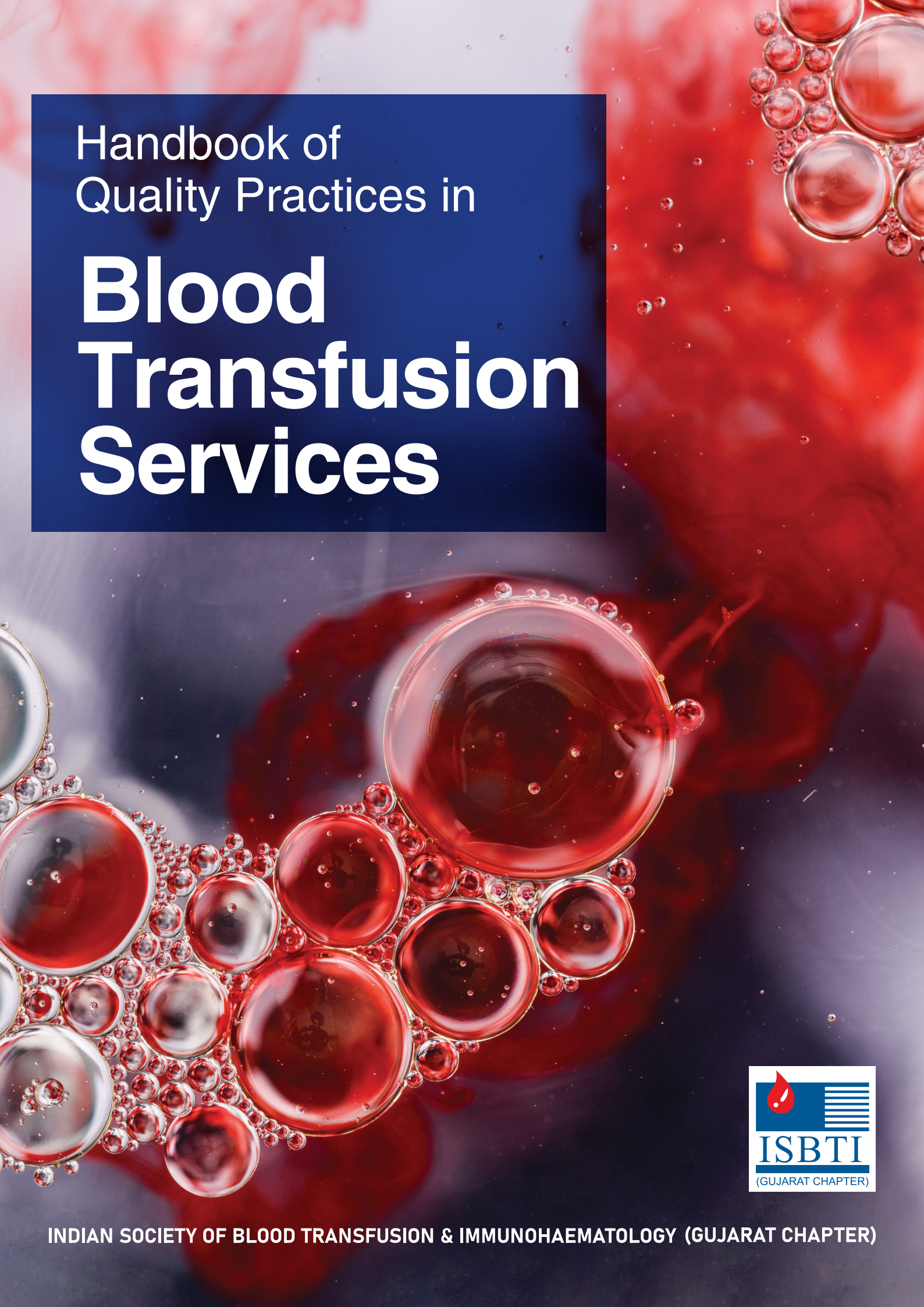
Handbook of
Quality Practices in

Blood Transfusion Services



ISBTI
(GUJARAT CHAPTER)

INDIAN SOCIETY OF BLOOD TRANSFUSION & IMMUNOHAEMATOLOGY (GUJARAT CHAPTER)

FOREWORD

Dear Friends,
Greetings from Organising Team of **GUJTRANSCON 2022!**

Gujarat has always been a Role Model for India, in overall Healthcare industry as well as Blood Banking. We have one of the finest Blood Centres in the country, both in private and public sector. Many accredited Blood Centres are there and many others are doing great Quality work.

To keep up the Quality Standards, on behalf of the organising Team, we present before you, a handbook of **"Quality Practices in BTS"**. This may not be the complete guidance document, but we hope that it would be of great help and a ready reckoner for maintaining Quality Practices in your Blood Centre.

Best Wishes,

Dr. Nidhi Bhatnagar
Chairperson,
ISBTI Gujarat Chapter

Dr. Ripal Shah
Secretary,
ISBTI Gujarat Chapter

CONTENTS

GENERAL

1. Starting of New Blood Centre

- Application for NOC from State Council of Blood Transfusion.
- Application made to State/Union Territory licensing authority as per guidelines under Drug and Cosmetic Act.
    1. Forwarding letter on letter head.
    2. Application form No 27-C.
    3. Challan of Rupees 7500 (6000 licence fees and 1500 inspection fees).
    4. Copy of approved blue print plan by Food & Drug Control Authority.
    5. Evidence of possession of building premises.
    6. Evidence of constitution.
    7. List of Equipment, Machineries and laboratory reagents (as per Schedule F).
    8. List of products with undertaking.
    9. Copy of draft label for each blood group.
    10. Undertaking for not to collect blood from professional donor.
    11. Clearance from State Blood Transfusion Council starting blood centre in particular area/location.
    12. Degree certificate, Registration copies, appointment letter, consent letter & experience certificate of medical officer, Technical Supervisor, Blood Centre technician and registered nurse.
    13. List of full name & address of each Directors/Trustees/Partners of the blood centre.
    14. Trust registration copy/memorandum of articles/partnership deed (whichever is applicable).
- Joint Inspection by State/Union Territory licensing authority.
- If condition and facilities are satisfying, State/Union Territory licensing authority will approve.
- The validity of license is 5 years from date of issue.
- The application for renewal is to be done at least 3 months prior to expiry.
- If applicant applies for renewal of licence after the expiry but within six months of such expiry, the fee payable for the renewal of licence shall be rupees 6000 and inspection fees of rupees 1500 and an additional fee at rate of rupees 1000 per month or a part thereof in additional to inspection fees.
- On receipt of the application for the grant or renewal of such licence, the licensing authority shall:
    1. Verify the statements made in the application form.
    2. Inspect the manufacturing and testing establishment in accordance with the provisions of rules 122-I.
    3. In case the application is for renewal of licence, information of past performance of the licensee shall be verified.
- 122-F: FORMS
    1. For application for Blood and its components: Form 27-C
    2. For license for Blood and its components: Form 28-C
    3. For renewal: Form 26-G
- Reference: http://nbtc.nco.gov.in/assets/resources/orders/Doc-7.pdf

2. Maintenance of Documents and Records

- The records from different areas of the blood centre are collected and kept according to the chronological numbers.
- The donor consent forms and patient requisition forms are sent for binding and then stored in separate racks year-wise.
- The registers should be given number according to the section.
- The first page of register should have: Effective from which date till date, Number of pages, Signature of Quality Manager.
- All register should have page numbers.
- Each register is given a unique identification number (Blood Centre/XXX/00n/year; where XXX stands for type of document, 00n stands for the serial number).
- The register for each year should be kept separately.
- All records are preserved for a period of 5 years as per Drugs and Cosmetics Act,1940. However, records related to plasma fractionation are preserved for 10 years.
- All record should have electronic backup, which should be validated and signed by Quality Manager.
- Record Disposal:
  - All records containing any type of patient or donor information are destroyed by shredding before disposal. This is done to ensure confidentiality.
  - No unauthorized person should have access to the confidential information that they contain.

3. Inventory Management

- A check list of all the consumables which are used in the Blood Centre is made.
- Define **critical limit** of each item, which can be calculated by formula:

  = (consumption of item/day x lead time) + buffer stock;

  - Lead time – Average duration of time in days between placing of order and receipt of supplies
  - Buffer Stock – quantity of materials set aside as safeguard against variation in demand and procurement.
- All the consumables are purchased after keeping a critical limit of minimum three months stock.
- A separate check list is maintained at each section.
- Physical stock is taken monthly.
- Any purchase to be done is discussed during monthly staff meeting and store in-charge is given permission to prepare purchase order and to take necessary steps to purchase process.
- Once new consumables is received, it has to be checked for-
  - Cold chain maintenance
  - Any breakage/leakage in package
  - Manufacturer, LOT no., Manufacturing & expiry date
  - Validation before use.

4. Hospital Transfusion Committee

- Fundamental role of HTC:
  - Ensure appropriate blood product use
  - Educating clinicians
  - Auditing blood use and adverse reactions

- Transfusion committee members:
  - Head of institute – Chairperson of Committee
  - Head of Blood Centre/IHBT – Secretory
  - Representees from various Department:
    1. Anesthesia
    2. Emergency Medicine
    3. Surgery
       a. Cardiothoracic
       b. Intensive care units
       c. Trauma
    4. Medicine
    5. Pediatrics/Neonatalogy
    6. Obstetrics and Gynecology
    7. Orthopedics

- Goal of HTC: To promote the safe and effective use of blood and its components.

- Agenda:
  - To review the usage of blood and its components by various clinicals units of the hospital
  - Review of work done in current year
  - Review of adverse events
  - Review of Errors/Wrong Practices
  - To promote transfusion reaction reporting practices along with reporting back safe transfusion
  - Create awareness for the use of blood components as well as Apheresis products
  - To invite suggestions for the promotion of voluntary blood donations
  - Suggestions or Grievances by the members to improve blood transfusion services
  - Any other matter with the permission of chair

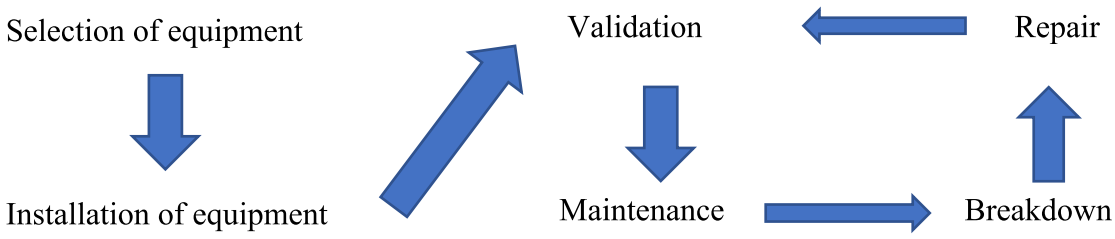
- Minutes of Meeting is filed for record keeping.

5. Maintenance of Equipment

- Equipment shall be maintained in a clean and proper manner.
- Operate the equipment in the manner for which it was designed.
- Equipments are observed, standardized and calibrated on a regularly scheduled basis.
- Regular training session for staff shall be done.
- Documentation should be done.

EQUIPMENT	PERFORMANCE	FREQUENCY	FREQ. OF CALIBRATION
Temp. recorder	Against calibrated thermometer	Daily	As often as necessary Once in 6 months
Refrigerated centrifuge	Observe speed and temp.	Each day of use	Tachometer, every 6 month & after repairs & shifting
General lab centrifuge	-	-	Tachometer, every 6 months
Automated Blood typing	Observe controls for correct results	Each day of use	-
Hemoglobinometer	Std against cyanmethemoglobin meter	Each day of use	-
Blood container weighing device	Std against container of known weight	Each day of use	As often as necessary
Waterbath	Observe temp.	Each day of use	As often as necessary
Autoclave	Observe temp	Each time of use	As often as necessary
VDRL shaker	Observe controls for correct results	Each day of use	Speed as often as necessary
Lab. Thermometer	-	-	Before initial use
Electronic thermometer	-	Monthly	-
Blood collection monitor	Weight of 1st container of blood filled for correct results	Each day of use	Std with container of known mass or volume before initial use & after repairs or adjustments
Tube sealer	Assessment of sealing of tubes	Each day of use	-

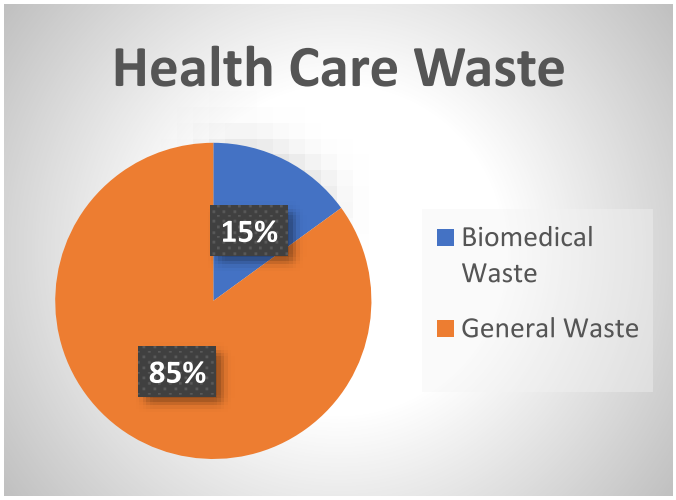
Equipment management cycle



- Breakdown of equipment:
  - Blood Centre shall have a procedure for replacement or repairing of defective equipments.
  - Whenever equipment is found to be defective, it shall be taken out of service, clearly labelled and appropriately stored until it has been repaired and shown to be calibrated to meet specified acceptance criteria.
  - The Blood Centre should have a policy and procedure for appropriate alternate storage where the blood/blood components shall be shifted in the event of breakdown of storage equipment.
  - Documentation of breakdown should be done.

6. Biomedical waste management

- "Bio-Medical waste" means any waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or research activities pertaining thereto or in the production or testing of biological or health camps.
- Generation and management of the waste is a very important aspect associated with health care Centres.
- Aim of BMW management:
  - To prevent transmission of diseases
  - To prevent injuries
  - To prevent exposure to harmful and serious health effects of BMW
  - To protect the environment
- It is the duty of the "Occupier" to take all steps to ensure that such waste is handled without any adverse effect to human health and the environment.
- Types of Waste:

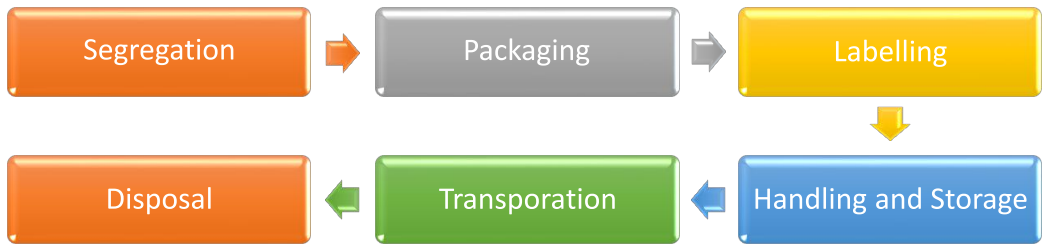


- BMW Management Rules. 2016 categories the biomedical waste generated from the health care facility into 4 categories based on the segregation pathway and colour code namely,
  - Yellow
  - Red
  - White
  - Blue

Categories of Biomedical Waste

Category	Type of Waste	Type of Bag or Container	Treatment and Disposal Options
YELLOW	Human & Animal Anatomical Waste	Yellow coloured non-chlorinated plastic bags	Incineration or Plasma Pyrolysis or Deep Burial
	Soiled Waste: Items contaminated with blood, body fluids, cotton swabs and bags containing residual or discarded blood and blood components		
	Expired or Discarded Medicines	Yellow coloured non-chlorinated plastic bags or containers	Incineration
	Chemical waste		Incineration or Plasma pyrolysis or Encapsulation
	Microbiology, Biotechnology and other clinical laboratory waste	Autoclave safe plastic bags or containers	Pre-treat to sterilize with non-chlorinated chemicals
RED	Contaminated waste (Recyclable)	Red coloured non-chlorinated plastic bags or containers	Autoclaving or micro-waving/hydroclaving followed by shredding or mutilation
WHITE (TRANSLUCENT)	Waste sharps including metals	Puncture proof, leak-proof, tamper-proof containers	Autoclaving or Dry Heat Sterilization followed by shredding/mutilation/ encapsulation in metal container
BLUE	Glassware	Cardboard boxes with blue coloured marking	Disinfection (by soaking the washed glass waste after cleaning with detergent and sodium hypochlorite treatment) or through autoclaving/microwaving/hydroclaving and then sent for recycling

BMW management Processes:



7. Spillage Management

A. Blood spillage

- In case of bag breakage or blood spills or accidents, the following steps are essential and to be followed.

