield
All urine / stool / body fluid handling	Yes	yes	No (routine)	No (routine)
Bone marrow aspiration and biopsy	Yes	Yes	Yes	No (Routine)
Referral Samples	Yes	Yes	Yes	Yes

10.2 Hand Hygiene

Hand hygiene must be performed diligently as per requirement and the task in hand by following one of the methods below:

Hand washing with soap and water

Hand washing with soap and water shall be performed in accordance with the WHO 'Five Moments & 7 steps for Hand Hygiene':

- Before touching the patient
- Before clean/aseptic procedure (venepuncture site preparation)
- After body fluid exposure risk (post-collection)
- After touching the patient
- After touching patient surroundings, also details mentioned in SOP: JDW/AMSIPC/01

Alcohol based hand rub (ABHR)

ABHR is the preferred method of hand hygiene in healthcare when hands are not visibly soiled. Use it at the WHO "5 Moments":

- Before touching a patient: on approach, before any contact.
- Before a clean/aseptic procedure: e.g., before catheter insertion, wound dressing, injections, or preparing sterile items.
- After body fluid exposure risk: after contact with blood, secretions, excretions, mucous membranes, non-intact skin, or wound dressings (even if gloves were worn).
- After touching a patient: on leaving the patient's side.
- After touching patient surroundings: bed, rails, equipment, or other surfaces in the patient zone.

Soap and water must be used when hands are visibly soiled, or for a unique identifier at JDWNRH (use soap and water only, ABHR is insufficient for *C. diff* spores).

10.3 Sharps Safety SOP:(JDW/AMSIPC/03)

- Use safety-engineered devices wherever available (retractable needles, safety lancets)
- Activate the safety mechanism immediately after needle withdrawal, before any other action

- Never resheath a needle with two hands single-handed scoop technique or safety device only
- Dispose of all sharps immediately into an approved sharps container at or near the point of use
- Never fill sharps containers beyond the fill line (75% capacity)
- Never leave sharps unattended on trolleys, beds, or surfaces

CRITICAL: Needlestick / sharps injury: Immediately squeeze site to encourage bleeding, wash with soap and water for 10 minutes, do not suck the wound, report to line manager immediately, attend Occupational Health / Emergency Department for post-exposure prophylaxis assessment. Document in the Incident Reporting System.

10.4 Specimen Handling and

- All samples are to be treated as potentially infectious
- Samples must be transported in closed, leak-proof primary containers, placed in sealed specimen bags

10.5 Spill Management

- In the event of a spill: Follow spill management protocol as per Infection control and medical waste management guidelines 2018.

11. Blood Collection - Venous (Venepuncture)

11.1 Equipment

- Safety vacutainer needle (21G standard; 23G for small/fragile veins) or butterfly needle (23G/25G)
- Appropriate blood collection tubes (see Section 11.4 and Appendix A)
- Tourniquet (single-use or disinfected between patients)
- 70% isopropyl alcohol swabs for site preparation
- 2% chlorhexidine in 70% alcohol or povidone-iodine (Betadine) for blood culture site preparation
- Sterile gauze and adhesive plaster/bandage
- Gloves, sharps container, specimen bag, request form, labels

11.2 Site Selection

Preferred venepuncture sites (in order of preference):

- Antecubital fossa - median cubital vein (first choice)
- Cephalic vein or basilic vein in the antecubital region
- Forearm veins
- Hand veins (adequate but more painful; avoid in coagulopathy)
- Foot or ankle veins (out of scope phlebotomy job description)

Avoid the following sites unless no alternative exists and with senior clinical approval:

- Arm with an IV infusion in progress always collect from the opposite arm; if impossible, stop infusion for at least 2 minutes, apply tourniquet distal to IV site, and document on request form
- Arm with AV fistula (dialysis access) take sample from opposite arm or refer to the specialist assistance if both arms have AV fistula
- Arm on the same side as mastectomy with axillary node clearance use opposite arm
- Oedematous or lymphoedematous limbs use unaffected limb
- Areas with haematoma, bruising, or skin infection
- Post-surgical limbs where lymphatics are compromised

11.3 Procedure - Standard Venepuncture

- Gather all equipment and check expiry dates before approaching patient
- Wash hands / ABHR; don gloves
- Introduce yourself, explain procedure, obtain verbal consent
- Perform three-point patient identification check (Section 8)
- Position patient comfortably with arm fully extended and slightly downward
- Apply tourniquet 7.5–10 cm above intended site; tourniquet must not be applied for more than 60 seconds total release before blood flow if prolonged
- Identify suitable vein by palpation do not tap or flick vein vigorously (causes haemolysis); ask patient to clench fist gently
- Clean site with 70% isopropyl alcohol swab from center outward; allow to dry completely (30 seconds minimum) do not re-palpate after cleaning without re-cleaning
- Remove needle guard; anchor vein; insert needle bevel-up at 15–30 degree angle into skin in one smooth movement
- When flashback is seen, push tube onto vacutainer holder fill to correct volume mark

- Release tourniquet as soon as blood flows freely (always before completing last tube)
- Fill tubes in order of draw (Section 11.4); gently invert each tube the correct number of times immediately after removal
- When last tube is filled, place gauze over needle site; withdraw needle smoothly; activate safety mechanism immediately
- Apply firm pressure to site for 3–5 minutes (longer if anticoagulated); do not bend arm at elbow
- Label all tubes at the bedside before leaving the patient
- Dispose of sharps immediately; remove gloves; perform hand hygiene
- Check puncture site for bleeding; apply adhesive dressing
- Complete the request form with time, date, and collector ID; place in specimen bag

11.4 Order of Draw and Blood Tube Reference

Follow Annex A for order of draw

> **NOTE:** The order of draw prevents additive carryover between tubes. Always follow the sequence above. If only one or two tubes are required, still maintain the relative order (e.g. citrate before EDTA).

11.5 Inversion Guide

Tube Type / Additive	Required Inversions	Purpose
Blood culture bottles	8-10 gentle inversions	Mix blood with culture broth
Sodium citrate (light blue)	3-4 gentle inversions	Mix with anticoagulant
Plain red (no additive)	0 inversions	Allow to clot undisturbed
SST gold (gel + activator)	5-6 gentle inversions	Activate clotting; mix gel
Lithium heparin (green)	8-10 gentle inversions	Ensure anticoagulation

Tube Type / Additive	Required Inversions	Purpose
EDTA (lavender/pink)	8-10 gentle inversions	Ensure full anticoagulation; prevent platelet clumps
Fluoride/oxalate (grey)	8-10 gentle inversions	Mix preservative

WARNING: Invert gently do NOT shake. Shaking causes haemolysis and will result in sample rejection.

