STANDARD OPERATING PROCEDURES FOR BLOOD TRANSFUSION

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FORWARD

To ensure safe blood, all the processes involved in blood collection up to transfusion to the patients require application of Standard Operating procedures (SOPs). There has been growing awareness about quality in blood transfusion services with the objective of releasing only those blood products and blood which fulfil the desired standards in terms of safety and efficacy. Consistency is the hallmark of quality and can be achieved only through the use of Standard Operating Procedures (SOPs) by all staff engaged in blood centres at all times. Use of SOPs has also become essential for licensing and accreditation. To ensure the quality in blood transfusion service, SOPs must be developed and practiced in all blood transfusion centres. Implementation of SOPs is mandatory as per Safe Blood transfusion ACT 2002.There is now an international unanimity on the framework of SOPs.

The Standard Operating procedures document has been prepared through series of consultative meeting with the stakeholder of public and private blood centres. Consensus was agreed among the experts of National blood transfusion service to develop the SOPs as per local facilities. SOPs that have been added here must be followed by each blood transfusion centres if the SOPs are matching with their requirement. Each centre, therefore encourage using these SOPs as guideline or may develop their own SOPs according to their resources, infrastructure and facilities and laboratory system. The developed SOPs were validated in 10 blood transfusion centres and finally modified as per results of validation. So, the SOPs which are described here already validated in different categories of blood transfusion centres shall be used in Medical College, district hospital and other institutes including the licensed blood transfusion centres. With collaboration of WHO, DGHS initiative has taken to publish and distribute some SOPs which are essential at this point of time. Other required SOPs may be prepared as per need of each blood transfusion centres. Some SOP for example TTI screening illustrated as example and each centre shall write SOP for TTI screening according to the types of kits they use and types of assay (rapid/EIS/CLIA/WB etc.) they practice. It is recommended that Medical Technologist (Lab) who has trained in SOPs writing should develop and write SOPs that are not covered by this document.

INTRODUCTION

Recently a comprehensive situation assessment was carried in blood transfusion centres including the private sector under WHO blood Safety project. The assessment was carried out by group of transfusion medicine experts and management personnel of DGHS of Ministry of Health. The data collected from the centres showed that a very few centres have SOPs developed for some processes that are routinely performed in the blood transfusion centres others centre neither have developed SOPs. In depth discussion with responder of the centres it was found that they were not oriented about the concept of development of SOPs, the process of writing and validation of SOPs. Most of the centres during assessment showed the flow chart of literature of the kits and reagent that were displayed in the laboratory as sample of SOPs of the process. It was also revealed during the assessment none of the centre could show any written procedure and instruction for any of the processes like blood donor selection, stock maintenance, supply of blood, donor care, pre-donation counselling and other routine serological procedures. Besides, the report also showed that there were no standard procedures followed during administration of blood in the clinical ward of different hospitals. So, under the circumstances, it is very much pertinent to assist blood centres to write SOPs as per WHO model guideline. Since the SOPs development and application are not familiar in the blood transfusion service, it is very much important that at the initial stage the experts of blood transfusion working in the centres be guided and assisted in writing the SOPs. Once the SOPs are developed the next stage is the validation and implementation. Because SOP is the written instruction for any process if it is followed, and therefore there is little scope for doing mistake in any laboratory process. When verbal instructions often are used for any laboratory process it may not be heard properly, the accent may be problem for verbal communication and instructions, the verbal instruction might be misunderstood if anything went wrong. Verbal instruction are Quickly forgotten and sometime ignored as well. So any laboratory, Policies, standards, processes, and procedures must be written down, approved, and communicated to all concerned.

Features of an SOP

What is Standard Operating Procedure (SOP) ?

An SOP is a written document of instruction to perform various operations in a testing site. It provides step-by-step instructions to ensure consistency, accuracy, quality of a laboratory process. An essential sub-element of a quality system required to ensure quality. Any written instruction is safe guard for those who uses it and it is a legal document. It is the pillar of all quality works. Without SOPs there is risk of error that endangers human life. SOP ensures reduction of variation, ensure consistency in procedure, ensure quality i.e. doing right thing every time to get right results. SOP is required for

- Quality System
- ISO
- Accreditation
- Audit
- Regulatory requirements

It gives confidence of reliability of report and confidence to the customer. Each of the process must have SOPs. Generally each SOP has six core processes which includes

- Scope & application
- Responsibility
- Reference
- Material Required
- Procedure
- Documentation

Each SOP document has two sections: one gives information about the location, subject, functions, distribution and genesis of SOP and the other is the technical section contained instructions for carrying out the specific activity.