Wear gloves

⇩

Cover the spillage with paper towel/absorbent material

⇩

Pour equal volume of freshly prepared 1% Sodium hypochlorite

⇩

Leave for 30 minutes

⇩

Clean the area

⇩

Wipe the surface with disinfectant again and mop up to leave a clean area.

Discard the mop and absorbent into red bag.

Essential requirements

1. Wear gloves, avoid direct contact of gloved hand with spill.
2. Sweep broken glass/fractured plastic with dustpan and brush.
3. Needle stick or other puncture wounds, cuts and skin contaminated with spill should be washed with soap and water, encourage bleeding.
4. Report immediately and document in the incident reporting register.
5. Maintain the record.
6. Damaged blood bag has to be discarded as per SOP.

B. Mercury spillage

1) Remove other things at the mercury spill site and switch off fan.
2) Remove ornaments.
3) Wear PPE (Mask, Cap, Gloves, Goggles).
4) Collect broken glass of broken instrument in paper towel (tissue paper) and put it in zip lock bag. Label it “contaminated with mercury, handle it with care”.
5) Collect small particles of mercury with card board. If particles are not visible, use torch.
6) Collect particles with syringe and drop it in plastic container with water. Put that container in zip lock bag and label “contaminated with mercury”.
7) The material used for cleaning and gloves, put in zip lock bag and label “Contaminated with mercury”.

8) All bags are handed over to pharmacist in drug store.
9) Wash the area with mercury neutralizing agents like 20% calcium sulphide or sodium thiosulphate solution (if the chemicals are available).
10) Wash your hands, face and any other areas of your body exposed to the mercury.
11) Keep the room well ventilated.
12) The records should be maintained in the incidence reporting register.

Contents of Mercury spill kit

Sr.no.	Items	Quantity
1.	Ziplock type plastic covers	5
2.	Plastic container with lid that seals	1
3.	Latex gloves	1 pair
4.	Tissue paper	10 nos
5.	Cardboard strips	5
6.	Eyedropper or syringe 10ml (without needle)	2
7.	Disposable apron	1
8.	Cap	1
9.	Face mask	1
10.	Goggles	1
11.	Torch	1
12.	Label/sticker	5 nos

8. NABH Accreditation

- Blood Centre should obtain a copy of NABH Standard from NABH office and other documents from NABH website (www.nabh.co).
- Conduct Gap Analysis of Blood Centre according to Standards.
- Blood Centre should prepare Quality Manual as per NABH standard and implement the requirements.
- The application form should be Submitted online.
- After the application is accepted, copy of internal audit and minutes of Management Review along with requisite fees should also be submitted.
- All the information should be filled. The e-mail ID provided in this form, should be used for all the future login.
- Once Blood Centre application form is received, NABH would activate login and provide password on the Blood Centre's e-mail.
- On using Blood Centre login and password, application form for Blood Centre should be filled, after which rest of the process would be through the software.
- After that, office will allot dates of assessment, assessment will be done and Non-Conformities are uploaded by assessor on website.
- All the Non-Conformities once addressed and clarified, NABH gives certificate.
- The validity of certificate is 3 years.
- The in-between surveillance is done once in 3 years.
- Blood Centre shall apply for reaccreditation before completion of 3 years.

9. Chemical Safety

1. **Hazardous Chemicals list** – Make list of hazardous chemicals (including reagents) used in blood centre.

2. **Material Safety Data Sheet (MSDS)** – Arrange "Material Safety Data Sheet" of all hazardous chemicals (used in Blood Centre) from company or google.

3. **NFPA Label: -** Label each hazardous chemical with NFPA (National Fire Protection Agency) label with scoring of hazards. Refer MSDS for scoring.

4. **Storage of hazardous chemicals: -** Store Hazardous chemicals at appropriate place preferably in metal box. It should have vent if chemical is inflammable.

5. **Quantity of hazardous chemicals: -** Define maximum quantity of hazardous chemicals to be stored.

6. **Training** – Train all staff members for chemical safety.

7. **Meaning of scoring (0 – 4) on NFPA sticker: -**

   - **Blue - health hazards -**
     4 - lethal (can lead to death)
     3- Severe or Permanent damage
     2- Temporary damage
     1- minor irritation
     0- no hazard

   - **Red - Inflammability -**
     4 - burn automatically at normal pressure and temperature
     3- burn with transient ignition
     2 - burn with moderate heat
     1 - burn when preheated several times
     0 - will not burn

   - **Yellow - instability and Reactivity-**
     4 - may explode at normal room temperature and pressure
     3- may explode with slight heat or pressure
     2- may explode with moderate heat or pressure
     1 - may explode

   - **White - Specific hazard - like-**
     Acid, Alkali, Radioactive, Corrosive, do not use water

8. **Health hazard routes and how to respond: -** Refer MSDS but following can be used generically

Route	How to respond
SKIN	Wash with plenty of water + Consult doctor + Inform HOD + raise Incidence report
EYE	Wash eye at eye wash station + Consult doctor + Inform HOD + Incidence report
INGESTION	Rinse mouth + Drink plenty of water + Consult doctor + Inform HOD + raise Incidence report
INHALATION	Go in open air + slow deep breath + Consult doctor + Inform HOD + raise Incidence report

9. **Chemical Spillage –** Define and arrange appropriate PPE for chemical spillage. Also define procedure of dealing with chemical spillage and train staff for it.

10. **Hazmat Kit: -** Define and make available Hazmat kit with defined contents. Maintain checklist of Hazmat kit with regular checking

11. **Eye wash station: -** Install eye wash station for eye splash. Define procedure to use it. Maintain checklist for regular checking.

10. Procedure of Internal Audit

1. Internal audit of all laboratories is done once in a year.

2. The incharge of a section becomes the auditee when he does the audit of another section. The auditor is qualified for doing the audit of respective lab.

3. Auditee is the incharge of the laboratory which is being assessed.

4. Audit plan is made by the Quality Assurance incharge for the year and the respective personnel are informed about it.

5. On the day(s) of audit, in the presence of lab incharge as the auditee, the procedures done by the lab personnel are observed.
   Format of 'audit of procedures' includes all SOPs of that lab.

6. All the documents and records are audited. Report is documented in the format of 'audit of documents'.

7. The equipment is audited. It is documented in the format of "Audit of equipment'.

8. Vertical audit is also done. Random donation ID numbers are selected and these are checked in the donor lab, grouping and testing lab and component lab.
   (The documents and computer entry are checked.).

9. Any nonconformance noted is given a NC No. Remarks/Recommendations are also noted.

10. Nonconformance, which is raised, is noted in the respective format by the auditor.

11. The auditee proposes the corrective action and tells the time period required to do the necessary corrections and the person responsible.

12. The auditee does the corrective actions and reports back to the auditor.

13. The auditor verifies the actions taken, and gives comments if required.
    The nonconformance and corrective action are mentioned in the format for the same. QA in-charge closes the non-conformances and signs it. A final management review is done after the audit.

14. Final audit report is made after the management review meeting.

11. Quality Indicators for Blood Centre

1. $$\text{TTI \%} = \frac{\text{Combined TTI cases (HIV + HBV + HCV + Syphilis + MP)}}{\text{Total no. of donors}} \times 100$$

2. **Adverse Transfusion Reaction Rate %** =

$$\frac{\text{No. of adverse transfusion reactions}}{\text{Total number of blood and component issues}} \times 100$$

(All major and minor reactions to be classified as per NHvPI and reported to blood bank)

3. **Wastage Rates** =

$$\frac{\text{No. of blood/ blood components discarded}}{\text{Total no. of blood/ blood components issued}} \times 100$$

4. **Turnaround Time (TAT) of Blood Issues** =

$$\frac{\text{Sum of the time taken}}{\text{Total number of blood and blood components cross matched/re served}}$$

(Time taken to be calculated from the time the request/ sample is received in the blood bank till the blood is cross matched/ reserved and available for transfusion. Blood Bank shall set upper limits for routine and emergency issues separately)

5. **Component QC failures (for each component)** =

$$\frac{\text{No. of component QC failures}}{\text{Total no. of component tested}} \times 100$$

6. **Adverse Donor Reaction Rate %**=

$$\frac{\text{No. of donors experienci ng adverse reaction}}{\text{Total no. of donors}} \times 100$$

7. **Donor Deferral Rate %** =

$$\frac{\text{No. of donor deferrals}}{\text{Total no. of donation + total no. of deferrals}} \times 100$$

8. **% of components** =

$$\frac{\text{Total component issues}}{\text{Total whole blood + component issues}} \times 100$$

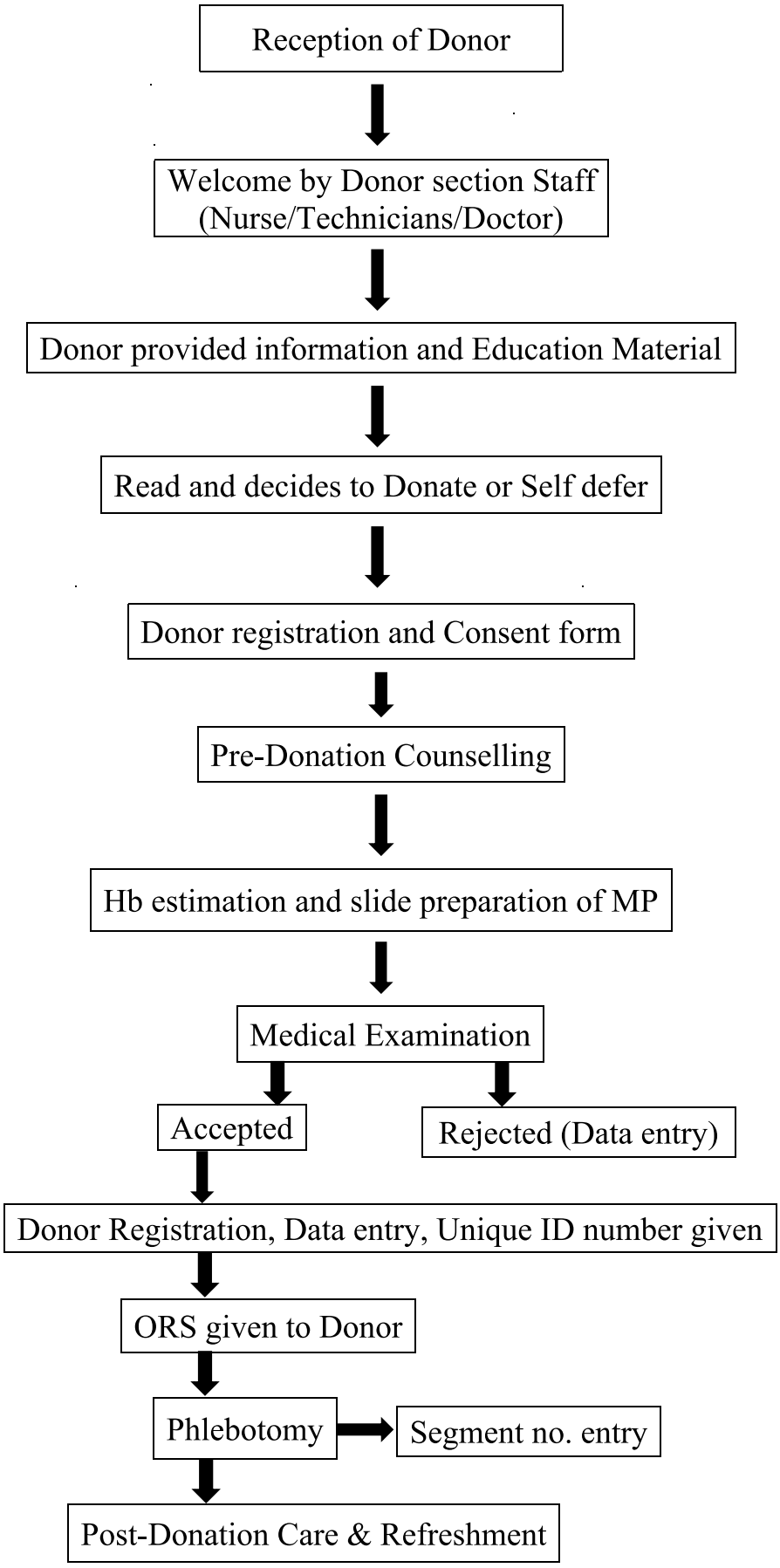
9. **TTI outliers % age** =

$$\frac{\text{No. of deviations beyond 2SD}}{\text{Total no. of batch assays}} \times 100$$

10. **Delays in transfusion beyond 30 min after issue-** sample audit by BB every month.

DONOR SECTION

1. Process flow – Donor



2.Hemoglobin estimation by Copper Sulphate method

Preparation of Copper sulphate Solution:

- Dissolve 159.6g of pure air-dried crystals of $CuSO_4.5H_2O$ in distilled water and make to 1000 ml at 25^0 C. The specific gravity of the solution must be 1.100 (Stock Solution).
- Pour distilled water in measuring cylinder till it is filled minimum $3/4^{th}$.
- Dip the bulb end of the hydrometer into the water.
- Let the hydrometer settle into the water.
- Note down the reading on the hydrometer corresponding to the upper meniscus of the water level.
- If the reading on the hydrometer corresponds to "1" (specific gravity 1.000), replace distilled water with stock solution of copper sulphate and measure the specific gravity as described above.

This stock solution is diluted to prepare solution of specific gravity 1052 to 1055 as specified below:

Specific Gravity	Stock Solution (ml)	Distilled Water (ml)	Hb equivalent (g/dl)
1052	51	49	12
1053	52	48	12.5
1054	53	47	13.0
1055	54	46	13.5

- The solution is stored at room temperature in tightly capped dark containers.

Principle:

This is a qualitative test based on specific gravity. The drop of donor's blood dropped into copper sulphate solution becomes encased in a sac of copper proteinate, which prevents any change in the specific gravity for about 15 seconds. If the haemoglobin is equal to or more than 12.5 gm/dL the drop will sink within 15 seconds.

Procedure

1. Medial side of the ring finger of the donor is cleaned with spirit swab and pricked using sterile lancet. Blood drop is taken with the help of micropipette after wiping first 1-2 drops with the help of swab. The lancet is discarded in a bowl of Hypochlorite solution.
2. Blood drop is dropped into a glass beaker/coplin jar containing $CuSO_4$ solution (height of solution should be at least 3 inches) of specific gravity 1.053 from a height of at least 1cm.
3. If blood drop sinks to the bottom of the solution, then Hb is more than 12.5 g/dl.
4. If blood drop floats for more than 15 seconds then Hb is less than 12.5 g/dl.
5. Donors with Hb more than 12.5 g/dl are accepted for donation.
6. $CuSO_4$ solution should be changed after every 25 donors screening & every day.

Interpretation:

1. If the drop of blood sinks within 15 seconds (i.e. donor's hemoglobin is more than or equal to 12.5gm/dL), the donor is accepted for blood donation.
2. However, if the blood drop sinks midway (i.e. hemoglobin level is less than 12.5gms/dL), and then comes up, the donation or donor is deferred.
3. If the drop sinks slowly, hesitates and then goes to the bottom of the jar, confirm the hemoglobin of this donor by other method.