11.6 Failed Venepuncture

If venepuncture is unsuccessful after two attempts by the same collector:

- Remove needle; apply pressure; dispose of needle safely
- Request a second experienced collector to attempt limit total attempts to two per collector
- After three failed attempts total, refer to senior clinician or specialist team (anaesthetics, IV team)
- Consider alternative sites or use of paediatric butterfly needles if available.

12. Blood Collection - Capillary (Fingerprick / Heelprick)

12.1 Indications

Capillary blood collection is indicated when:

- The patient is an infant or young child (heel prick is the primary method for neonates)
- Venous access is extremely poor and the test volume requirement permits capillary collection
- Point-of-care testing requires capillary blood (glucometer, HbA1c POCT, bilirubin meter, Micro hemato-centrifuge and hemoglobinometer)
- Repeated sampling from fragile veins would cause unacceptable harm

12.2 Site Selection

Patient Group	Preferred Site	Contraindicated Sites
Neonates (heel prick)	Lateral plantar surface of heel - medial or lateral aspect only	Posterior curvature of heel (calcaneus); fingers in neonates <1 year
Infants and toddlers	Lateral plantar heel; finger (>1 year)	Ear lobe; big toe
Children and adults	3rd or 4th fingertip of non-dominant hand (lateral aspect)	Thumb (highly vascular), 5th finger, areas with poor circulation or oedema

12.3 Heel Prick Procedure (Neonates)

- Warm heel with warm damp cloth or heel warmer for 3–5 minutes to increase blood flow
- Perform hand hygiene; don gloves
- Three-point patient identification (check wristband, maternal record, cot card)
- Clean lateral heel surface with 70% isopropyl alcohol; allow to dry
- Hold foot firmly; use automated safety lancet (depth appropriate for neonate 1.0 mm max depth)
- Wipe away first drop with dry sterile gauze (diluted by tissue fluid)
- Collect subsequent drops into capillary tube or onto filter paper do NOT squeeze excessively
- Apply gentle intermittent pressure only; avoid 'milking' (dilutes sample with tissue fluid)
- Fill capillary tubes/cards to required mark; label immediately
- Apply gentle pressure with gauze; do not apply adhesive directly to neonatal skin if fragile
- For further detail Refer to Capillary SOPs No: JDW/LAB/BIO11

WARNING: Excessive squeezing of the heel causes haemolysis and dilution of the sample. Use gentle intermittent pressure only.

12.4 Fingerprick Procedure (Adults / Older Children)

- Warm finger if cold;

- Select 3rd or 4th finger of non-dominant hand; do not use oedematous or previously punctured areas
- Clean with alcohol; allow to dry
- Use appropriate safety lancet; puncture lateral aspect of fingertip in one motion
- Wipe away first drop; collect subsequent drops into appropriate microcollection device
- Dispose of sharps

13. Urine Collection

13.1 Urine Collection Methods

Collection Type	Indications	Instructions
Midstream Urine (MSU)	Routine urinalysis, culture & sensitivity, PCR(protein creatinine ratio) and urine electrolyte	Start voiding and let initial stream pass; without interrupting urination collect midstream into sterile container
First-void Urine (FVU)	STI PCR (Chlamydia, Gonorrhoea)	Patient must not urinate for ≥1 hr prior; collect first 20-30 mL of stream
24-Hour Urine	Creatinine clearance, protein, hormones, oxalate	Start after discarding first morning void; collect all urine for 24 hrs; -Refer to SOPs:JDW/LAB/BIO/09
Catheter Specimen (CSU)	Catheterised patients	Aspirate from with syringe after clamping; (Refer SOP for catheterised patient- IPD) Note: *never collect from drainage bag*
Suprapubic Aspirate (SPA)	Neonates, ambiguous culture results	Clinicians to perform procedure under ultrasound guidance;
Early Morning Urine (EMU)	TB culture, orthostatism, casts	First void on waking; concentrated specimen; 10-20 mL (EMU).

Collection Type	Indications	Instructions
Paediatric Bag Urine	Young children (Routine urinalysis only)	Bag urine unreliable for culture; contamination risk; use SPA or CSU for cultures

13.2 Midstream Urine (MSU) - Patient Instructions

Provide the following instructions to the patient, preferably in written form in Dzongkha and English:

- Wash hands thoroughly with soap and water
- Female patients: Start voiding and let initial stream pass; without interrupting urination collect midstream into sterile container
- Male patients: Start voiding and let initial stream pass; without interrupting urination collect midstream into sterile container
- Begin urinating into the toilet; after passing the first portion of urine, collect the midstream portion directly into the sterile container provided without stopping the flow
- Fill the container at least halfway (minimum 3 mL); recap tightly; hand to staff immediately

NOTE: Urine collected for culture must reach the laboratory within 2 hours of collection if kept at room temperature, or within 24 hours if refrigerated at 4°C. Boric acid preservative containers extend stability to 48 hours at room temperature.

13.3 24-Hour Urine Collection

- Instruct to collect in a clean, leak proof clean container
- Day 1, Morning (e.g. 07:00): Patient empties bladder into toilet this is DISCARDED (this is time zero)
- From this point, collect ALL urine voided (including the first morning void on Day 2 at 07:00) into the collection container

- Storage container should be at cool and dark place throughout the 24-hour collection period until patients bring it back to the laboratory
- Complete the collection at the same time the following morning; record exact start and end times on label
- Mix gently; measure and record total volume; send aliquot to laboratory with total volume noted on request or Refer to SOPs:JDW/LAB/BIO/09.

14. Cerebrospinal Fluid (CSF) and Other Body Fluids

14.1 CSF

CSF may be collected by lumbar puncture or through external ventricular drain by clinicians or nursing staff. Laboratory staff are responsible for correct sample receipt and processing. . The collecting clinical staff are responsible for:

- Obtaining informed consent
- Observing strict aseptic technique throughout
- Collecting CSF/ External Ventricular Drain (EVD) into sequentially numbered, sterile screw-capped containers as required for the requested tests. Refer Annex -- for CSF/EVD
- CSF parameters such as glucose and protein are best interpreted with blood glucose and protein levels. Therefore it is recommended to take blood samples for protein and glucose at the time of lumbar puncture / CSF collection.
- Transporting immediately to the laboratory never refrigerate CSF

Note : requires good coordination and communication between the clinical and laboratory staff.