The instruction part of SOP shall have following components:

- Name of the blood transfusion centre
- Subject of SOP
- Function of SOP
- Distribution of SOP
- Unique Number of SOP
- Version and revision
- Date from which SOP shall be effective and the period after which it has to be reviewed
- Number of pages and No of copies (Quality Manager or designated official shall keep a record of those whom SOP has been distributed)
- Name and signature of the author
- Name and signature of the person who has been authorized to approve SOP
- Name and signature of the person who is to authorize the use of SOP from effective date.

The technical part SOP shall have following components

- Scope and application
- Responsibility
- References for technical content, if any
- Materials required to perform the procedure
- Various steps of procedure
- Interpretation criteria
- Quality assurance
- Documentation

Format of SOP

The format of SOP must have all the information and instruction that covers the whole purpose of particular activities. It should include all the relevant information and directions to complete a laboratory process from starting to end including the step by step process, role and responsibility of person involved in the process and the delegate authority of each persons to complete the process in the laboratory.

Each laboratory process must have one SOP. A SOP should not have multiple processes. But for particular laboratory process or activity, reference could be made as sub activities related to the process. Since equipment, reagents, methodology and kits used may vary in different blood centre, it is important for every blood transfusion centre to have its own SOP. Each SOP must be given a unique identity number along with the revision number, if any. Information about the procedure, location where the SOPs will be used, its function and distribution list; date from which it will be effective and signatures of the author(s) and the person from top management who can authorize the use of SOP from the effective date must precede the technical details. Prior to their use, the SOPs must be validated to demonstrate their utility in the setting of the respective blood transfusion centre. Before use, SOP needs to be validated and periodic review (usually after one year or whenever there is a change in methodology or material) should be undertaken to bring about revisions, if necessary.

Format of an SOP varies. But commonly includes:

- Purpose *
- Responsibilities *
- Scope and application *
- References*
- Definitions
- Materials required (including associated controlled documents) *
- Procedure*
- Results
- Interpretation
- Documentation*
- Appendices

** Minimum requirements*

Format of SOP

No	Effective Date	Pages	Author	Authorized by
SOP/…..		4		
Version	**Review period**	**No of copies**	**Approved by**	**Date**

LOCATION	SUBJECT
FUNCTION	**DISTRIBUTION**

1. PURPOSE
2. SCOPE AND APPLICATION
3. RESPONSIBILITY

4. REFERENCES:
5. MATERIALS REQUIRED
    a. Equipment:
    b. Specimen:
    c. Reagents:
    d. Glassware:
    e. Miscellaneous:
6. PROCEDURE:
    a. Principle:
    b. Steps:
    c. Flow Chart
    d. Results:
    e. Interpretation:
7. DOCUMENTATION:

Description of Information part of SOP

No. (number): In a given laboratory there are various types of SOP available and followed routinely. So, any SOP developed must have serial number for keeping record. The serial number may be provided as unique number with any number of digits and types of SOP may be stated as abbreviation for the title of the process. For example if a SOP is for ABO grouping and, it is the SOP for serological section, we can use the no. as SOP/SS/001. 001 for ABO grouping. Then SOP/SS/002 will be for RhD typing in serology section. SS indicates serology section. So, SOP/SS/003 will indicate SOP for Compatibility. For SOP of rapid assay of HBsAg testing will have no as SOP/TTI/001 and so on.

Effective date: Generally for any new process SOP is be written first, then it is to be validated to have desired result which will meet the purpose for which the SOP is developed. Once the SOP is successfully validated then the authority will decide from which date the SOP will be effective and implemented**.**

Page Number: Any approved document must have page number specially when it is an instruction. Each SOP thus has page number which is to be mentioned.

Author : SOP is written by who uses SOP.

Approved by: The person usually the head of the department or in charge of blood transfusion centre or experts as designated. Otherwise it is quality manager who approves SOP.

Authorized by: Usually it is the institutional head where the blood transfusion centre is located. Since SOP indicates the responsibility of medical officer, medical technologist and other laboratory staff, the document needs authorization from the local authority to ensure the responsibility described in the SOP. However, with respect to the current context, the departmental head or in charge may authorize SOP in consultation with institutional head or authorization may be given by both. Before, distribution SOP shall be signed by who approves and authorize it.

Version: For a given process, if it is necessary to include certain steps in the process without changing the entire SOP which has already validated, SOP version changes accordingly for that particular process. So, SOP may have different version. Once the new version is introduced the old version needs to be withdrawn from laboratory.