Quality Control of $CuSO_4$:

The purpose of quality control checks is to ensure that the results obtained are accurate, consistent and reproducible. It also ensures compliance with statutory requirements such as of Drugs Controller of India or international agencies (AABB). A copper sulphate solution (1.053 specific gravity) is used for semi-quantitative estimation of Hb concentration (12.5 gm/dl) in a prospective blood donor. Periodic QC check of copper sulphate solution are necessary to prevent false acceptance of anemic blood donors or false rejection of normal donors.

It is the responsibility of the technician in the donor area to perform quality checks as per the SOP and document the results. If any unexpected results are obtained, the Faculty I/C donor area and/or Quality manager are to be informed.

Measurement of specific gravity

- Pour distilled water in measuring cylinder till it is filled minimum 3/4th.
- Dip the bulb end of the hydrometer into the water.
- Let the hydrometer settle into the water.
- Note down the reading on the hydrometer corresponding to the upper meniscus of the water level.
- If the reading on the hydrometer corresponds to "1" (specific gravity 1.000), replace distilled water with working / stock solution of copper sulphate and measure the specific gravity as described above.

Validation of function of copper sulphate solution

- Obtain at least three blood samples of known Hb conc. preferably between 12 to 13 g/dl.
- Arrange all blood samples in a plastic rack.

Check the Hb level of all these blood samples using working solution of copper sulphate

Note:

- *Check the copper sulphate solution against a light source for the presence of precipitate / cloudiness before QC check*
- *If the specific gravity of DW is not 1.000, it indicates poor quality DW or faulty hydrometer.*
- *Specific gravity measurement should be done at room temperature 22^{0}C*
- *EDTA blood samples with known Hb levels must be as fresh as possible.*

3. Performance check of BCM

Principle:

It is important to check whether equipment is giving proper results, hence daily performance check needs to be done.

Procedure:

- Switch on the Blood Collection Monitor (BCM).
- Press the start button to check if plate is properly working.
- Place the known weight on the plate.
- Check the display whether it is showing the same weight.
- If it is showing weight outside the range (+/- 10 % of weight), BCM has to be calibrated.
- Document the above in daily Performance Check Sheet.

4. Donor Notification & Follow Up

- It is important to reveal the infectious disease of blood donor. WHO defines counselling as a dialogue between a client and a care provider (doctor/social worker/nurse) aimed at enabling the client to cope up with stress and to take personal decision relating to TTI and HIV/AIDS in particular the evaluation of personal risk and the facilitation of preventive behaviour.
- It is the responsibility of a counselor in the Blood Centre or medical officer in-charge.

HIV infection

Donor is referred to ICTC Centre or in case of donor of outside the city, he can be referred to the Centre which is nearby to his place.

The counselor does not disclose the reactive status of HIV in any circumstances.

Hepatitis B/C

Donor is referred to the physician/gastroenterologist for decision regarding further management.

The donor is to be convinced for not to donate his blood in future.

Not to share his personal blade, razor, nail cutter, tooth brush with anyone.

To inform his doctor about the reactive status, to test his wife and children for HBsAg and if they are negative, vaccinate them against hepatitis B.

Syphilis

Syphilis positive cases are referred to the STD clinic.

Malaria

Malaria positive cases are referred to the medicine department.

- The counselor should maintain all the records of TTI positive cases and their follow up. They should be entered in the counselling register. Telephonic calls should be documented.

5. Common Adverse Donor Reaction Management

- Immediate steps to be taken if adverse reactions occur:
  1. Remove /deflate tourniquet & withdraw needle from donor's vein at the first sign of a reaction. Put sterile swab at the venipuncture site and apply pressure with thumb.
  2. If possible, remove donor to an area where he/she can be attended to in privacy.
  3. Call for assistance from other personnel, if required.
- After recovery, post donation instruction must be given to the donor and some additional instructions depending upon severity of the reaction. Record of such reactions must be mentioned in donor adverse reaction form and copy of one form is given to donor.
- Observation is very essential. Keep donor under observation in rest room. If needed, assistance should be provided to accompany the donor to his place to avoid any injury or accident.

1. Giddiness/Syncope (vasovagal syndrome):

SYMPTOMS: Sweating, weakness, dizziness, pallor, loss of consciousness, convulsions, involuntary passage of urine & faeces, Skin is usually cold, BP falls & pulse becomes thready.

REASON: May be caused by the sight of blood, by watching others giving blood, or by individual or group excitement or unexplained reasons.

MANAGEMENT:

a. Raise feet and lower head end.
b. Loosen tight clothing (belt, tie etc.)
c. Ensure adequate airway.
d. Check pulse and blood pressure.
e. Apply cold compresses to forehead and back.
f. Administer inhalation of spirit of ammonia if needed. The donor should respond by coughing which will elevate the blood pressure.
g. If there is bradycardia and hypotension-
   Administer inj. Atropine 1 ml IM, if bradycardia continues for more than 20 minutes.
   Administer IV normal saline or dextrose saline infusions if hypotension is prolonged.

2. Convulsions:

Keep the head tilted to the side; prevent the tongue bite; keep the airway patent by inserting a tongue blade or gauze between the teeth.

3. Vomiting

Usually this provides relief. If the donor feels nauseous or if vomiting is severe, inject Antiemetic (Stemetil).

Usually subsides on its own.

4. Tetany/Muscular spasm /Twitching :

SYMPTOMS: Tingling, twitching or muscular spasms. (1 in 1000)

REASON: Anxiety or deep breathing may cause the excited donor to loose excess of CO_2, which may cause tetany.

MANAGEMENT:

These are usually due to hyperventilation in an apprehensive donor. Ask the donor to breath in a paper bag, which provides prompt relief. Do not give oxygen.2

5. Haematoma :

Release the tourniquet/pressure cuff immediately.

Apply pressure on the venepuncture site and withdraw the needle from the vein.

Raise the arm above the head for a few minutes.

Apply Thrombophob ointment gently around the phlebotomy site after about 5 minutes.

Advice the donor to apply ice if there is pain and inform about the expected change in skin colour.

6. Eczematous reactions of the skin around venepuncture site:

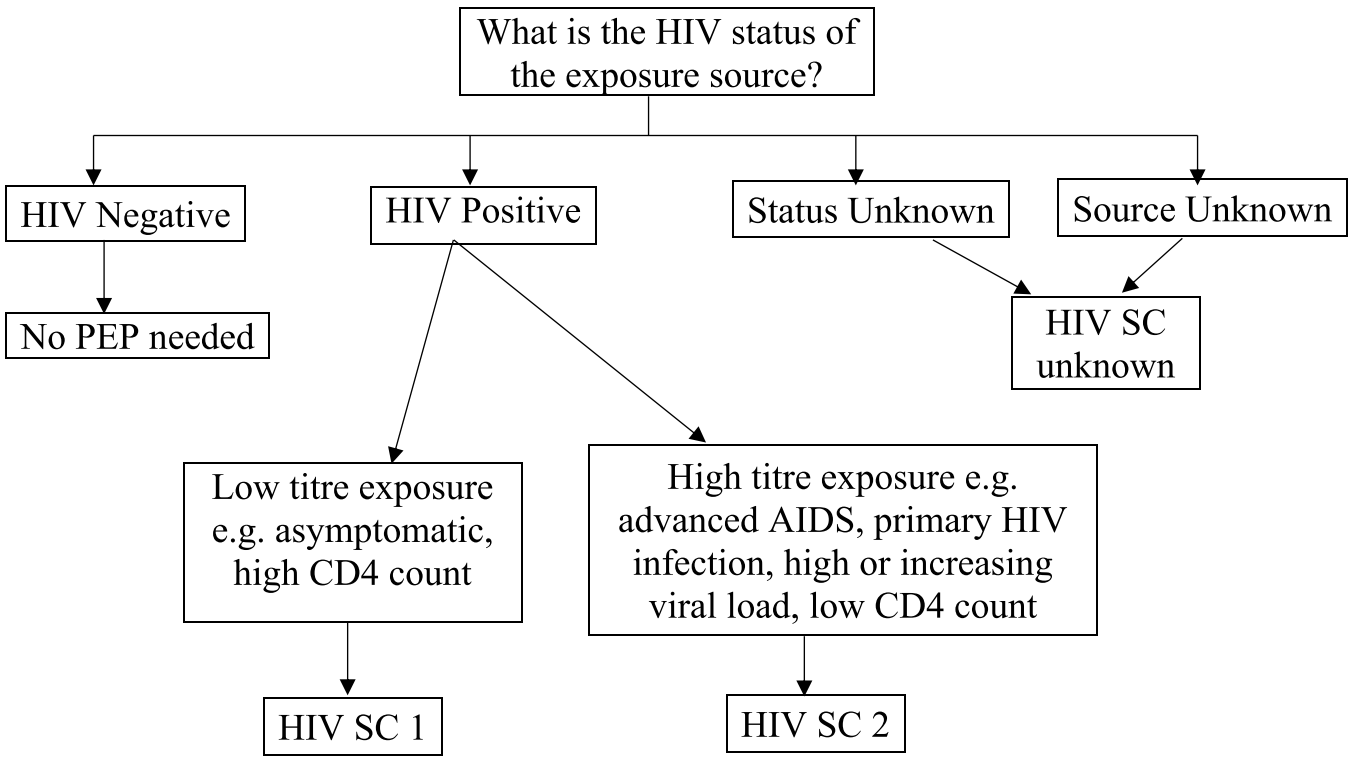
Apply steroid ointment.

7. Delayed syncope

These may occur as late as 30 minutes to 1 hour after donation, usually after the donor has left the blood Centre. Permanently defer any donor who gives history of such attacks more than twice.

6. Needle Stick Injury & Post Exposure Prophylaxis

- In the event of needle stick injury wash with soap under running water.
- Do not squeeze the site.
- Let the blood flow.
- Wash the site with plenty of water.
- Report to the Faculty In-charge, who in turn will report to the head of the department/ centre.
- Destroy the disposable needle and syringes and discard it in a puncture resistant container containing hypochlorite.
- Test the blood samples of the source as well as the staff who got needle stick injury.
- Refer concerned staff to ICTC/ emergency room.
- If both the samples are negative, repeat the test after 3 months on the sample from the concerned staff.
- If source positive,



*EC= Exposure Code, SC= Status Code

Make entry in incident reporting form.

EC	HIV SC	PEP Recommendation
1	**1**	**PEP may not be warranted.** Exposure type does not pose a known risk for HIV transmission. Whether the risk for drug toxicity outweighs the benefit of PEP should be decided by the exposed HCW and treating physician.
1	**2**	**Consider basic regimen.** Exposure type poses a negligible risk for HIV transmission. A high HIV titre in the source may justify consideration of PEP. Decision should be taken by the exposed HCW and treating physician.
2	**1**	**Recommended basic regimen.** Most HIV exposures are in this category. No increased risk for HIV transmission has been observed but use of PEP is appropriate.
2	**2**	**Recommended expanded regimen.** There is an increased risk of HIV transmission.
2/3	**Unknown**	**Consider basic regimen.** If the source (in case of an unknown source), and the setting where the exposure occurred suggests a possible risk for HIV exposure, PEP basic regimen can be considered.

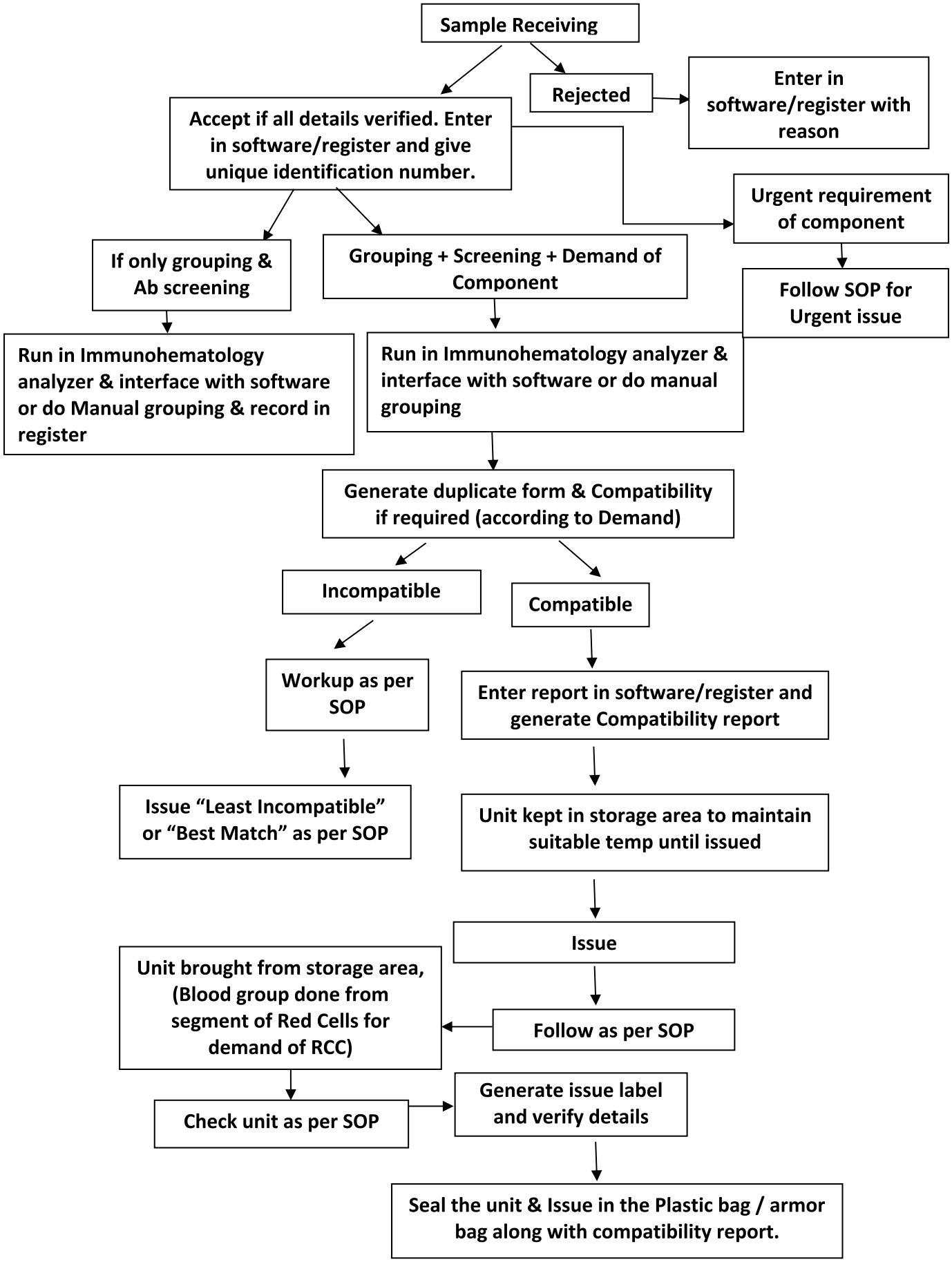
Revised ARV regimen for PEP for HIV infection:

Exposed person	Preferred regimen for PEP	Alternate Regimen (if preferred regimen is not available or contraindicated)
Adolescents and Adults (>10 years and >30 kg weight)	Tenofovir (300 mg) + Lamivudine (300 mg) + Dolutegravir (50 mg) (FDC – 1 tablet OD)	Tenofovir (300 mg) + Lamivudine (300 mg) (FDC – 1 tablet OD) + Lopinavir (200 mg)/Ritonavir (50 mg) (2 tablets BD) OR Tenofovir (300 mg) + Lamivudine (300 mg) + Efavirenz (600 mg) (FDC – 1 tablet OD)
Children (≥ 6 years and ≥ 20 kg weight)	Zidovudine + Lamivudine (dosage as per weight band) + Dolutegravir (50 mg) (1 tablet OD)	If Hb <9 gm/dl: Abacavir + Lamivudine (dosage as per weight band) + Dolutegravir (50 mg) (1 tablet OD)
Children (<6 years old or <20 kg weight)	Zidovudine + Lamivudine + Lopinavir/Ritonavir (dosage as per weight band)	If Hb <9 gm/dl: Abacavir + Lamivudine + Lopinavir/Ritonavir (dosage as per weight band)

- The first dose of PEP should be administered immediately (preferably within 2 hours) and maximum within 72 hours of exposure.
- Duration of PEP is 28 days, irrespective of regimen.