14.2 CSF (Extraventricular drain)

To collect a cerebrospinal fluid (CSF) sample from an External Ventricular Drain (EVD), use strict aseptic technique to clean the CSF Sampling Port, gently aspirate 1–2 mL of fluid into a syringe, and transfer it into a sterile specimen container.

Step-by-Collection Procedure

- Clamp the drain: If allowed by your hospital protocol and ordered by the physician, clamp the EVD tubing briefly (e.g., 10–15 minutes) before collection to help accumulate CSF if drainage is low.
- Clean the port: Scrub the needleless luer-lock sampling port (typically the proximal port closest to the patient) vigorously with an antiseptic wipe and let it dry completely.
- Attach the syringe: Connect a sterile syringe securely to the sampling port.
- Aspirate gently: Keep the clamp on or open it slowly depending on your specific device instructions, and gently attempt to aspirate. Never use excessive force or rapid suction, as this risks damaging brain tissue or collapsing the ventricle.
- Withdraw the sample: Collect only the required amount, usually 1 to 2 mL of CSF.
- Transfer to container: Empty the fluid from the syringe directly into a sterile, labeled specimen container. Do not send the sample in a syringe due to the risk of spills or contamination.

CSF Tube No.	Volume	Examination
Tube 1	≥1 mL	Chemistry: protein, glucose, LDH, other biochemistry transfer in Red cap vial (note: tube 1 may have RBCs from traumatic tap)
Tube 2	≥1 mL	Microbiology: Gram stain, culture, sensitivity, cryptococcal antigen, AFB microscopy & Xpert (PCR).
Tube 3	≥1 mL	Cell count and differential (use tube 3 least contaminated with blood) , xanthochromia
Tube 4 (if available)	≥0.5 mL	Special studies: viral PCR, cytology, oligoclonal bands,

CRITICAL: CSF must NEVER be refrigerated. Transport to the laboratory immediately at room temperature. For culture, maintain at 37°C if any delay is anticipated. Xanthochromia samples must be protected from light.

14.2 Pleural Fluid

Collected by thoracocentesis by clinicians,

- Tube 1: Biochemistry (protein, LDH, glucose, amylase, cholesterol, triglycerides)transfer in Red cap vial
- Tube 2: Microbiology inoculate directly into blood culture aerobic/anaerobic bottles at the bedside if bacterial infection is suspected (increases yield significantly)
- Tube 3: Cell count and differential
- Tube 4: Send to the Cytology lab in the appropriate container as per physician request for malignancy.
- Heparinised tube if coagulation analysis needed

14.3 Ascitic Fluid

For spontaneous bacterial peritonitis (SBP) screening: inoculate blood culture bottles at the bedside (10 mL each into aerobic and anaerobic bottles). Also send ≥5 mL Transfer in plain tube or Red cap vial for biochemistry (albumin, protein, LDH, , glucose,) and EDTA tube for cell count. Send maximum sample volume (if possible) in a container for cytology.

14.4 Synovial Fluid

Sterile container for culture (or blood culture bottle inoculated at bedside);Transfer plain tube or Red cap vial for biochemistry and crystal analysis; EDTA tube for cell count. Crystal analysis must be performed within 4 hours; do not refrigerate if crystal microscopy is required. Also sent to cytology for malignancy.

14.5 Pericardial Fluid

Collected by pericardiocentesis. Same split allocation as pleural fluid. Notify the laboratory in advance of all pericardial fluid samples; these are high-priority.Also sent to cytology for malignancy.

14.6 Bone marrow

Bone marrow aspiration and trephine biopsy samples shall be collected by an authorized healthcare professional with appropriate training and competency. Laboratory personnel may assist with specimen collection and handling. Refer SOP/JDW/LAB/HAEM/11 for bone marrow aspiration collection and smear preparation.

Specimen	Examination	Container/Preparation	Handling and Transport
Bone marrow aspirate	Morphological examination	Direct smears and smudge smear preparation	Direct bed side preparation
	Flow cytometry	EDTA and Heparin	
	Cytogenetic/molecular studies	EDTA and Heparin	Confirm requirements before collection and transport promptly
Trephine biopsy	Histopathological examination	Appropriate fixative in 10% neutral buffered formalin	Handle gently and place in fixative promptly
	Imprint preparation	Clean glass slides	Prepare imprints at the bedside before placing the biopsy in fixative

14.7 **Whole blood**

Should be collected in EDTA anticoagulant vials with appropriate blood -to anticoagulant ratio. Underfilling can cause cell shrinkage and significant artefact.

Sample type	Test	Temperature	Max time to Lab	Special requirements
Venous blood	Peripheral blood smear examination (PBS)	Room Temperature	6 Hours	CBC should be requested alongside

				PBS. Clinical history of a patient is required
	Malaria	Room Temperature	6 Hours	Sample collection from the capillaries is preferred due to parasite sequestration.
	Filarial Worm Microscopy	Room Temperature	6 Hours	Collection time: Night time Collection (10 p.m to 2 a.m) for *Wuchereria bancrofti* and *Brugia malayi*- Daytime collection (10 a.m to 2 p.m)- Loa Loa. For other filarial worms- any time
	Leishmania Donovi (LD bodies) microscopy	Room Temperature	6 Hours	

Refer SOP JDWNRH/LAB/HAEM/2 and SOP JDWNRH/LAB/HAEM/7 for blood smear preparation.

14.8 **FNAC Sample collection**

Fine Needle Aspiration Cytology (FNAC) is an invasive diagnostic procedure used to obtain cellular material from palpable lump for Cyto pathological / bacteriological examination, Refer to JDW/Lab/Cyto/09 for more details.