Review period: It is period or date when certain SOPs needs to be changed as per need.

No. of copies: Like any other official document number of copies of SOP issued from department must have record.

Date: it is the date when it is authorized.

Location: An ideal blood transfusion centre has different section which includes Donor section, serology section, TTI section, blood component section, Storage area etc. In the format of SOP

particular section is to be mentioned. The section may be illustrated as abbreviation. Serology Section as SS, TTI section as TTI, Blood component as BC etc.

Subject: It is the title of SOP of particular process. For example ABO grouping, HBsAg testing

Function: It is the technique how particular SOP would be done. For example if the subject of SOP is RhD typing then how RhD typing will be done –by Tube Test for Rh D Typing or slide method is to be mentioned under function.

Distribution: It is the status of distribution of total number of copies of particular SOP. It is to be noted that one document is to be kept as mater file and other copies are available as per location.

Description of technical part of SOP.

Following are the section of a SOP.

1. PURPOSE
2. SCOPE AND APPLICATION
3. RESPONSIBILITY
4. REFERENCES:
5. MATERIALS REQUIRED
   a) Equipment:
      i) Specimen:
      ii) Reagents:
      iii) Glassware:
      iv) Miscellaneous:
6. PROCEDURE:
   a) Principle:
   b) Steps:
   c) Flow Chart
   d) Results:
   e) Interpretation:
7. DOCUMENTATION:

Following are the list of SOPs that can be developed in each blood transfusion centre and Hospital.

A. Donor Issues

1. Criteria for donor selection
2. Donor Screening
3. Qualifying test for blood donation
4. Haemoglobin estimation of donor
5. Preparation of copper Sulphate solution
6. Blood collection: Preparation for blood collection

7. Selection of bangs
8. Blood Collection
9. Blood Collection: Post donation care
10. Blood Collection: Management of adverse reactions in a donor
11. Traceability of blood bags

B. Component Separation

1. Blood component separation

C. Immunohaematology (Red Cell Serology/ Blood Grouping)

1. ABO blood group
2. Rh D Typing
3. Preparation of red cell suspension
4. Antibody screen
5. Detection of incompatibility between patient and donor
6. Antiglobulin cross match
7. Investigation of transfusion reaction
8. Reliability and reproducibility of blood group results

D. Screening of transfusion transmissible infections

1. HBsAg testing
2. Anti HIV testing
3. Anti HCV testing
4. Syphilis testing
5. Malaria Testing

E. Labelling, preservation and storage of blood and components

1. Labelling of blood bags and blood components
2. Preservation of blood and blood components
3. Inventory of blood bags and blood components
4. Supply of safe blood for transfusion
5. Issue of blood for transfusion

F. Quality Assurance

1. Optimum quality assurance
2. Equipment maintenance: Preventive maintenance
3. Equipment maintenance: calibration
4. Incident report

Clinical SOPS

A. Clinical SOPs are the responsibility of the hospital

- Administering the blood, including final patient identity check at the bedside
- Recording the transfusion
- Records of the monitoring of the patient, including adverse reactions
- Management, reporting and investigation of adverse reactions

B. Patient Identification

- SOP on checking patient identification
- Identification at the bedside
- Staff performing identification
- Identifiers used
- Identification of all samples collected

C. Product Identification

- Identification of each product
  - Cross-check with patient details
  - Product details: e.g. type, number, integrity
  - Staff checking the product identification

D. Recording the Transfusion

- Transfusion details: record in patient's case notes / file
  - Type and volume of product
  - Donation number of each product / unit
  - Blood group
  - Time at which transfusion started and stopped
  - Signature of person responsible for transfusion

E. Records of Monitoring the Patient

All monitoring activities performed

- Record details in patient's case notes /file
- Baseline patient information
  - Temperature
  - Pulse
  - Respiratory rate
- Change of administration sets
- Evidence of improved clinical status

F. Recording Adverse Events

Record symptoms / signs of reactions

- Immediate or delayed
- Action taken and outcome of action
- Transfusion reaction report form completed

STANDARD OPERATING PROCEDURE

PREPARATION OF CUSO4 SOLUTION

Number	Effective Date	Pages	Author	Authorized by
SOP/…….		2		
Version	**Review Period**	**No. of Copies**	**Approved by**	**Date**
01	Biennial			

LOCATION	SUBJECT
Donor Room	Haemoglobin Estimation
FUNCTION	**DISTRIBUTION**
Preparation of $CuSo_4$ solution	– Donor Area – Medical Officer In charge – Master File

1. Purpose

Estimate the haemoglobin of blood donor before donation

2. SCOPE & APPLICATION:

The Specific gravity of 1.053 is equivalent to 12.5 g/dl haemoglobin. Hence $CuSO_4$ solution of Specific Gravity 1.053 is used for pre-donation Hemolglobin test in case of male and 1.051 in case of female which is equivalent to 11.5 g/dl.