Immuno-hematology

1. Process Flow Of Red Cell Serology Lab



2. Quality Control of Antisera

PURPOSE

The acceptance criteria for Anti-A, Anti-B, Anti-AB, Anti-D (IgM) and Anti-D (Blend) blood grouping reagents for parameters like potency, intensity, avidity, prozone, rouleaux, specificity and physical appearance given in this manual are intended to help the Blood Transfusion Services to strengthen their Quality Control Department.

There should be no reaction with negative control, no cross reactive and no rouleaux or prozone phenomena. The reagent should be clear on visual inspection daily.

Test Procedure for Anti-A (Monoclonal), Anti-B (Monoclonal) and Anti- A, B (Monoclonal), Anti-D (IgM) and Anti-D (Blend) Blood Grouping Reagent

MATERIAL AND EQUIPMENT REQUIRED:

1. Red Blood Cells suspension: 2-5% cells
2. Normal Saline
3. Test tubes 5ml
4. Test tubes 10ml
5. Glass Slides
6. Cover Slips
7. Centrifuge
8. Microscope
9. Micropipettes
10. Incubator for 37°C
11. Stop watch
12. Refrigerator

PRE-TEST PREPARATION

Bring all the reagents to be evaluated at room temperature.

1. Check the reagent for physical appearance and color and document observations in Proforma.
2. Prepare protocol for procedure to be followed for Titre, Specificity, Avidity, Intensity, Rouleaux and prozone testing.
3. Use normal saline or any other diluent as per standard instructions.
4. Arrange and label the test tubes for the reagent to be tested in separate test tube stands.
5. Use fresh pipette tip for each dilution to avoid carryover of reagent to next higher dilution.

➢ **TITRATION AND SPECIFICITY:**

1. Arrange and label one row of test tubes from 1:1 to 1:1024 for dilution of antisera under evaluation.
2. Beginning with the undiluted antisera, prepare two-fold master serial dilutions (1:2, 1:4 etc. till 1:1024) for test.
   A. Add 200µl of Normal Saline to each tube except tube no.1 and 400µl of antisera to tube no.1.
   B. Transfer 200µl reagent to tube no.2.
   C. Mix and transfer 200µl to the next tube.
   D. Repeat step C till tube no.11 as below.
   E. Add 100µl of 2-5 % suspension of corresponding pooled red cells.
   F. Arrange and label the tubes for negative control cells for specificity testing of reagent under test. Use undiluted (100µl) reagent for specificity testing for each negative control cell.
   G. Dispense 100µl of reagent red blood cell suspension of positive control cells and negative control cells to the respective rows of tubes for reagent under test
   H. For Anti-D (Blend), incubate tubes at 37°C for 30-45 minutes.

I. Gently shake test tube stand to mix the contents thoroughly.
J. Centrifuge for 1 minute at 1000 rpm.
K. For specificity, observe all the negative control tubes under the microscope for clear-cut negative reaction.
L. Gently dislodge the cell buttons of each test tube and examine grade of reaction macroscopically and record the readings.

200µl 200µl 200µl 200µl 200µl 200µl 200µl 200µl 200µl 200µl

Master Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Tube No	1	2	3	4	5	6	7	8	9	10	11

➢ **AVIDITY & INTENSITY TESTING BY SLIDE METHOD (at room temperature)**

1. Pooled Red Cell Preparation: Prepare 40-50% of red cells suspension.
2. Dispense an equal volume of antisera under test (20-50µl) and pooled red blood cells (40-50%) on clean glass slide, adjacently.
3. Mix antisera and cells rapidly in a circular manner using a tooth pick and spread over 1-3 mm diameter area on slide.
4. Observe and measure the time for appearance of the first visible agglutination.
5. For each antisera repeat steps 2 to 4 three times and calculate mean of three observations.
6. Record the grade of the reaction.

➢ **REACTIVITY**

HAEMOLYSIS: Observe all tubes for absence of haemolysis.

ROULEAUX: Check the contents of all the negative control tubes microscopically for absence of rouleaux. Place about 5µl of the mixed contents on a slide and cover with cover slip and observe under the microscope.

➢ **PROZONE: TESTING BY TUBE METHOD**

1. Arrange and label 3 tubes for each antisera to be tested, “15 Minutes”, “30 Minutes” and “60 Minutes” respectively.
2. Add 100µl of neat antisera to all tubes or as per manufacturer’s instructions.
3. Add 100µl of 2-5 % suspension of corresponding pooled red cells to respective tubes.
4. Mix and incubate at room temperature for the duration indicated in the tube.
5. Centrifuge at 1000 rpm for 1 minute.
6. Record the grade of the reaction as in the case of titration.
7. At least a 2+-reaction grade should be obtained with all samples at all incubation times.
8. Interpretation of the test:

NO PROZONE is present - <u>if the reaction grades are the same or increase as the incubation time increases,</u>

A PROZONE is present - <u>If the reaction grade decreases as the incubation time increases.</u>

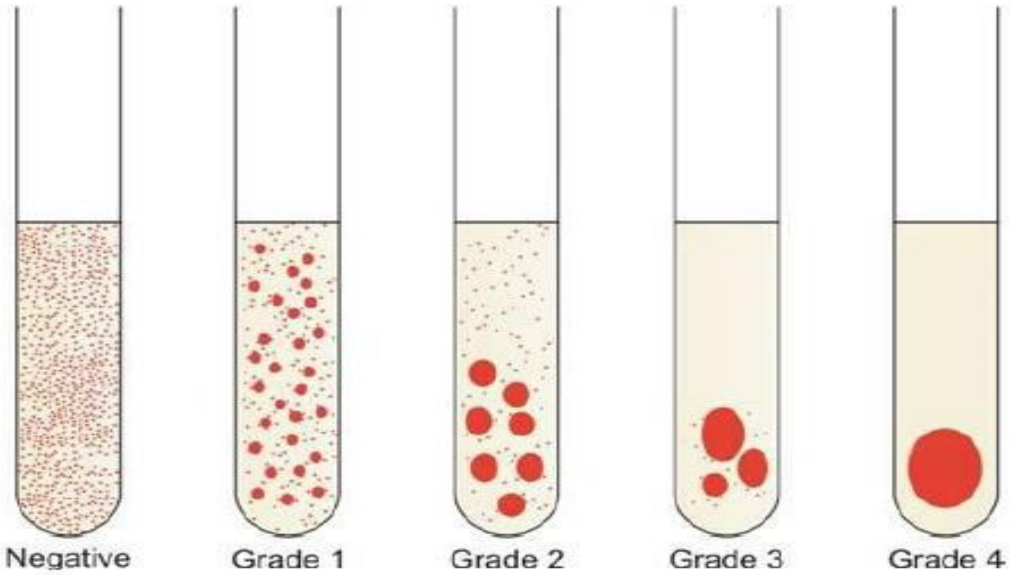
Acceptance Criteria for Titre, Specificity and Avidity for Anti-A (Monoclonal), Anti-B (Monoclonal) and Anti-AB (Monoclonal) Reagent

Name of the Reagent	Type of the Reagent	Physical Appearance and Color	Type of Red Cells	Titre	Avidity (time in Seconds)	Intensity	Specificity	Reactivity (Rouleaux Haemolysis Prozone)
Frequency		Each day		Each new lot.	Daily and each new lot.		Daily and each new lot.	Each new lot.
Anti- A	Monoclonal	Clear, No turbidity, precipitate, particles or gel formation by visual inspection and blue coloured liquid	A1 A2 A2B B O	≥1:256 ≥1:128 ≥1:64 --- ---	3 - 4 sec 5 – 6 sec 5 – 6 sec --- ---	3+ 2+ to 3+ 3+ to 4+ --- ---	Positive Positive Positive Negative Negative	Absent
Anti- B	Monoclonal	Clear, No turbidity, precipitate, particles or gel formation by visual inspection and yellow coloured liquid	B A1B A1 O	≥1:256 ≥1:128 --- ---	3 – 4 5 – 6 --- ---	4+ 2+ to 3+ ---- ---	Positive Positive Negative Negative	Absent
Anti-A,B	Monoclonal	Clear, No turbidity, precipitate, particles or gel formation by visual inspection and colourless or cherry coloured liquid	A1 B A2 Ax O	≥1:256 ≥1:256 ≥1:128 --- ---	3 - 4 sec 3 – 4 sec 5 – 6 sec --- ---	4+ 4+ 3+ --- ---	Positive Positive Positive Positive Negative	Absent

Anti-D (IgM)	Monoclonal	Clear, No turbidity, precipitate, particles or gel formation by visual inspection and colourless liquid	O +ve R1 r (or) R1R2	IS - 1:64 – 1:128 37^0C x 30” - 1:128 – 1:256	5 - 10 sec	3+	Positive	Absent
			Rh-negative (IAT)	----	----	----	Negative	
Anti-D (IgG+ IgM) Blend	Monoclonal	Clear, No turbidity, precipitate, particles or gel formation by visual inspection and colourless liquid	O +ve R1 r (or) R1R2	IS - 1:32 – 1:64 37^0C x 30” 1:128 – 1:256	10 - 20 sec	3+	Positive	Absent
			Rh-negative (IAT)	----	----	----	Negative	

Grading agglutination reactions for titre

Grade	Appearance
Complete or 4+	A single agglutinate. No free red cells detected.
$4+^{w}$ or $3+^{s}$	Strong reaction with a large agglutinate and 1or 2 small agglutinate. No free red cells detected.
3+	Strong reaction. A number of large agglutinates. No free red cells detected.
$3+^{w}$ or $2+^{s}$	Strong reaction with a number of small and large agglutinates. No free red cells detected.
2+	Large agglutinates in a sea of smaller clumps, no free red cells.
$2+^{w}$	Many agglutinates-medium and small no free red cells.
$1+^{s}$	Many medium and small agglutinates and free red cells in the background.
1+	Many small agglutinates and a background of free red cells.
$1+^{w}$	Many very small agglutinates with a lot of free red cells.
± Macro*	Weak granularity in the RBC suspension. A few macroscopic agglutinates but numerous agglutinates microscopically.
(+) Micro**	Appears negative macroscopically. A few agglutinates of 6-8 red cells in most fields.
(0^{R})Rough	Rare agglutinates observed microscopically.
0	An even red cells suspension. No agglutinates detected.
***Macro = Macroscopic**	****Micro = Microscopic**



3. Quality Control of Reagents

Standardization of blood group reagents is carried out by the manufacturers and they should meet established requirements. The quality control at the user laboratory should be done to check new batches to ensure that they comply with all biological standards.

Almost all laboratories use reagents red cells, ABO and Rh typing sera, anti-human globulin (AHG), one or more proteases, bovine serum albumin (BSA), isotonic saline and low ionic strength salt solution (LISS). Their quality control is very essential.

- **Quality Control of Reagent red blood cells**

Parameters	**Quality requirements**	**Frequency of controls**
Appearance	No hemolysis or turbidity in supernatant by visual inspections	Each day
Reactivity and Specificity	Clear cut reactions with known sera against red blood cells antigens	Each day

If reagent red cells are slightly hemolysed, the cells can be washed once with saline. If the supernatant becomes clear after one wash, and they are reactive, the cells are acceptable for use. Hemolysed and discoloured red cells are discarded.

- **Quality Control of AHG Antisera**

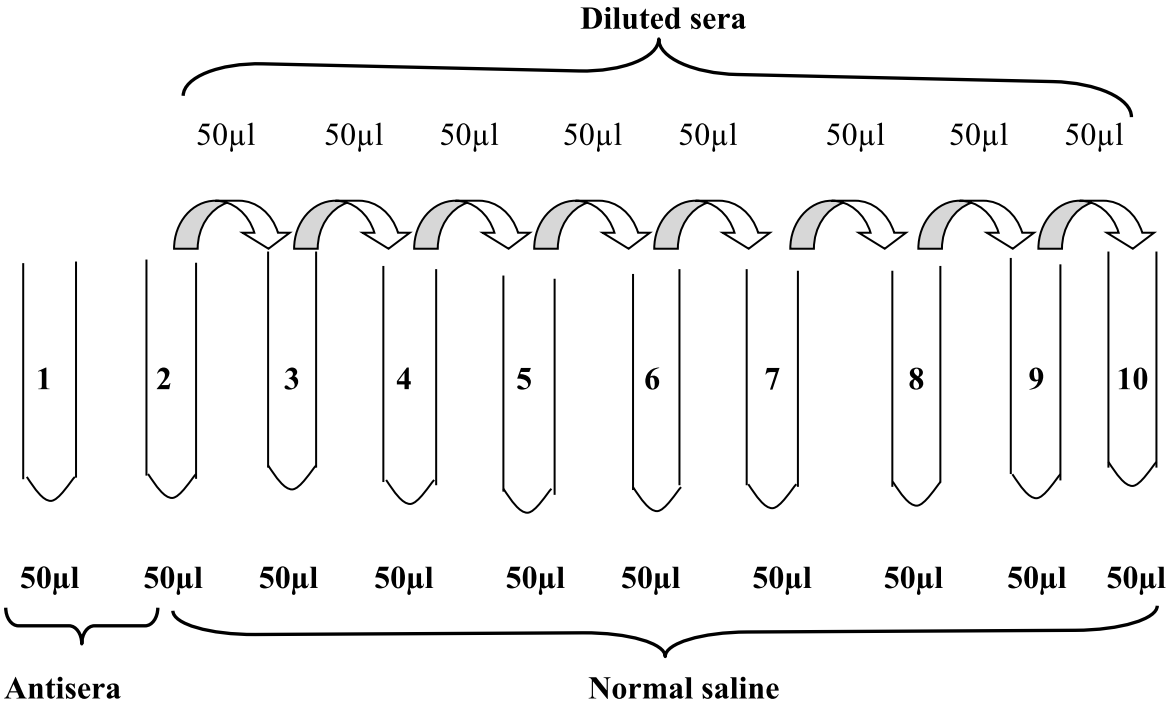
Method 1:

1. Put 1 drop/100 µl of normal saline in each tube from tube no 1 to 10.
2. Now add 2 drops/200 µl sera in 1st tube from a known ICT positive patient, wipe off the excess serum from pipette with gauge piece.
3. Mix the mixture thoroughly with the help of pipette and transfer 1 drop/100 µl of mixture in 2nd tube and continue this procedure up to tube no 10. From the last tube discard 1 drop/100 µl.
4. Further add 1 drop of 5% saline cell suspension of O positive cells.
5. Incubate at 37^{0}C in water bath for 30-40 minutes.
6. Take out all the tubes from water bath and wash with normal saline 3 times and then add Coombs sera.

Method 2:

1. Label 10 test tubes according to the AHG dilution (starting from 1:1, 1:2, 1:4, 1:8 upto 1:512) as shown in Figure for AHG to be checked.

2. Deliver one volume of saline to all test tubes except the first tube.
3. Add an equal amount of AHG to each of the first two tubes.
4. Using a clean pipette mix the contents of the 1 in 2 dilution several time and transfer one volume into the next tube as shown in following figure.



Scheme for serial dilutions for titration of antisera

- Continue the same process for all the dilutions, using a clean pipette to mix and transfer each dilution.
- Remove one volume of diluted serum from the final tube and save it for use if further dilutions are required.
- Add 1 drop of sensitized group 'O' cells (check cells) (5%) into each test tube.
- Mix well and keep these test tubes at room temperature for at least 15 minutes.
- Centrifuge all these test tubes at 1000 rpm for 1 minute.
- Examine test results macroscopically; grade and record the reactions.