15. Microbiology Specimen Collection

15.1 General Principles for Microbiology samples

- Collect microbiology samples BEFORE starting antimicrobial therapy wherever clinically safe to do so
- Use the correct transport medium for the specimen type .Dry swabs are not acceptable for routine bacterial culture
- Collect sufficient volume/quantity, small volumes and insufficient samples are the leading cause of false-negative culture results
- Label all samples with patient details, sample site, and clinical diagnosis
- Notify the laboratory of special or high-priority samples in advance
- samples for unusual organisms (Brucella, Leptospira, Vibrio, etc.) must be discussed with the microbiologist before collection

15.2 Microbiology Sample collection guide

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
Blood culture (adult)	Aerobic + grey, green & blue	5–10 mL each (As per manufacturer insert)	Aseptic skin prep; 2 sets from 2 sites
Blood culture (paediatric neonate & infant)	Paediatric bottle Yellow & pink	1–3 mL(As per manufacturer insert)	Single set if volume limited
Blood culture (children)	Yellow & pink	3-5 mL (As per manufacturer insert)	Single set if volume limited
Urine (MSU)	Sterile container	≥3 mL	Midstream clean-catch; clean periurethral area first
Urine (catheter)	Sterile syringe via port	≥3 mL	Aspirate from catheter port; never from bag

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
Throat swab	Amies/charcoal swab	1 swab	Swab posterior pharynx and tonsils; avoid uvula
Urine (AFB microscopy & Rapid molecular testing (Xpert)	Sterile screw-cap container	20-50 mL	AFB-3 consecutive days, Xpert – 1 spot (Early morning first void)
Ear swab	Amies & sterile wide mouth container (screw cap) or sterile dry swab	1 Swab each	The specimen of choice is an aspirate from behind the tympanum (eardrum)
Eye swab	Amies & sterile wide mouth container (screw cap) or sterile dry swab or slide	1 Swab each	Onsite collection: A moistened swab with isotonic normal saline is used to collect the sample from lower conjunctiva avoiding eyelid border and lashes. Offsite collection: A moistened swab is used over lower Conjunctiva and placed in transport medium. Fungal KOH: Corneal scrapping (preferred) by ophthalmologist.
Wound swab (superficial)	Amies medium swab & sterile widemouth	1–2 swabs	Clean wound edge; swab base; use Levine technique

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
	container (screw cap)		
Wound swab (deep/pus)	Sterile syringe or swab	≥1 mL pus or 2 swabs	Aspirate pus preferred over surface swab
HVS (genital female)	Amies swab	1 swab	From posterior fornix; note clinical details
Urethral swab (male)	Amies swab	1 swab	Rotate in urethra 2–4 cm; patient must not urinate 1 hr prior
Sputum (routine culture)	Wide-mouth sterile container	≥1 mL (3–5 mL preferred)	Early morning; deep cough; not saliva
Sputum (AFB microscopy / TB Xpert)	Wide-mouth sterile container	≥5 mL AFB microscopy: 2-5 mL Xpert: 1-4 mL	AFB microscopy- Spot sputum & morning sputum (before meal) Xpert- Spot/morning sputum. (Please follow National TB guidelines 8th edition 2024)
BAL (bronchial washing)	Sterile container	≥5 mL	Via bronchoscopy; notify lab in advance
Stool (routine C&S)	Stool pot with spoon lid Sterile screw-cap container with spoon lid.	5 mL / pea-sized sample R/E: 4-5 g (4-5 mL; watery stool) C/S: 1-2 g or 2-2 mL	Fresh formed or liquid; avoid contamination with urine
Stool (ova & parasites)	fresh	2–5 g	Three samples on alternate days for O&P

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
Stool (C. difficile toxin)	Sterile container (liquid stool only)	Liquid stool, ≥5 mL	Only test liquid/unformed stool; do not freeze
Stool (AFB microscopy & Xpert)	Sterile screw-cap container with spoon lid.	2-3 g (Xpert & AFB)	AFB microscopy- 3 samples for 3 consecutive days Xpert- 1 sample (Please follow National TB guidelines 8th edition 2024)
CSF (meningitis)	3 sterile screw-cap tubes	Tube 1: chemistry/protein, Tube 2: micro (Xpert-minimum 0.1 mL), Tube 3: cell count	Sequential collection; label in order of draw
Pleural fluid	Sterile container + blood culture bottle	10-20 mL for micro; inoculate bottle at bedside, Xpert:5-10 mL (more the better)	Anaerobic + aerobic inoculation at bedside
Joint/synovial fluid	Sterile container + culture bottle	≥2 mL; 0.5-1 mL minimum	Inoculate blood culture bottle at bedside
Ascitic fluid	Wide-mouth sterile container	C/S: >50 mL (if obtainable) Xpert: 5-10 mL (more the better)	Sterile free-flowing sample
Peritoneal fluid	Wide-mouth sterile container	C/S: 2-4 mL	Sterile free-flowing sample

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
		Xpert: 5-10 mL (more the better)	
Pericardial fluid	Wide-mouth sterile container	C/S: 2-4 mL Xpert: 5-10 mL (more the better)	Sterile free-flowing sample
Tissue (biopsy)	Sterile container dry or saline-moist	Full biopsy specimen Xpert: 0.5-1 g (collect as much as possible)	Do NOT place in formalin if culture needed
Bone	Wide-mouth sterile container	Xpert: 0.5-1 g (collect as much as possible)	Do not add formalin for xpert test
FNAC	Wide-mouth sterile container	0.5-1 mL (minimum)	Do not add formalin. Normal saline is accepted.
Gastric aspirates	Wide-mouth sterile container	AFB microscopy: 1-5 mL Xpert: 1-4 mL	AFB Microscopy- 1 GA sample (before feeding/meal) or 3 consecutive early morning samples. Xpert- 1 GA sample (spot/early morning)
Pus	Wide-mouth sterile container	0.5-1 mL (minimum)	Xpert: Free-flowing sample (needed for dilution); swab sample not accepted. C/S: accepted in sterile swab stick.

15.3 Blood Culture Collection

Follow the manufacturer's instructions or the laboratory approved SOP for the collection of blood culture samples.

CRITICAL: Do NOT refrigerate blood culture bottles. Transport to the laboratory within 2 hours and load into the blood culture incubator immediately. Delay or refrigeration will reduce organism recovery and extend time to detection.

15.4 Fungal KOH sample collection

Sample types like skin scraping, nail clipping, hair and other samples such as body fluids and CSF and sputum should be collected as described in SOP no. JDW/LAB/MICRO/TB/11 for more details.

15.5 Hansen test /SSS (Slit skin smear) sample collection

- Specimen Collection by laboratory staff (Slit and scrape Method):
- Explain the procedure to the patient (or parent if the patient is a child).
- The smears are generally collected from at least 3 sites. Both ear lobes, chin, forehead.
- Smear from lesions, nasal and nodules are to be taken on the advice of a physician. Refer SOP no. JDW/LAB/MICRO/TB/12 (SOP on Laboratory Diagnosis of Leprosy) for more details.

16. Paediatric and Neonatal Sample Collection

16.1 Special Considerations

Paediatric sample collection requires modification of standard adult techniques. The following principles must be applied:

- Always involve a parent or guardian in the procedure; obtain verbal consent and explain the procedure in age-appropriate terms to the child
- Use the smallest possible needle gauge: 23G butterfly for most paediatric venepuncture; 25G for neonates and infants
- Use microcollection tubes (paediatric tubes) to minimise blood volume avoid iatrogenic anaemia from over-collection
- Apply topical anaesthetic at least 60 minutes before elective venepuncture in children aged 1 month and above
- Use non-nutritive sucking (pacifier with sucrose) for neonatal pain management during heel prick

- Apply tourniquet for the minimum time possible in neonates
- Distraction techniques and play therapy should be offered for children aged 3 and above

16.2 Minimum Blood Volumes - Paediatric

Age Group	Approximate Blood Volume	Maximum Single Collection	Common Tube
Neonate (<28 days)	80–100 mL/kg	1–2 mL per episode (2.5% of total blood volume max)	Microcollection / neonatal blood card
Infant (1–12 months)	75–100 mL/kg	2–4 mL per episode	Paediatric 2 mL EDTA / mini SST
Toddler (1–3 years)	70–80 mL/kg	5–10 mL per episode	Paediatric / small adult tubes
Child (3–12 years)	70–80 mL/kg	10–20 mL per episode	Standard adult tubes appropriate volumes
Adolescent (>12 years)	65–75 mL/kg	Adult volumes applicable	Standard adult tubes

WARNING: Cumulative blood volume removed from neonates and infants over a 24-hour period must not exceed 2.5–5% of total estimated blood volume without medical officer approval. Consult the attending paediatrician if multiple tests are requested.

16.3 Neonatal Screening (Blood Spot / Guthrie Card)

- Collect between 48–72 hours of age (heel prick) not before 48 hours post-delivery
- Apply blood to all designated circles on the filter paper card; allow blood to soak through; do NOT layer multiple drops each circle should be filled with a single application
- Allow to air-dry horizontally for a minimum of 3 hours at room temperature do not stack wet cards
- Do NOT expose to direct sunlight, heat sources, or moisture

- Complete all demographic fields on the card; send with completed demographic form to National Newborn Screening Laboratory

17. Critical Care, Emergency, and Perioperative Collections

17.1 Intensive Care Unit (ICU) and High Dependency Unit (HDU)

ICU patients often have indwelling arterial or venous lines. The following applies to line-drawn samples:

- Arterial lines: draw and discard a dead-space volume (usually 2–3x the catheter dead space, or per manufacturer instruction) before collecting blood; use the discard volume to avoid heparin contamination; do not draw coagulation samples from heparinised lines without protocol
- Central venous catheters: avoid if possible for routine laboratory samples (higher haemolysis and dilution risk); if necessary, document line used and dead space discarded
- Never collect from the same arm as a running infusion
- For ABG collection from arterial lines: purge line with fresh blood (discard ≥3 mL), collect ABG anaerobically, analyse within 15 minutes or place on ice

17.2 Arterial Blood Gas (ABG) Collection

4. Gather: pre-heparinised ABG syringe, 23G needle or arterial line adapter, ice slurry if time to analysis >5 minutes, gloves
5. Perform Allen's test before radial artery puncture (compress radial and ulnar arteries; release ulnar if palm flushes within 5 seconds, collateral circulation is adequate)
6. Palpate radial artery; clean with alcohol; allow to dry
7. Insert needle bevel-up at 30–45 degrees; allow syringe to fill passively from arterial pressure (1–3 mL)
8. Remove needle; apply firm pressure for minimum 5 minutes (10 minutes if on anticoagulants)
9. Immediately expel all air bubbles; cap syringe; mix gently 5 times; label
10. Analyse within 15 minutes at room temperature, or within 60 minutes if stored in ice slurry

CRITICAL: Air bubbles in ABG samples cause falsely high pO2 and falsely low pCO2. Expel ALL air immediately after collection and before capping.

17.3 Emergency Department (ED) Collections

In the Emergency Department, the clinical urgency may necessitate expedited collection. However, the following non-negotiable requirements apply even in emergencies:

- STAT samples must be telephoned ahead to the laboratory before transport
- Critical STAT analytes (troponin, lactate, K+, ABG, glucose, cross-match) must be delivered to the laboratory within 15 minutes of collection
- Cross-match and blood group samples for emergency transfusion require two-person patient identification verification and must be collected in separate tubes labelled at the bedside by the collecting clinician

17.4 Biopsy Collection

- Post operative samples (tissue for histology, fluid for culture) must be labelled immediately and transported with a completed request form to the relevant laboratory. More than one biopsy/specimen for the same patient, label the specimen with letter A,B,C,D etc (e.g. A-tissue from right cheek,B- Tissue from left cheek) and clearly mention it on the requisition form.
- If multiple samples are submitted in a single container (which is less is less ideal) there needs to be some method of identification (i.e. suture) to denote respective anatomical sites.
- Tissue for the frozen section or intraoperative must be taken fresh (not in formalin). The concerned surgeon should discuss the case in advance with the pathologist and inform the histopathology staff to put on cryostat one day prior to surgery. Cryostat takes a minimum of 5 hours to prepare prior to the procedure (The chamber should be in between -20- to -30 °C). (Refer SOP JDW/Lab/HISTO/09)
- Renal biopsy is collected from the interventional radiology department.The renal biopsy specimen is generally divided and collected in two vials,appropriately labelled; one for H&E (fixed on 10% neutral buffered formalin) and one for direct immunofluorescence examination (transported in Michel's transport medium).The completed requisition form along with sample is send to Ex-country.
- Routine biopsy is collected from respective clinical departments ,procedure rooms,endoscopy unit.dental unit ,or other designated areas . Biopsies are placed in properly -labelled ,leak-proof containers containing adequate 10% neutral formalin and transported with a completed requisition form to the relevant laboratory. Refer SOP JDW/LAB/HISTO/O1

18. Point-of-Care Testing (POCT)

Point-of-care testing (POCT) conducted at JDWNRH is governed by ISO 22870:2016 and the JDWNRH POCT Policy (JDWNRH-LAB-POL-004). For POCT, the following applies with respect to pre-examination:

- Patient identification is mandatory before POCT same three-point check as for laboratory samples
- POCT operators must be trained and assessed as competent on each device before independent use
- POCT devices must have current quality control (QC) within acceptable ranges before patient testing do not use device outside QC
- Results from POCT must be documented in the patient record and, where required, in the laboratory information system
- Capillary blood for POCT: follow the fingerprick or heelprick procedure in Section 12
- POCT device maintenance records must be kept per the manufacturer's schedule

19. Sample Transport

19.1 General Transport Requirements

All samples transported within or from JDWNRH must comply with the following requirements, consistent with ISO 20658:2023 Clause 9:

- Samples must be placed in a sealed primary container (the collection tube or container), which must then be placed in a sealed, leak-proof secondary container (specimen bag)
- The request form must accompany the sample but must be placed in a separate compartment of the specimen bag, not in contact with the primary sample
- The secondary container (bag) must be placed in a rigid, clearly labelled transport box or carrier for transfer to the laboratory
- All transport containers must be clearly labelled with the biohazard symbol
- Transport containers must be kept upright at all times

19.2 Internal Transport (Within JDWNRH)

- Samples should be transported to the laboratory within 30 minutes of collection for routine samples, and within 15 minutes for STAT/urgent samples

- Samples may be hand-carried by the phlebotomist/laboratory assistant from the sample collection unit
- Pneumatic tube systems, if installed, may only be used for sealed, non-breakable containers glass tubes and open containers must not be sent via pneumatic tube
- Transport trolleys must be cleaned and disinfected at the start and end of each working day
- Spills in transit must be managed per Section 10.4; the incident must be documented

19.3 Temperature and Time Requirements

Sample Type	Temperature	Max Time to Lab	Special Requirements
Blood culture bottles	Room temp (20–25°C)	Immediate / if delay <2 hrs	Do NOT refrigerate; keep upright
Coagulation (citrate)	Room temp (18–25°C)	<4 hours	Do not freeze; separate plasma ASAP if delay
Serum (biochemistry)	Room temp	<8 hours (unseparated)	Centrifuge and separate within 2 hrs. maintain sample to anticoagulant ratio.
EDTA (haematology CBC)	Room temp (18–25°C)	<24 hours	Mix gently x8
EDTA (blood film / morphology)	Room temp	Make film within 1–2 hrs of collection	Sample to anticoagulant ratio.
Glucose (fluoride)	Room temp	<24 hours	Inhibits glycolysis; still analyse promptly
Ammonia (green heparin)	Ice slurry (0–4°C)	<15 minutes	Critical: on ice immediately; avoid haemolysis
Arterial Blood Gas (ABG)	Ice slurry	<15 minutes	Anaerobic; expel air bubbles immediately

Sample Type	Temperature	Max Time to Lab	Special Requirements
CSF	Room temp / 37°C incubator	<1 hour	Do NOT refrigerate; split aliquots for micro/chem
Urine (routine)	Room temp / 4°C refrigerated	<2 hrs room temp; <24 hrs refrigerated	Boric acid preservative if >2 hrs
Urine (culture)	4°C refrigerated	<24 hours	Boric acid or direct refrigeration
Stool (culture/ova/parasite)	Room temp	<2 hrs fresh; or preservative	SAF/formalin fixative for O&P
Swabs (microbiology)	Room temp in transport medium	<2 hrs (or 24 hrs in transport medium)	Charcoal or Amies medium
Sputum (AFB microscopy/Xpert / routine C/S)	Room temp	<1 hr; or refrigerate <24 hrs Xpert; up to 72 hrs at room tempt (>72 hrs refrigerate at 2-8°c)	Tight-cap container
Tissue (histopathology)	At room temperature immersed in 10% neutral buffered formalin	Immediate fixation	Volume of formalin should be 10 times the tissue volume
Blood group / cross matching	Room temp	<72 hours	Dedicated EDTA tube for Blood group, for cross matching serum tube; properly labelled (discuss with NBB)

Sample Type	Temperature	Max Time to Lab	Special Requirements
Bone marrow aspirate	Room temp / special fixative	<1 hour	Fresh smears at bedside; notify lab in advance
	Room temp (18–25°C)	<24 hours	For the flow cytometry. Inform the lab in advance

19.4 External Transport (Inter-Facility / Reference Laboratory)

Samples sent to external reference laboratories (including regional hospitals or international reference labs) must comply with:

- Triple packaging requirement: primary container + absorbent material + secondary container + outer rigid packaging
- UN 3373 Biological Substance Category B labelling for transport of diagnostic samples
- IATA Packing Instruction P650 for air transport (if applicable)
- Cold chain documentation for temperature-sensitive samples (chain of custody record)
- All required documentation: chain of custody form, test requisition, patient consent (if applicable)
- Prior arrangement with the reference laboratory before sending any sample
- Specimens requiring investigations not available at JDWNRH ,such as specialized IHC, are referred to Ex-country.
- National inteer-facility transport
- Any external health centers referring clinical samples to DPLS, JDWNRH should follow this Sample collection and transport manual and the relevant unit of DPLS advice and provide technical assistance and guidance to ensure sample integrity.

19.5 Shipment to National Reference Laboratory:

Refer SOP no. JDW/LAB-01 (Surveillance Sample Collection & Transportation to RCDC)

20. Non-Conforming Samples - Rejection Criteria and Actions

20.1 Sample Acceptance and Rejection Criteria

All samples received by the laboratory are evaluated against acceptance criteria upon receipt. Samples not meeting acceptance criteria are non-conforming and shall be managed according to the procedures below.

Criterion	Examples	Action
Unlabelled sample	No label on primary container	Reject, do not process and return non-conforming sample to ward/OPD with documentation
Mislabelled / discrepancy	Name/CID/UHID mismatch between label and request	Reject; do not process; notify requester, with documentation
Insufficient/over filled volume	Less/more than required volume	Reject or partial prioritise testing after consulting referring ward/unit/department, with documentation.
Haemolysed sample	Pink/red serum affects K+, LDH, enzymes	Reject, do not process and inform to ward/OPD/patient with documentation,
Lipaemic sample	Turbid serum affects spectrophotometric assays	Reject or dilution protocol/Fasting: document
Icteric (extreme)	Deep yellow interferes with specific assays	Document; check assay interference limits
Clotted anticoagulant tube	Visible clots in EDTA/citrate tubes	Reject, do not process and inform to ward/OPD/patient with documentation
Wrong tube used	e.g. EDTA used for coagulation test	Reject, do not process and inform to ward/OPD/patient with documentation
Incorrect anticoagulant ratio	Citrate tube under/over filled (±10%)	Reject, do not process and inform to ward/OPD/patient with documentation

Criterion	Examples	Action
Sample beyond stability limit	Exceeds time/temperature threshold in transit	Reject document transit delay
Broken / leaking container	Compromised primary container	Biohazard reject; notify sender
Unlabelled additive mismatch	Additives not matching test requirements	Reject recollect
Missing/incomplete request	No patient ID, no test specified	Hold contact requester for clarification
Receiving of tissue/biopsy sample in non-fixatives	Biopsy received in alcohol, water ,or non-fixatives.	Reject for routine histopathology and notify sender and clinician for re-biopsy if possible.