Note:

- *There is no need to change microtips between tubes if micropipette is being used for serial dilutions.*
- *QC of **Anti Human Globulin (polyspecific)** shall include physical examination and titration in AHG phase against known DAT positive cells (check cells).*

Now check the validity of coombs AHG sera with the help of check cells (check cells should give agglutination in case of negative test result).

- **Quality Control of Coomb's cards**

1. Prepare check cells or use commercially available check cells.
2. Perform DAT.
3. The result of DAT should be at least 2+.
4. The quality control of Coomb's cards should be done on each day of use and documented in register.

- **Quality Control of Antihuman Globulin (AHG)**

Parameters	Quality Requirements	Frequency of control
Appearance	No precipitation, turbidity or gelling	Each day
Reactivity	No hemolysis, rouleaux formation or Prozone phenomena	Each new lot
Specificity	No agglutination of unsensitized red cells.	Each day
	Agglutination of red cells sensitized with anti D having lgG	Each new lot
	Agglutination of red cells sensitized with complement binding Ab	Each new lot
	Agglutination of red cells coated with C3d or C3b but no agglutination with C4 coated cells	Each new lot
Avidity	The polyspecific AHG should give minimum 1:16 titre	Each new lot

Note:

- *If any of this QC parameter gives non-conforming results, it should not be used and this should be informed to faculty in-charge/ Quality manager.*

DOCUMENTATION

1. Record the details of antisera such as Manufacturer name, lot No, Date of Expiry in the registers/computer.
2. Record all results such as physical check, specificity, titre, avidity and score in the register.

- **Quality Control of Normal Saline**

Preliminary

- Take bottle of normal saline.
- Examine the normal saline for followings and note down
    - Color/clarity: No turbidity or particles.
    - Any visible contamination: No.

Determination of pH

Take 200 ml normal saline into a beaker. Measure the temperature of normal saline and then measure it's pH. It should be between 6.0 - 8.0.

Determination of NaCl content

It should be around 0-154 mol/l (=9 g/l) and should be checked with each batch of normal saline.

Determination of quality

1 ml of red cells should be mixed with equal volume of normal saline and should be centrifuged, check for hemolysis. If hemolysis present, it should not be used.

- **Quality Control of Distilled Water**

Preliminary

- Take Distilled Water.
- Examine the DW for followings and note down
    - Color/clarity: No turbidity or particles.
    - Any visible contamination: No.

Determination of pH

Take 200 ml DW into a beaker. Measure the temperature of DW and then measure the pH of DW. It should be between 6.0-7.0.

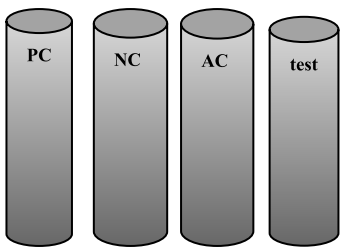
➢ Quality control of Albumin

Preliminary

- Take one vial of each reagent for quality control testing from the new lot.
- Examine the albumin for followings and note down
    - Color / clarity
    - Any visible contamination
    - Any flocculation
    - It should be free from nonspecific agglutination and should not react with saline suspension of O, A, B, cells and should give positive result with Rh positive coated cells with incomplete anti D serum (Coomb's control cells).
- Test the antisera for determination of specificity, avidity and titre as described below.

Determination of specificity

- Label four [2 test tubes for O cells (first O +ve & second O Neg) for positive and negative control, one auto control (cells and serum from same sample) and one with patient serum and donor cell (test)] clean glass test tubes for each to be used using marker pen.
- Put 1 drop of corresponding 5% red cell suspension of known ABO group in respective tubes.
- Add 1 drop of respective antisera to each tube.
- Add 1 drop of albumin to each test tube
- Incubate the tubes at temperature (37^0C) for 15-30 minutes.
- Centrifuge the tubes at 1000 rpm X 1 min in the table top centrifuge
- Observe, grade and record the results



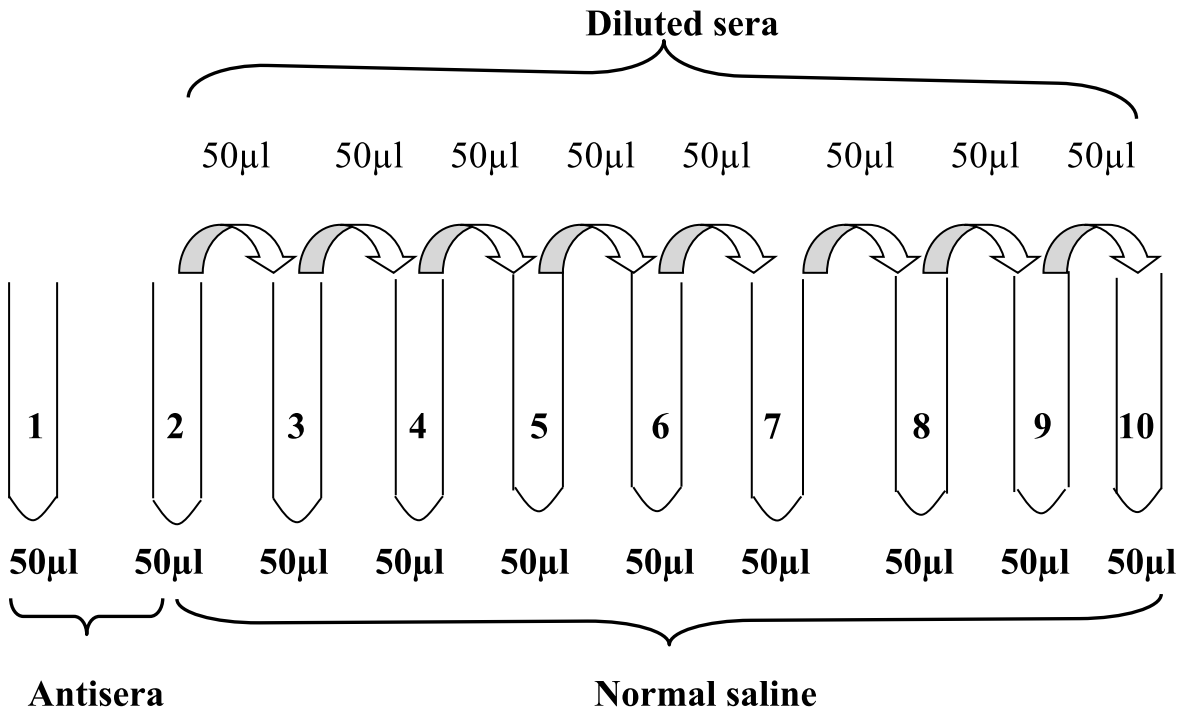
Scheme for determining specificity of albumin

Determination of Avidity

- Label a clean test tube for each antisera to be used using marker pen.
- Put 1 drop of 5% red cell suspension of respective known group.
- Put 1 drop of respective antisera with the red cell suspension.
- Mix and add albumin to it.
- Start the stop watch simultaneously
- Observe and note the time required for visible agglutination

Titration of Bovine Serum Albumin

- Label 10 test tubes according to the anti-serum dilution (starting from 1:1, 1:2, 1:4, 1:8 up to 1:512) preferably Anti-D antisera.
- Deliver one volume of saline to all test tubes except the first tube.
- Add an equal amount of anti-serum to each of the first two tubes.
- Using a clean pipette mix the contents of the 1 in 2 dilution several time, and transfer one volume into the next tube as shown in following figure.



Scheme for serial dilutions for titration of antisera

- Continue the same process for all the dilutions, using a clean pipette to mix and transfer each dilution.

- Remove one volume of diluted serum from the final tube and save it for use if further dilutions are required.
- Add 1 drop of the corresponding red cell suspension (5%) into each test tube.
- Mix well and add albumin to the test tube. Keep these test tubes at 37^0C temperature for at least 45 minutes.
- Centrifuge all these test tubes at 1000 rpm for 1 minute.
- Examine test results macroscopically; grade and record the reactions. Titration should be at least 1:32 to 1:64.

INTERPRETATION :

Physical Examination:

- If the physical examination detects any abnormality in the form of color change, visible contamination No precipitation or gelling etc, the quality of that antisera is suspect and should not be used.

Specificity:

- Agglutination /hemolysis with cell and serum mixture denotes positive reaction
- No Agglutination /hemolysis with cell and serum mixture denotes negative reaction.
- There should be no prozone phenomenon or agglutination of unsensitized red cells.

Avidity

- Undiluted antisera should give visible agglutination within 3 to 5 seconds for ABO antisera and 5 to 10 sec for anti-D antisera.

Titre

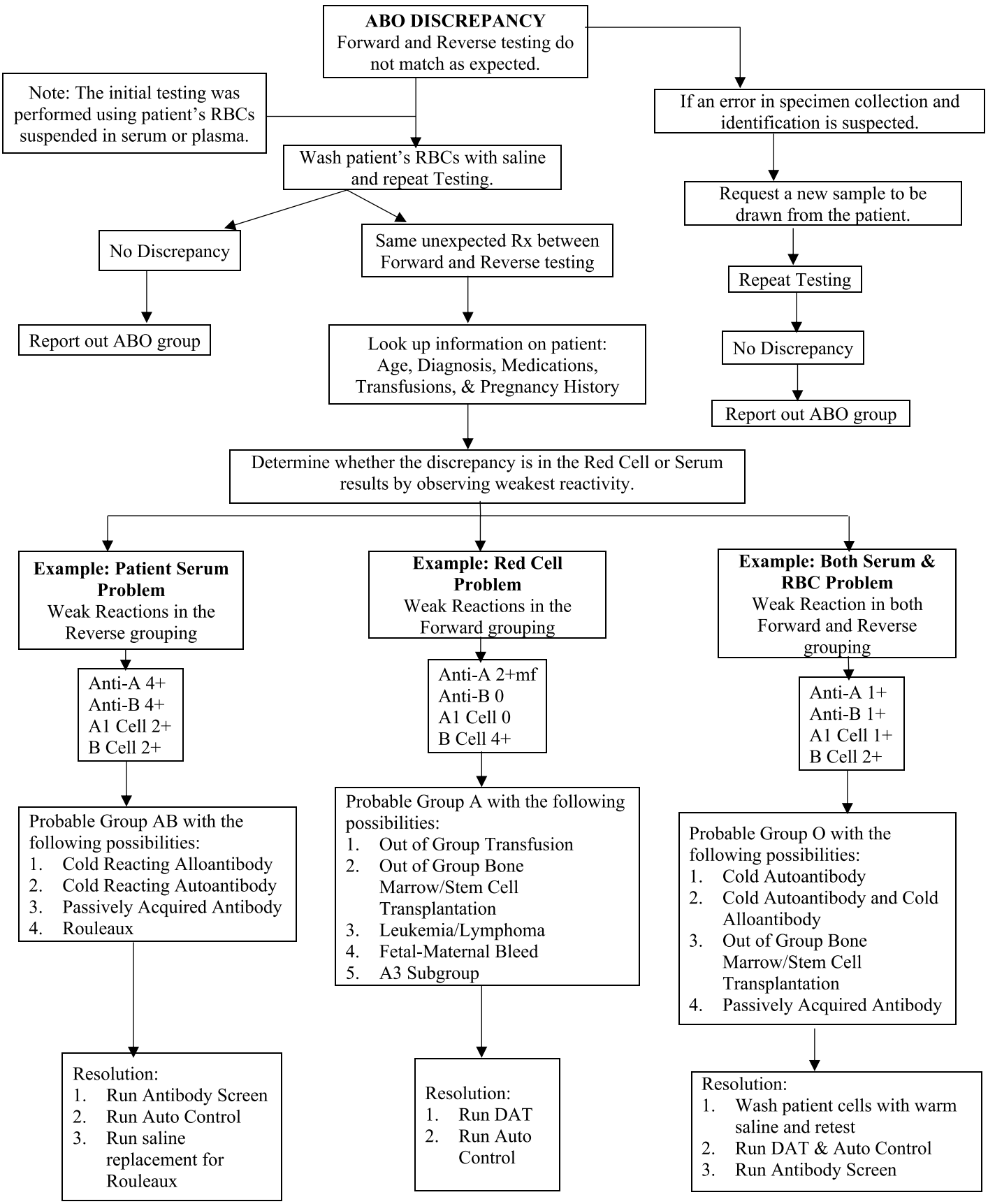
- It should give titre of 1:32 to 1:64 with R1R1 cells.

Purity: > 98% Albumin by serum electrophoresis

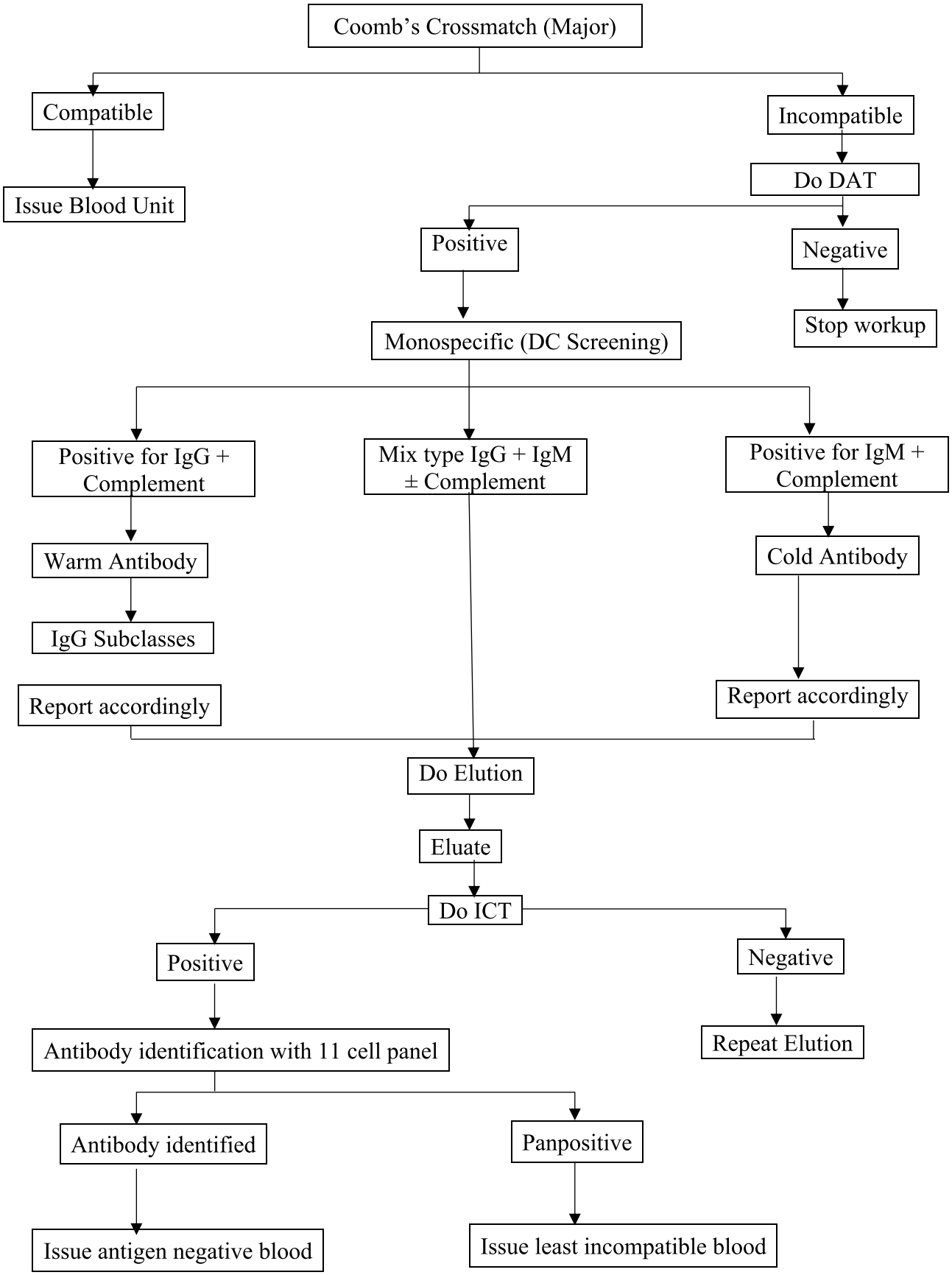
Note:

- ***If any of these QC parameter gives non-conforming results, it should not be used.***
- *Record the details of antisera such as Manufacturer name, lot No, Date of Expiry in the registers and/or computer.*
- *Record all results such as physical check, specificity, titre, avidity and score in the same register.*

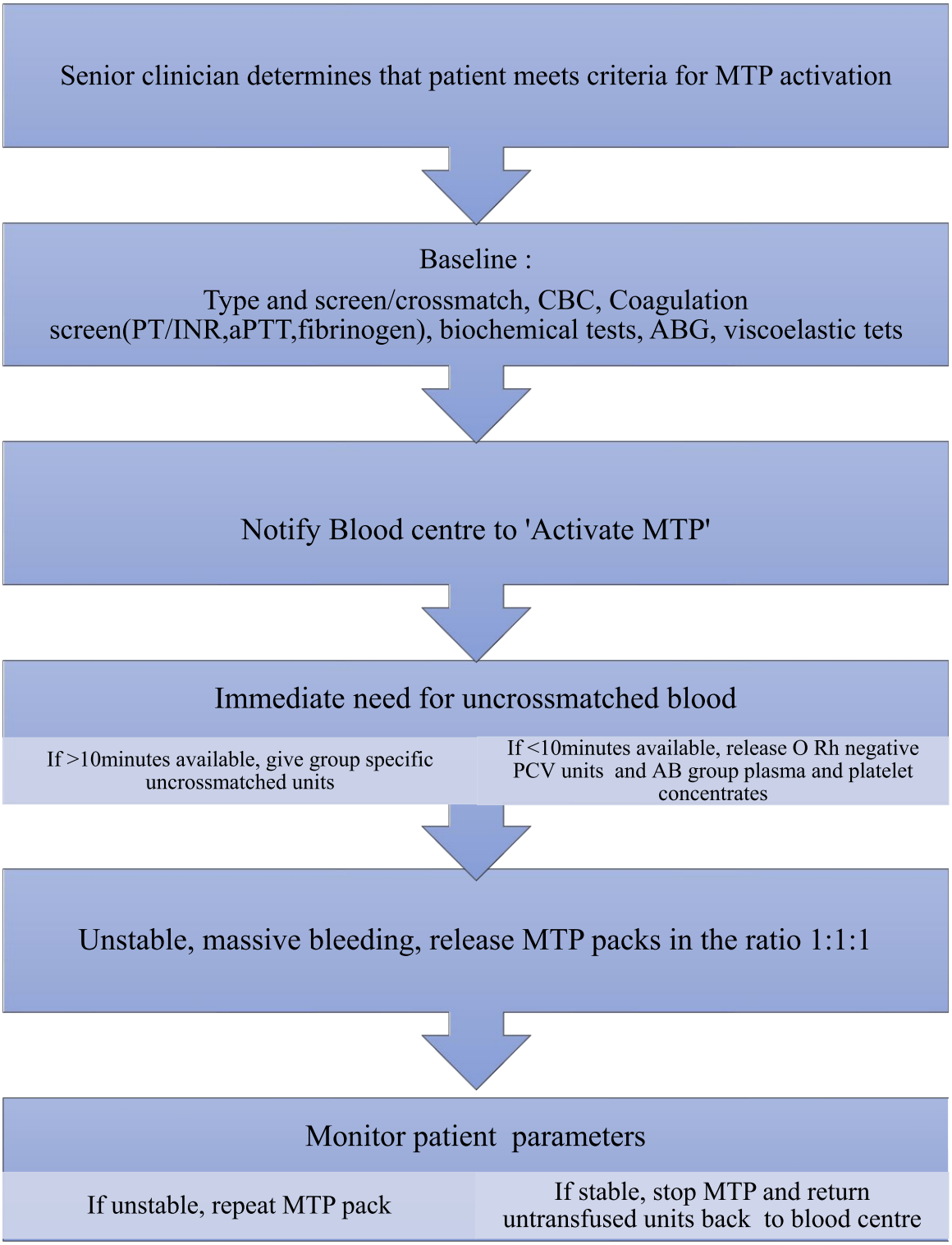
4. ABO Discrepancy – Flow Chart



5. Flow Chart For Workup Of AIHA



6. Massive Transfusion Protocol



7. Transfusion Reaction work-up

Principle:

A transfusion reaction is any unfavourable event occurring during or shortly following transfusion of blood components, which can be related to that transfusion. The purpose of this procedure is to delineate the steps taken in the evaluation of a reported transfusion reaction.

Responsibility:

- It is the responsibility of the resident in-charge of Serology laboratory to perform complete work-up of suspected transfusion reaction.
- Technician in the Serology laboratory shall ensure that reaction form with complete details and requisite samples and/or blood bag are received and handed over to the Resident.
- In case of transfusion reaction reported in emergency hours, it will be the responsibility of Resident on duty to perform preliminary investigation to rule out major mismatch and informed to Faculty In-charge.

Procedure:

- When a report of transfusion reaction arrives with post transfusion patient sample and other requisite forms, obtain full clinical details of patient including signs and symptoms, diagnosis, transfusion history, medication history etc. as mentioned in the Reaction Work-up Form.
- Check the time of issue from the Blood Centre, time of starting and stopping the transfusion and volume of blood transfused.
- Examine the implicated unit for any change in color, clots, leakage.
- Examine the color of blood in blood bag as well as in administration set.
- Perform an **initial investigation** to rule out acute hemolytic transfusion reaction.
    - Ensure that blood samples meet the labelling requirements and check for any clerical or identification errors.
    - Look for hemolysis (pink color) in the supernatant plasma in the post transfusion sample.
    - Repeat ABO/Rh grouping on pre-transfusion, post-transfusion sample of the patient and implicated blood unit.
    - Repeat cross match of the implicated unit with both pre and post transfusion samples as per SOP.
    - Perform DAT on the pre and post transfusion sample as per SOP.
- If the **initial investigation is positive** (i.e., hemolysed plasma supernatant, DAT positive only on post transfusion sample or increase in the DAT strength or the clerical error shows definite mismatch), continue with further investigations.
    - Serological investigations:
        - Perform antibody screen on both pre and post transfusion sample of the patient as per SOP.
        - If antibody screen is positive, perform antibody identification as per SOP.

- Biochemical investigations:
  - Perform or obtain the results of LDH test, retic count and serum bilirubin from the corresponding department, if available.
  - Post transfusion urine sample of the patient can be examined for free red cell, haemoglobinuria or hemosiderinuria using appropriate test.
  - If the investigations suggest acute hemolytic reaction, coagulation profile (PT, APTT, Fibronectin, Platelet count) may be required to rule out impending disseminated intravascular coagulation.
- Microbiological investigations:
  - Send a sample from the implicated donor unit to microbiology department for bacterial culture.
  - If donor unit reveals bacteraemia, get the patient's blood culture done.

Note:

- *It is important to know if any medication or intravenous fluid has been added to the blood unit as it may result in hemolysis in the bag, giving rise to transfusion reaction.*
- *If the post transfusion patient sample has been withdrawn more than 24 hours after reaction, interpret the results of DAT and hemolysis carefully, as all sensitized cells may have undergone hemolysis misleading to negative DAT even in blood group mismatch.*
- *If any labelling or clerical error has occurred, another patient's sample may also have been incorrectly labelled. In this case, to prevent any mishap to other patients, it would be helpful to check all the units that are cross-matched on the given date for any labelling or clerical error.*
- *Detailed investigation as above may not be required for reaction due to transfusion of FFP and / or platelets because FNHTR are very common with these components.*
- *Subsequent transfusion should be withheld for 24 hours unless urgent.*

Interpretation:

- Interpret the initial investigations for hemolysis, where a negative means no evidence of hemolysis and a positive means presumptive evidence of hemolysis.

Interpretation of results of initial investigations

Interpretation	Positive	Negative
Post-transfusion DAT	More positive in strength than pre transfusion sample	Negative or same strength as pre transfusion sample
	OR	**AND**
Post-transfusion plasma supernatant color	More pink or red than pre transfusion sample	Not pink or red, or same color as pre transfusion sample

Acute hemolytic transfusion reaction

Non-hemolytic reaction (either FNHTR or allergic)

- When post transfusion sample is hemolysed (pink plasma supernatant) but DAT is negative, it could be due to reasons such as pseudo hemolysis due to transfusion of already hemolysed blood unit (bacterial contamination, thermal damage, physical damage), non-immunological hemolysis (bacterial sepsis) or late withdrawal of post transfusion sample.
- If repeat ABO & Rh grouping demonstrates discrepancy either between pre transfusion and post transfusion patient sample or grouping of implicated blood donor unit, it indicates identification or labelling error.
- If repeat cross match with post transfusion sample is incompatible, it may be due to development of alloantibodies, which were missed at the time of pre transfusion testing.
- If post transfusion IAT is positive, it indicates DHTR in which case implicated antibody shall be identified and antigen negative blood shall be provided.
- Biochemical investigations will give an idea of the severity of in vivo hemolysis.
- Deranged post transfusion coagulation profile indicates impending DIC due to intravascular hemolysis.
- Positive bacterial culture of the implicated unit indicates septic reaction. For confirmation of this, the implicated organism should also be identified in the recipient.

<u>Documentation</u>:

- When AHTR has occurred, inform the Faculty I/C of Serology laboratory and the Consultant I/C of the patient for immediate actions to be taken.
- Record the results of investigations in the BTR file.
- Upload BTR details on monthly basis on the Hemovigilance Programme of India Website (HVPI).
- One copy of the report shall be sent to the Consultant I/C of the patient.
- Report positive findings to the Faculty I/C serology lab and Quality Manager.

8. Cold Chain Maintenance

The blood cold chain is a process that begins at the time of collection and continues until the unit is transfused.

COLLECT → PROCESS → STORE → TRANSPORT → TRANSFUSE

It is a series of interconnected activities involving equipment, personnel and processes that are critical for the safe storage and transportation of blood from collection to transfusion.

Handling of blood and blood products

Care must be taken when handling blood and blood products.

1. Minimal physical handling of all blood and blood products must be practiced to ensure safety of products which are kept within their recommended temperature ranges.
2. Ensure that while handling any red blood cell products, it should not be exposed to temperature outside refrigeration specifications for longer than 30 minutes.
3. Ensure that while handling any frozen blood products outside storage conditions, it is kept on dry ice or frozen ballast within a container to prevent temperature changes.
4. The blood and blood product bag and/or packaging integrity must be inspected before sending to another health provider or facility.

Harmful effects of Improper Storage

- Decreased component potency.
- If blood is stored or transported outside of the set temperatures for long, it loses its ability to transport oxygen or carbon dioxide to and from tissues respectively upon transfusion.
- Other factors of serious concern are the risk of bacterial contamination if blood is exposed to warm temperatures.
- Conversely, blood exposed to temperatures below freezing may get hemolysed and can lead to a fatal transfusion reaction.

Elements of Cold Chain Management

Policy → Process → Procedure → Personnel → Equipment

Transport of Whole Blood/ Red cells

- Whole blood / Red cells should always be stored at 2-6^{0}C in a Blood Centre Refrigerator with in-built temperature monitoring and alarm devices and a cooling fan to ensure even distribution of cold air throughout the equipment.
- Temperature maintenance is essential to maintain oxygen carrying ability of blood, to minimize growth of any bacterial contamination and to prevent hemolysis of red cells. Hemolysed cells if transfused can cause renal failure & fatal bleeding problems.
- Whole Blood & Red Cells should be issued from the Blood Centre in the blood transport box or Insulator carrier that will keep the temperature under 10^{0}C.
- Once issued, Red Cells should be transfused within ½ hour of release from Blood Centre. If not required, it should be sent back to Blood Centre immediately.
- Blood and Blood components collected at Blood Centre or outdoor sessions should be transported between 2^{0}C and 10^{0}C (except when PC/PRP is to be prepared) to blood centre in validated transport boxes in appropriate conditions of temperature, security and hygiene.

- Blood unit should be transported from the collection site to the component preparation laboratory as soon as possible and time should not exceed 6 hours for component preparation.

Condition	Temperature Range	Storage
Transport of pre-processed blood	+ 20^0C to + 24^0C	Less than 6 hours
Storage of pre-processed blood or processed blood	+ 2^0C to + 6^0C	35 days or 42 days
Transport of processed blood	+ 2^0C to + 10^0C	Less than 24 hours

Transport of Plasma

- Fresh Frozen Plasma is stored in Blood Transfusion Centre at -40^0C or colder. If not stored at this temp, the coagulation factors as Factor VIII and Factor V deteriorate and the amount is greatly reduced.
- FFP should be transported in a blood transport box in which the temperature is maintained between 2-6^0C. This can be achieved with a suitable quantity of wet ice or dry ice in well-insulated containers or standard shipping cartoons lined with insulating materials.
- Once thawed, FFP should be infused within 30 minutes. If not transfused, thawed plasma can be stored at 2-6^0C for 24 hours.

Transport of Platelets

- Platelet are prepared by manual as well as automated methods and then stored at 22-24^0C in platelet agitator cum incubator to maintain platelet function.
- Whole blood from which platelet is to be separate should be kept at 20-24^0C before processing as lower temperatures affect platelet function and its separation.
- Platelets should be transported in a blood transport box that keeps the temperature in the correct range of about 20-24^0C.
- Platelets should never be refrigerated and should be transfused as soon as possible.

Transport Conditions for Platelets

Process	Temperature Range	Storage
Storage	+ 20^0C to + 24^0C	Upto 5 days
After issue, before transfusion	+ 20^0C to + 24^0C	30 minutes
Open system and/or pooled platelet prepared in open system	+ 20^0C to + 24^0C	4 hours
Pooled platelets prepared in closed system	+ 20^0C to + 24^0C	5 days

Packing Blood Components for Transportation

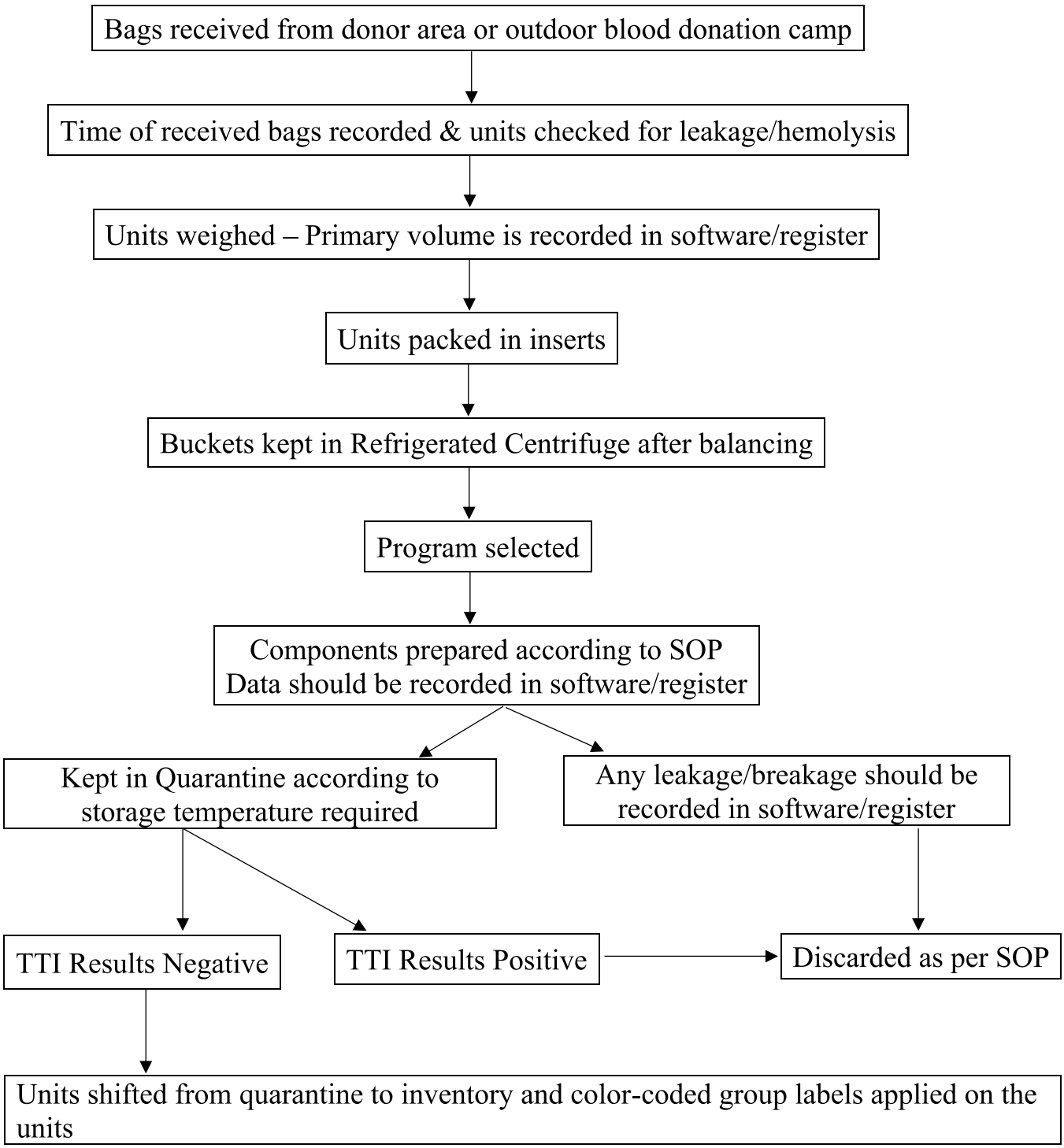
Red Cells: Ice should be placed above the blood because cool air moves downward. Red cells should not come in direct contact with ice because red cells may freeze and haemolysed.