20.2 Procedure for Rejected Samples

- Immediately contact the requesting clinician or ward, verbally and in writing, with the specific reason for rejection
- Record the rejection in the laboratory non-conformity log (including patient MRN, reason, action taken, and responsible clinician notified)
- If the test is clinically critical and recollection is not immediately possible, proceed with testing under documented caveat report result with a statement of limitation
- Follow up to confirm a corrected sample has been collected and submitted
- Trend analysis of rejection reasons shall be reviewed monthly by the Laboratory Quality Manager as a pre-analytical performance indicator

20.3 Pre-Analytical Performance Indicators

Indicator	Target	Monitoring Frequency
Sample rejection rate (all causes)	<1% of total samples received	Monthly
Mislabelled / unlabelled sample rate	<5%	Monthly
Haemolysis rejection rate	<5%	Monthly
Incorrect tube	<5%	Monthly
Patient identification incidents	Zero tolerance target	Per incident

21. Special Considerations

21.1 Difficult or Anxious Patients

- Always explain the procedure clearly; anxious patients may experience vasovagal syncope
- Collect blood with patient seated or lying down never standing
- For patients with a history of needle phobia or vasovagal reactions: collect supine; have resuscitation equipment accessible; do not leave patient unattended post-collection
- Vasovagal episode management: lay patient flat, elevate legs, maintain airway, cold compress to forehead, monitor pulse; if unresponsive, call for emergency assistance

21.2 Elderly Patients

- Use the smallest appropriate gauge needle (23G) for fragile veins
- Avoid aggressive tourniquet pressure apply loosely for the minimum time
- Fragile skin tears easily do not use aggressive tape removal; consider a foam pad under the tourniquet
- Dehydration is common warm the arm, use gravity, and ensure the patient is well-hydrated if possible before elective collection

21.3 Oncology and Immunocompromised Patients

- Strictly avoid venepuncture on the same side as a peripherally inserted central catheter (PICC) or implanted port use the opposite arm

- Line draws from PICC or Hickman lines must follow specific institutional protocols and shall be performed by trained IV team/oncology nurses only
- Document all access sites and any complications

21.4 Patients Receiving Anticoagulants

- Apply firm pressure for at least 10 minutes post-venepuncture longer if INR >3 or APTT >2.5x normal
- Coagulation tests (PT/INR, APTT) require citrate tubes filled to exactly the fill line under-filling invalidates results
- Ensure the correct timing of therapeutic drug monitoring samples relative to dose (trough levels: just before next dose; peak levels: 60 minutes after IV dose)

21.5 HIV(Human Immunodeficiency Virus)Hepatitis B/C and Other Bloodborne Pathogen Precautions

- Standard (universal) precautions apply to ALL patients do not modify technique based on known or suspected bloodborne pathogen status
- Double gloves for known VHF (viral haemorrhagic fever) risk, multi-drug resistant organism (MDRO) precautions, or active bleeding
- Clearly label samples with 'Biohazard' do not use special labels that identify the patient's diagnosis; all samples are handled identically under universal precautions

22. Clinical Waste Disposal

All waste generated during sample collection must be disposed of in accordance with the JDWNRH Waste Management Policy infectious control and medical waste management guidelines (JDWNRH-FM-POL-003) and Bhutan's Biomedical Waste Management Guidelines:

Waste Type	Disposal Container	Colour Code
Sharps (needles, lancets, broken glass)	Rigid puncture-proof sharps container	Yellow container/White
Bloodstained gauze, gloves, swabs	Clinical waste bag	Red

Waste Type	Disposal Container	Colour Code
Sample tubes (after analysis)	Biohazardous waste bag (laboratory)	Red
Specimen bags and packaging	Clinical waste bag	
Empty (uncontaminated) packaging	General waste	Green
Large volumes of body fluid	Clinical waste do not drain without authorisation	Red
Excess blood/urine (small volumes)	Urine-Down sink with running water after treating with 5% sodium hypochlorite or as per IPC protocol Blood- Autoclave and discard as per IPC protocol.	N/A

WARNING: Sharps containers must never be filled beyond the stated fill line (75% capacity). Overfilled containers are a sharp injury risk and must be reported as a safety incident.

Appendix A: Blood Tube Quick Reference Guide

Draw Order	Tube Colour	Additive	Volume (mL)	Common Tests
1	Yellow & pink (Paediatric), grey, blue & green (Adult)	Blood Culture (Aerobic)- Culture broth/medium & SPS	5–10, 3-5 & 1-3	Blood cultures
2	Light Blue	Sodium Citrate (3.2%)	1.5/ 2.7 / 4.5	PT, APTT, INR, D-dimer, fibrinogen, citrate platelet count.
3	Red	None (serum)	5–10(1- 10)	Biochemistry, serology, immunology, hormones
4	Gold / SST	Gel + clot activator	5–8.5(3-10)	Serum chemistry, TFT, lipids
5	Green	Lithium Heparin	3–6	Plasma chemistry, ammonia, STAT biochemistry, flow cytometry and cytogenetics
6	Lavender / Purple	EDTA K2/K3	1.5/ 2–4	CBC, ESR, HbA1c, blood film, reticulocytes, flow cytometry and Hb electrophoresis/ HPLC
7	Pink / Royal Purple	EDTA (cross-match)	6	Blood group, cross-match, DAT
8	Grey	Fluoride / Oxalate	2–4	Glucose, lactate

Draw Order	Tube Colour	Additive	Volume (mL)	Common Tests
9	Dark Blue	No additive / EDTA	6	Trace metals, toxicology

Appendix B: Sample Rejection Criteria Summary

Criterion	Examples	Action
Unlabelled sample	No label on primary container	Reject - return request to ward/OPD
Mislabelled / discrepancy	Name/MRN mismatch between label and request	Reject - do not process; notify requester
Insufficient volume	Less than minimum required volume	Reject or partial testing with documentation
Haemolysed sample	Pink/red serum - affects K+, LDH, enzymes	Reject for affected tests; document cause
Lipaemic sample	Turbid serum affects spectrophotometric assays	Reject or dilution protocol; document
Icteric (extreme)	Deep yellow interferes with specific assays	Document; check assay interference limits
Clotted anticoagulant tube	Visible clots in EDTA/citrate tubes	Reject - recollect required
Wrong tube used	e.g. EDTA used for coagulation test	Reject - recollect in correct tube
Incorrect anticoagulant ratio	Citrate tube under-filled (<90%)	Reject for coagulation tests
Sample beyond stability limit	Exceeds time/temperature threshold in transit	Reject - document transit delay
Broken / leaking container	Compromised primary container	Biohazard - reject; notify sender
Unlabelled additive mismatch	Additives not matching test requirements	Reject - recollect
Missing/incomplete request	No patient ID, no test specified	Hold - contact requester for clarification

Appendix C: Sample Transport Temperature and Time Guide

Sample Type	Temperature	Max Time to Lab	Special Requirements
Blood culture bottles	Room temp (20–25°C)	Immediate / <2 hrs	Do NOT refrigerate; keep upright
Coagulation (citrate)	Room temp (18–25°C)	<4 hours	Do not freeze; separate plasma ASAP if delay.
Serum (biochemistry)	Room temp	<8 hours (unseparated)	Centrifuge and separate within 2 hrs
EDTA (haematology CBC)	Room temp (18–25°C)	<24 hours	Mix gently x8; analyse within 4 hrs for platelets
EDTA (blood film / morphology)	Room temp	Make film within 1–2 hrs of collection	-
Glucose (fluoride)	Room temp	<24 hours	Inhibits glycolysis; still analyse promptly
Ammonia (green heparin)	Ice slurry (0–4°C)	<15 minutes	Critical: on ice immediately; avoid haemolysis
Arterial Blood Gas (ABG)	Ice slurry	<15 minutes	Anaerobic; expel air bubbles immediately
CSF	Room temp / 37°C incubator	<1 hour	Do NOT refrigerate; split aliquots for micro/chem
Urine (routine)	Room temp / 4°C refrigerated	<2 hrs room temp; <24 hrs refrigerated	Boric acid preservative if >2 hrs
Urine (culture)	4°C refrigerated	<24 hours	Boric acid or direct refrigeration
Stool (culture/ova/parasite)	Room temp	<2 hrs fresh; or preservative	SAF/formalin fixative for O&P

Sample Type	Temperature	Max Time to Lab	Special Requirements
Swabs (microbiology)	Room temp in transport medium	<2 hrs (or 24 hrs in transport medium)	Charcoal or Amies medium
Sputum (AFB / culture)	Room temp	<1 hr; or refrigerate <24 hrs	Tight-cap container
Tissue (histopathology)	10% neutral buffered formalin	Immediate fixation	Volume of formalin 10x tissue volume
Blood group / cross-match	Room temp	<72 hours	Dedicated EDTA tube; properly labelled
Bone marrow aspirate	Room temp / special fixative	<1 hour	Fresh smears at bedside; notify lab in advance
	Room temp (18–25°C)	<24 hours	Collected bedside for flow cytometry and cytogenetics

Appendix D: Microbiology Specimen Collection Reference

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
Blood culture (adult)	Aerobic + anaerobic bottles	8–10 mL each	Aseptic skin prep; 2 sets from 2 sites
Blood culture (paediatric)	Paediatric bottle	1–3 mL	Single set if volume limited
Urine (MSU)	Sterile container	≥3 mL	Midstream clean-catch; clean periurethral area first
Urine (catheter)	Sterile syringe via port	≥3 mL	Aspirate from catheter port; never from bag
Throat swab	Amies/charcoal swab	1 swab	Swab posterior pharynx and tonsils; avoid uvula
Nasal swab (MRSA screen)	Dry/charcoal swab	1 swab each nostril	Rotate 5 times in each anterior naris
Wound swab (superficial)	Amies medium swab	1–2 swabs	Clean wound edge; swab base; use Levine technique
Wound swab (deep/pus)	Sterile syringe or swab	≥1 mL pus or 2 swabs	Aspirate pus preferred over surface swab
HVS (genital — female)	Amies swab	1 swab	From posterior fornix; note clinical details
Urethral swab (male)	Amies swab	1 swab	Rotate in urethra 2–4 cm; patient must not urinate 1 hr prior
Sputum (routine culture)	Wide-mouth sterile container	≥1 mL (3–5 mL preferred)	Early morning; deep cough; not saliva

Specimen	Container / Medium	Volume / Amount	Collection Method Summary
Sputum (AFB / TB)	Wide-mouth sterile container	≥5 mL	3 consecutive early morning samples; S-E-S protocol
BAL (bronchial washing)	Sterile container	≥5 mL	Via bronchoscopy; notify lab in advance
Stool (routine C&S)	Stool pot with spoon lid	5 mL / pea-sized sample	Fresh formed or liquid; avoid contamination with urine
Stool (ova & parasites)	SAF fixative or fresh	2–5 g	Three samples on alternate days for O&P
Stool (C. difficile toxin)	Sterile container (liquid stool only)	Liquid stool, ≥5 mL	Only test liquid/unformed stool; do not freeze
CSF (meningitis)	3 sterile screw-cap tubes	Tube 1: chemistry/protein, Tube 2: micro, Tube 3: cell count	Sequential collection; label in order of draw
Pleural fluid	Sterile container + blood culture bottle	≥5 mL for micro; inoculate bottle at bedside	Anaerobic + aerobic inoculation at bedside
Joint/synovial fluid	Sterile container + culture bottle	≥2 mL	Inoculate blood culture bottle at bedside
Tissue (biopsy)	Sterile container - dry or saline-moist	Full biopsy specimen	Do NOT place in formalin if culture needed

Appendix E: Urine Collection Method Summary

Collection Type	Indications	Instructions
Midstream Urine (MSU)	Routine urinalysis, culture & sensitivity	Clean periurethral area; discard initial stream; collect midstream into sterile pot
First-voided Urine (FVU)	STI PCR (Chlamydia, Gonorrhoea)	Patient must not urinate for ≥1 hr prior; collect first 20–30 mL of stream
24-Hour Urine	Creatinine clearance, protein, hormones, oxalate	Start after discarding first morning void; collect all urine for 24 hrs; refrigerate throughout
Catheter Specimen urine (CSU)	Catheterised patients (micro)	Aspirate from sampling port with syringe after clamping; never collect from drainage bag
Suprapubic Aspirate (SPA)	Neonates, ambiguous culture results	Physician procedure under ultrasound guidance; sterile syringe collection
Early Morning Urine (EMU)	TB culture, orthostatism, casts	First void on waking; concentrated specimen
Paediatric Bag Urine	Young children (urinalysis screening only)	Bag urine unreliable for culture; contamination risk; use SPA or CSU for cultures

— End of Document —

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