Plasma: There should be at least as much wet ice in the cold box as there is plasma. If possible, they should have been placed in cardboard boxes before freezing to protect the bags from developing small cracks.

Platelets: Containers for transporting platelets should be equilibrated at a temperature of + 20^0C to + 24^0C before use. If outdoor temperature is extremely high, special chemical coolant pouches and containers with a power source are available that maintains temperature between + 20^0C to + 24^0C.

COMPONENT SECTION

1. Process Flow – Component Section



2. Sample Collection for Quality Control

Principle:

The laminar airflow principle involves double filtration of air. Atmospheric air is drawn through a pre-filter and is made to pass through highly effective HEPA (high-efficiency particular air) filters having efficiency as high as 99.99% with cold DOP and 99.97% with hot DOP test, thus retaining all DOP particles of size 0.3 micron and larger.

Procedure:

- Before starting the laminar airflow, the electrical supply of the unit must be turned off.
- The door of the cabinet is opened from the lower half and fixed with the magnet.
- Then the main switch of the power supply is turned on.
- Fluorescent light is turned on followed by the motor from the control panel.
- Clean the work-table surface with spirit/isopropyl/alcohol during the running of airflow and keep all the required materials are kept inside the work table.
- Then motor & florescent light are switched off and the door is closed.
- After that, switch on the U.V. light by the switch for approximate 20-25 minutes, and after that switch off the U.V. light.
- The door is kept half-open for use and switch on the fluorescent light & motor.
- Now the work can be started on the work surface by choosing the appropriate unit.
- Strip the terminal segment five to six times while gently shaking the unit.
- Using hand sealer, two segments are made of approximately 10 centimetres each.
- Use one segment for sterility testing and one for quality parameters testing.
- Complete the work on the work surface within 30 mins of switching off the UV light.
- If work is not completed in 30 mins, repeat the cleaning and disinfecting process as described above.

3. Quality Control of Blood and Blood Components

- For QC of red cell component and whole blood (WB) following parameters are checked namely volume (varies according to 350 or 450 ml bag), hematocrit, and sterility testing.
- Frequency of check is weekly.

a) Preliminary-

1. 1 % of the total RBCs prepared and total whole blood collected in a single blood bag system should be tested for Quality Control. Every week 5 to 6 RBC units/ whole blood are selected randomly from the inventory based on Outdoor camp or In-house collection. On the label, check the date of expiry & infectious disease status.
2. Document the unit number in the stock register.
3. Estimate the volume of PCV/Whole Blood units with the help of weighing balance using the following formula:

   Wt. of PCV = wt. of PCV bag – wt. of empty bag

   Volume of PCV= Wt. of PCV /Specific gravity of red cell (1.096)

   Wt. of whole blood = wt. of WB bag – wt. of empty bag

   Volume of WB= Wt. of WB /Specific gravity of red cell (1.05)

4. The tubing of the PCV bag/whole blood bag is stripped at least thrice and mix the red cells/whole blood and take representative sample from the segment. Label all the test tubes.

b) Estimation of Hematocrit by Cell counter.

Run the test sample of the donor unit in the cell counter as per SOP and record result of hematocrit.

c) Sterility of PCV/Whole blood

Strip the bag segment 5/6 times and completely mix the red cell/WB. Now take the sample from the bag in the laminar flow in the sterile tube and send it for bacterial culture.

- **Quality Parameter of Whole Blood**

Parameter	Quantity Requirement	Frequency of Control
Volume	350/450 ml ± 10 %	1% of all units
PCV (HCT)	>30%	1% of all units or at least 4 units per month (Whichever is more)
HbsAg	Negative by ELISA	All units
Anti-HCV	Negative by ELISA	All units
Anti-HIV ½	Negative by ELISA	All units
Syphilis	Negative by Screening test	All units
Malaria	Negative	All units
Sterility	By culture	Periodically (1% of all units)

- **Quality Parameter of red cell in preservative solution prepared from 450 ml whole blood (ADSOL/SAGM)**

Note: Same as for whole blood except

Parameter	Quantity Requirement	Frequency of Control
Volume	300-400 ml	1% of all units
PCV (HCT)	55-65%	Periodically (1% of all units)

- **Quality Parameter of red cell in preservative solution prepared from 350 ml whole blood (ADSOL/SAGM)**

Note: Same as for whole blood except

Parameter	Quantity Requirement	Frequency of Control
Volume	245-325 ml	1% of all units
PCV (HCT)	55-65%	Periodically (1% of all units)

- **For QC of Platelets:**

The bags for quality control testing are selected from the available inventory preferably after first, third and fifth day of preparation. 1 % of the total collection should be tested for quality control purposes.

Swirling: Check the swirling movement of platelets whether it is present or not.

Volume of the bag = weight of platelet bag - empty bag/1.03

Sterility of platelets: Strip the bag segment 5-6 times and completely mix the product. Now take the bag to the laminar air flow and take the sample in sterile tube and send for bacterial culture.

Platelet count/ leucocyte Count/ RBC Count: Strip the bag segment 5-6 times and completely mix the product. Take the bag to the laminar air flow and collect the sample in EDTA tube and check all the counts by cell counter.

pH of platelets: Empty platelets in plain glass beaker. Note the temperature.

Dip pH electrode in beaker having PC sample.

Note pH reading after adjusting temp.

OR

Take a pH strip

Dip pH strip in platelet sample in tube.

Compare the colour of pH strip with reference color code

given on the strip and note the pH.

- **QC of platelet concentrate prepared from 350/450 ml of whole blood**

Parameter	Quality Requirement	Frequency of control
Volume	50-70ml	All units
Platelets count	$\geq 3.5/4.5 \times 10^{10}$	4 units per month
pH	>6.0	4 units per month
RBC contamination	<0.5ml	4 units per month
WBC contamination	5.5×10^{7} - 5×10^{8}	4 units per month

- **QC of platelet concentrate prepared from Buffy coat**

Parameter	Quality Requirements	Frequency of control
Volume	70 - 90 ml	4 units per month
Platelet count	$>6 \times 10^{10}$	4 units per month
pH	> 6.0	4 units per month
WBC contamination	$> 5.5 \times 10^{10}$	4 units per month
RBC contamination	Traces to 0.5 ml	4 units per month

On visual inspection unit which does not have a pink or red discoloration may be assumed to contain insufficient red cells to cause immunization.

- **QC of platelet concentrate prepared from Apheresis**

Parameter	Quality Requirements
Volume	200-300 ml
Platelets count	$\geq$ 3.0-7.0 x 10^{11}
pH	>6.0
Residual leucocytes	<5.0 X 10^6
Red cells	Traces to 0.5 ml

- **Quality control of fresh frozen plasma (FFP)**

1 % of the total collection should be checked for quality control. 75% units checked should meet the quality standards.

Vol. of the bag = wt. of FFP bag-empty bag/1.03

Sterility: Thaw FFP at 37^0C temp in waterbath. Then take the bag and strip segment for 5-6 times and mix with bag properly. Now take the FFP from segment in sterile test tube under laminar air flow for culture.

Checking of parameters: Thaw FFP at 37^0C temp. in waterbath. Then take the bag and strip segment for 5-6 times and mix with bag properly. Now take the FFP from segment in the test tube under laminar air flow for checking of following parameters in the semi- automated coagulometer.

Parameter	Quality Requirements	Frequency of control
Volume	200-220 ml	4 units per month (or 1%of total components prepared)
Factor VIII	0.7 units/ml	4 units per month (or 1%of total components prepared)
Fibrinogen	200-400 mg/bag	4 units per month (or1%of total components prepared)

- **Quality Control of Cryoprecipitate:**

  1 % of the total collection should be checked for quality control. 75% units checked should meet the quality standards.

  Vol. of the bag= wt. of cryoprecipitate bag-empty bag/1.03

  Sterility: Thaw CRYO at 37^0C temp in waterbath. Then take the bag and strip segment for 5-6 times and mix with bag properly. Now take the CRYO from segment in sterile test tube under laminar air flow for culture.

  Checking of parameters: Thaw CRYO at 37^0C temp. in waterbath. Then take the bag and strip segment for 5-6 times and mix with bag properly. Now take the CRYO from segment in the test tube under laminar air flow for checking of following parameters in the semi- automated coagulometer.

Parameter	Quality Requirements	Frequency of control
Volume	10-20 ml	4 units per month (or 1% of total components prepared)
Factor VIII	80-120 units/bag	4 units per month (or 1% of total components prepared)
Fibrinogen	150-250 mg/bag	4 units per month (or 1% of total components prepared)

4. Maintenance and Performance Check of Refrigerated Centrifuge

- Switch on the stabilizer which is indicated by green light.
- Switch on the machines power – switch on the front light and wait for the appliance identification display.
- Wait for the lid symbol to light up.
- Press the key. The lid unlocks. Lift it by hand.
- Uncover the rotors and check that they are securely fixed and also can be rotated fully.
- Operation is only permitted when all the six buckets (black colour) are inserted and are of the same type.
- The inserts (white colour) must be symmetrically loaded (i.e. radially or tangentially balanced blood bags).
- Finally, close the lid but don't allow it to fall, the open display disappears when the lid is correctly closed.
- The present program number can be displayed flashing by pressing the "store mode" key.
- Press the "start" key.
- After the completion of a program time segment, the appliance automatically stops and beeps continuously (beeps can be stopped by pressing any key).
- The lid can be opened as previously described.
- Take out the blood bags- close the lid.
- Switch off the machine's main power.

Quality Check:

- Speed check with the tachometer.
- Temperature recorded with digital thermometer.
- Acceleration and Deceleration time recorded with stop watch.
- Cleaning of inserts with a spirit and hypochlorite or with mild hot detergent water monthly should be done.
- Greasing at the hinge region with tube every 6 months by the service engineer.

5. Alarm Test

Principle:

The purpose of an alarm check is to ensure that the results of the storage refrigerator for blood components are working efficiently. It also ensures compliance with statutory requirements such as of Drugs Controller of India or international agencies (AABB). To achieve consistently high-quality blood transfusion services, it is imperative that all Refrigerators used during processing and storage are maintained in good working order. Operating Manuals supplied with refrigerators usually contain detailed instructions on how to maintain the same.

Procedure:

1) Alarm test is necessary for the following Refrigerators:

a. **Blood Centre refrigerator (2-6^0 C)**
b. **Deep freezer (-40^0 C)**
c. **Deep freezer (-80^0 C)**

2) For a 2-6^0 C Blood Centre refrigerator, a high alarm can be tested by putting the sensor of the digital display of equipment in a bowl having distilled water at room temperature. As soon as the temperature goes beyond the upper set temperature, it gives an alarm.

3) For 2-6^0 C refrigerator, the low alarm can be tested by putting the sensor of the digital display in a bowl having chilled water/ ice, it gives an alarm.

4) In Deep freezers (-40^0 C and -80^0 C), provision is given to test the alarm by pressing low and high "alarm Test" buttons. By pressing and holding these buttons, we can change the set points, so the equipment gives an alarm.

6. Validation of Laminar Airflow

Procedure:

Note: 6 blood agar plates are required.

1. **Start-up of Biosafety cabinet:**
    - The inner surface of the cabinet (both horizontal & vertical) is cleaned & disinfected with 70% alcohol. Then, the shutter is closed.
    - The UV light is switched on for half an hour.
    - The airflow is switched on for 10 min. before opening the shutter.
    - The UV light is switched off and the simple light is switched on before starting the work.
    - Then the shutter is opened.

2. **Exposure of plates and incubation:**
    - Five blood agar plates are labeled according to the position (left, right, middle, front & rear) where it has to be placed in the cabinet, and one is put as a control.
    - The plates are placed in the cabinet according to the label and the lids of blood agar plates are opened. The cabinet is closed and plates are kept for 30 minutes.
    - After 30 minutes, the shutter of the cabinet is opened and the lid of the blood agar plates is closed.
    - The plates are kept in an incubator at 37°C.
    - The results are taken after 24hrs and after 48hrs.

3. **Shut Down of Biosafety cabinet:**
    - The inner surface of the cabinet is cleaned and decontaminated with 70% alcohol.
    - The shutter is then closed.
    - The simple light & airflow are switched off.
    - UV light is switched on for 10 minutes.
    - The UV light is then switched off.

Interpretation:

The presence of growth is considered positive and suggestive of contamination of laminar airflow.

7. Standardization of Refrigerated Centrifuge for Platelet Separation

Principle:

Successful preparation of platelet concentrates requires adequate but not excessive centrifugation; the equipment used must perform in a consistent and dependable manner.

Procedure:

Step	Action
	For Preparation of PRP
1	Perform a platelet count on the anticoagulated specimen.
2	Calculate and record the number of platelets in the Whole Blood unit: platelets/µL × 1000 × mL of whole blood = number of platelets in whole blood.
3	Prepare PRP at a selected speed and time.
4	Place a temporary clamp on the tubing so that one satellite bag is closed off. Express the PRP into the other satellite bag. Seal the tubing close to the primary bag, leaving a long section of tubing, or the "tail." Disconnect the two satellite bags from the primary bag. Do not remove the temporary clamp between the satellite bags until the platelets are prepared
5	Strip the tubing and "tail" several times so that they contain a representative sample of PRP.
6	Seal off a segment of the "tail" and disconnect it so that the bag of PRP remains sterile.
7	Perform a platelet count on the sample of PRP in the sealed segment. Calculate and record the number of platelets in the bag of PRP: platelets/µL × 1000 × mL of PRP = number of platelets in PRP.
8	Calculate and record the percentage of yield: (number of platelets in PRP × 100) divided by (number of platelets in whole blood) = % yield.
9	Repeat the above process three or four times with different donors, using different speeds and times of centrifugation; compare the yields achieved under each set of test conditions.
10	Select the shortest time and lowest speed combination that results in the highest percentage of platelet yield without unacceptable levels of red cell content in the PRP.
11	Record the centrifuge identification, the calibration settings selected, the date, and the identity of the person performing the calibration.

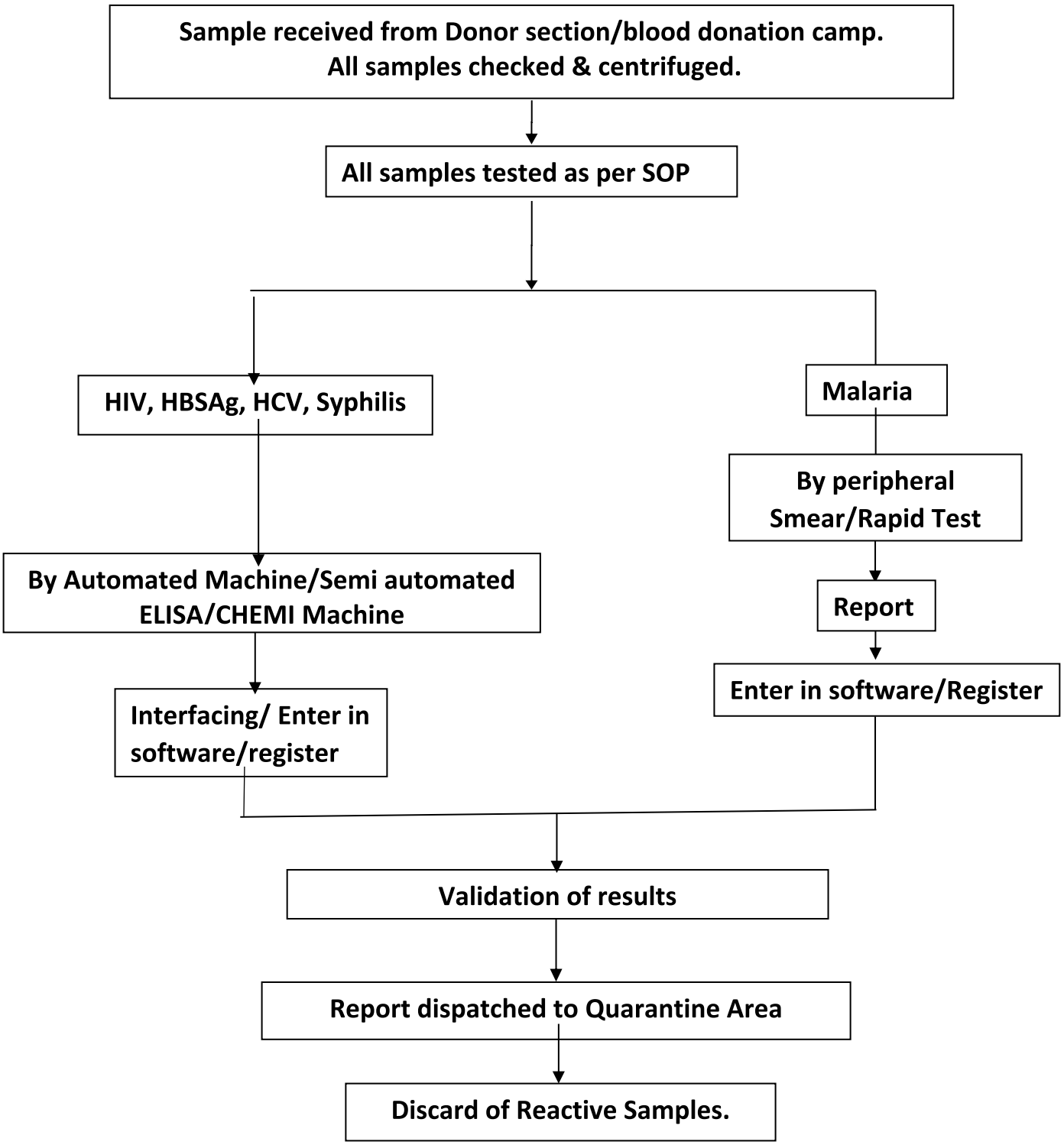
Step	Action
	For Preparation of Platelets
1	Centrifuge the PRP (as prepared above) at a selected time and speed to prepare platelets.
2	Remove the temporary clamp between the two satellite bags, and express the supernatant plasma into the second attached satellite bag, leaving approximately a 55- to 60-mL volume in the platelet bag. Seal the tubing, leaving a long section of tubing attached to the platelet bag.
3	Allow the platelets to rest for approximately 1 hour.
4	Place the platelets on an agitator for at least 1 hour to ensure that they are evenly resuspended. Platelet counts performed immediately after centrifugation will not be accurate.
5	Strip the tubing several times, mixing tubing contents well with the contents of the platelet bag. Seal off a segment of the tubing and disconnect it, so that the platelet bag remains sterile.
6	Perform a platelet count on the contents of the segment.
7	Calculate and record the number of platelets in the concentrate: platelets/μL × 1000 × mL of platelets = number of platelets in platelet concentrate.
8	Calculate and record the percentage of yield.
9	Repeat the above process with PRP from different donors, using different speeds and times of centrifugation; compare the yields achieved under each set of test conditions.
10	Select the shortest time and lowest speed combination that results in the highest percentage of platelet yield in the platelet concentrate.
11	Record the centrifuge identification, the calibration settings selected, the date performed, and the identity of the person performing the calibration.

Notes:

1. *It is not necessary to perform functional recalibration of a centrifuge unless the instrument has undergone adjustments or repairs, or unless component quality control indicates that platelet counts have fallen below acceptable levels. However, timer, speed, and temperature calibrations of the centrifuge should occur on a regularly scheduled basis.*
2. *Each centrifuge used for preparing platelets must be calibrated individually. Use the conditions determined to be optimal for each instrument.*
3. *Each centrifuge used to prepare platelets should be calibrated upon receipt and after adjustment or repair. Functional calibration of the centrifuge for both the preparation of platelet-rich plasma (PRP) from whole blood and the subsequent preparation of platelet concentrates from PRP can be performed during the same procedure.*

TTI SECTION

1. Process Flow Of TTI Section



2. Preparation of External QPC

1. Preparation of dilutions of ELISA positive sample with negative serum

Arrange tubes for serial dilution, 1:2, 1:4, 1:8, 1:16, 1:32….

Add 100 µl of diluent (Negative serum) in all the tubes.

Add 100 µl of ELISA positive donor serum in the first tube.

Mix and transfer 100 µl of solution from tube 1 to tube 2, and discard the microtip.

Repeat this step for remaining tubes, with a new microtip each time.

Run the ELISA as per the kit insert

Run each serial dilution in ELISA in duplicate. Along with the kit positive and negative controls (PC & NC).

Calculate the mean (OD) of duplicate values for each dilution. Calculate the 'Cut off' value as per kit instructions.
Calculate E ratio = OD/Cut Off, for each dilution.

2. Selection of a dilution

- The dilution selected should be such, that it's OD falls between the OD of kit positive control and the cut off value, preferably little above the cut off value i.e. Borderline reactive sample should be selected as suitable dilution

3. Production of larger batches of EPC

- If, for e.g. 1:128 gave us the desired dilution, large batch production of EPC can be done by taking: 127 µl of non-reactive serum + 1 µl of ELISA positive serum.
- Batch validation is done & batch is accepted if the reconstituted batch after aliquoting yields the targeted titre with minimal inter-aliquot variation (mean, SD & CV of E - ratios of total number of aliquots are calculated)
- Less CV (<20%) suggests little inter-aliquot (batch) variation

4.Storage of EPC

- Label the sample as Biological Hazard, positive for TTI (name), vial no., date of preparation and expiry date (12 months from the date of collection)
- These vials can be stored at -40º C for 1 year.
- A vial once opened should be stored at 2º-8º C and discarded after 7 days.

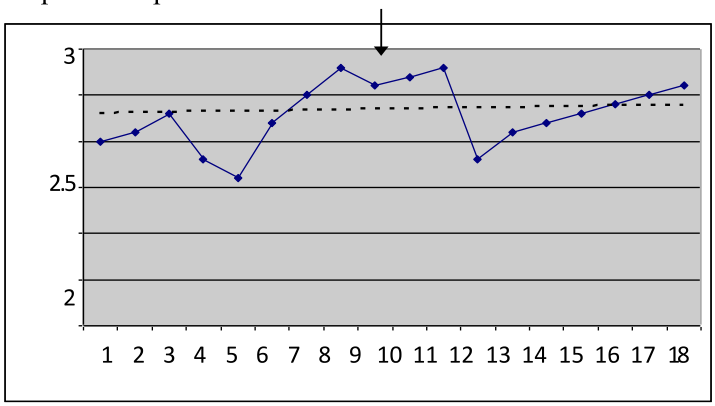
3. Interpretation of LJ Chart

Shift (systematic shift): Control value of consecutive runs fall on one side of the mean

Inference: When analytical method suffers a sudden development of problem **SHIFT**

Possible reasons:

1. Switching to a new lot of kits
2. New reagents
3. Changes in incubation temperature
4. New technician



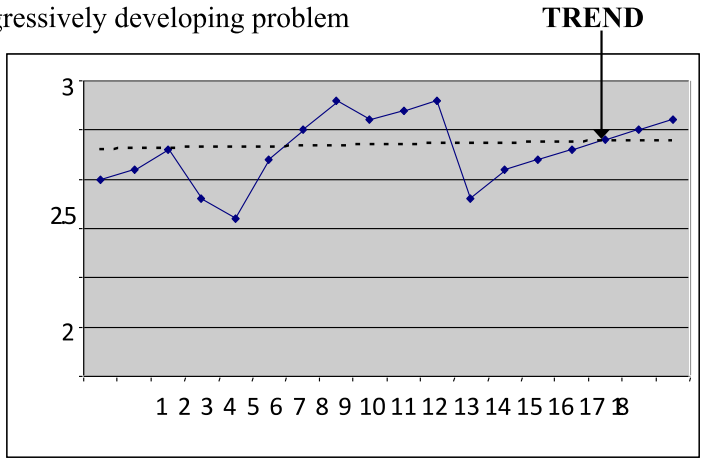
Trend (systematic drift):

Successive points distribution in one direction (towards either higher or lower results) within the acceptable limit

Inference: When analytical method suffers a progressively developing problem

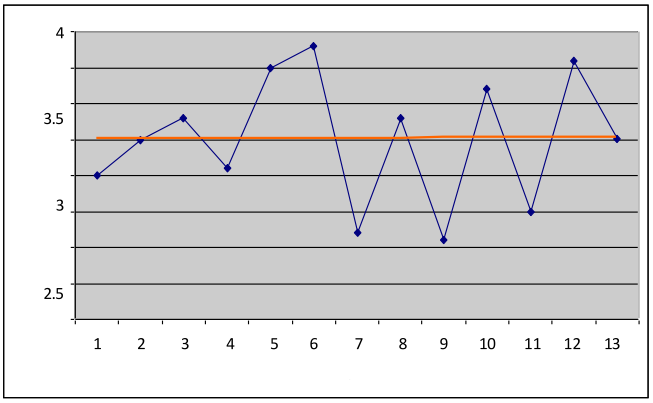
Possible reasons:

1. Deteriorations of reagents
2. Slowly faltering equipments e.g. pipette



Dispersion: Increase frequency of both high & low outliers

Inference: Increased dispersion is observed when random error or imprecision increases



Random error:

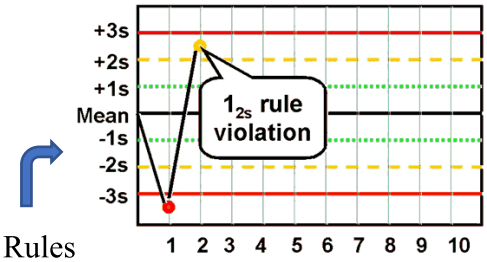
Common causes of random error include by

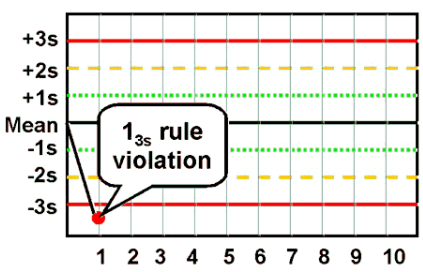
a. Transcription errors
b. Sample mix up
c. Poor pipette precision
d. Poor mixing of sample
e. Reader not calibrated
f. Washing ineffective or not consistent

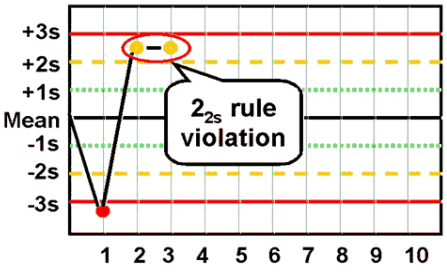
Application of Multiple (Westgard) rules in daily run

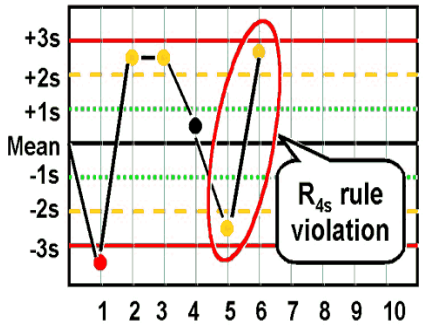
1_{2s}	One observation exceeds 2SD
1_{3s}	One observation exceeds 3SD
2_{2S}	Sequential values >2SD on same side of mean
R_{4S}	Difference between 2 values >4SD
10_X	Ten sequential values on same side of mean

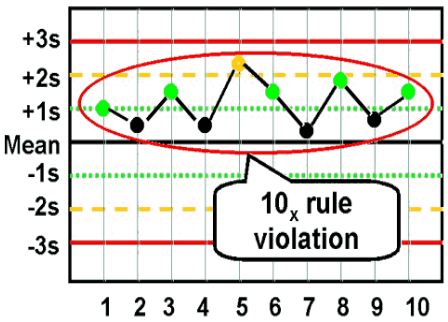
Warning Rules











4. Calibration of Micropipettes (In-House)

1. **PURPOSE OF EXAMINATION**: In-House calibration of Micropipettes used in testing of samples.
2. **STAFF RESPONSIBLE AND AUTHORISED TO PERFORM THE PROCEDURE**: Technician
3. **PRINCIPLE OF PROCEDURE USED FOR EXAMINATION**: In calibrated analytical balance distilled water is weighted by setting the volume of micropipettes. Then, the coefficient of variation is calculated. Water is used because 1 microliter of water is 1 micrograms.
4. **PROCEDURE**:
   - **a.** Place beaker on the pan of analytical balance & tare it to zero.
   - **b.** For Fixed volume micropipettes
     - i. Set it to minimum
     - ii. Dispense the volume by reverse pipetting in beaker
     - iii. Take the reading
     - iv. Calculate the mean & Standard deviation & CV
     - v. Repeat the procedure for 10 times
     - vi. Set it to maximum
     - vii. Repeat the same procedure for this setting
   - **c.** For variable micropipettes
     - i. Set it minimum, maximum and one value in between minimum and maximum
     - ii. Repeat the same procedure as describe in 4.b
   - **d.** Frequency of calibration: Every 3 months
5. **MATERIALS AND EQUIPMENT USED**: Calibrated analytical balance, micropipettes, glass beaker & distilled water
6. **STANDARD USED**: Calibrated analytical balance, calibrated in NABL Accredited Calibration Lab.
7. **PRECAUTIONS TAKEN**:
   - **a.** Take care to tare the balance and set it to zero after putting the beaker
   - **b.** Use the small tip for all deliveries during the calibration procedure, whether the pipette is used for repetitive dispensing.
8. **REVIEW, REMARKS & RECOMMENDATION:**
   Results are recorded & In house calibration certificate is produced authorised by the Assistant Quality Manager & Maintained in Calibration file in each section. If the coefficient of variation in results is >2%, call the company person for repair, again calibrate it after repair, again if the CV >2% replace the pipette.

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