2. RESPONSIBILITY:

The Medical Technologist (Lab) in the donor area.

3. REFERENCE:

Bangladesh gazette, extra, 7^{th} may 2005, pate no.- 2492 Model SOP for Blood Transfusion service by WHO 2002.

4. Material Required

5. PROCEDURE:

Stock solution is made as follows and kept in a jar or battle-

- Dissolve 170g crystalline $CuSO_4$, $5Hu_2$ in 1000 ml distilled water. Working solution for SP gr 1.053
- Every morning prepare fresh solution.
- Add 51 ml stock solution to 49 ml distilled water.
- Cheek Specific Gravity which should be 1.053. If not adjusting it using either stock solution or Distilled Water.

6. DOCUMENTATION:

Record the volume of stock and working solution prepared on the register

STANDARD OPERATING PROCEDURE

CRITERIA FOR DONOR SELECTION

Number	Effective Date	Pages	Author	Authorized by
SOP/…..		3		
Version	**Review period**	**No. of Copies**	**Approved by**	**Date**
0	1 Year	5		

LOCATION	SUBJECT
Donor Room	Criteria for donor selection
FUNCTION	**DISTRIBUTION**
Assessing suitability of for blood donation	- Medical officer in charge of donor area - Master file

1. Purpose

To assess the suitability of blood donor

2. SCOPE AND APPLICATION:

This SOP describes the donor selection criteria for blood donation, one of the most important steps in protecting the safety of the blood supply. The process is intended to identify elements of the medical history and behaviour or events that put a person at risk for transmissible disease or at personal medical risk, either temporarily or permanently.

3. RESPONSIBILITY:

A qualified Medical Officer must determine the eligibility of donors. Donor selection is based on a medical history questionnaire and a limited physical examination done on the day of donation including the results of pre-donation screening tests.

4. REFERENCES:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; P 99 – 104, 110 –115.
- SRO-145, Bangladesh National Blood Transfusion services 2008

5. MATERIAL REQUIRED:

- Donor Questionnaire
- Donor Card

6. PROCEDURE:

CRITERIA FOR SELECTION OF BLOOD DONORS

A. Accept only voluntary / replacement non-remunerated blood donors if following criteria are fulfilled:

The interval between blood donations should not be less than three months. The donor shall be in good health, mentally and physically fit and shall not be a person having multiple sex partners or a drug-addict or a jail inmate. The donors shall fulfil the following requirements, namely:-

(a) Age: donor should be in age group of 18 to 60 years;
(b) Weight: should not be less than 45 kg / 100 lbs.;
(c) Temperature: below 99.5 ^{0} F;
(d) Pulse: 60 – 100 / min;
(e) Blood pressure: Systolic pr. – 100 to180 mm Hg & Diastolic pr. – 60 to 100 mm Hg without medication.
(f) Hemoglobin: should not be less than 75% (or in case of Male 12.5 g/dl, in case of female 11.5g /dl)
(g) Donor should be free from acute respiratory distress;
(h) Donor should be free from any skin disease specially at the site of phlebotomy;
(i) Donor should be free from transfusion-transmitted diseases as far as possible determined by history and examinations mentioned above.
(j) There should not be any puncture site or scar mark on arms or forearms indicative of professional donors or intravenous drug abusers.

B. Deferral of the donor for the period mentioned below:

CONDITIONS	**PERIOD OF DEFERMENT**
(a) Abortion	6 months
(b) History of blood transfusion	12 months
(c) Surgery	12 months
(d) Typhoid	12 months after recovery
(e) History of Malaria duly treated	3 months endemic area 3 years non endemic area
(f) Tattoo	6 months
(g) Immunization (Cholera, Typhoid, Diphtheria, Tetanus)	15 days
(h Rabies vaccination	1 year after vaccination
i) Hepatitis in family or close contact	12 months
(j) Immunoglobulin	12 months
(k) Tooth extraction	14 days
(l) Eczema	After recovery
(m) After child birth	6 months
(n) Local infection	After recovery

C. Deferral of the donor permanently suffering from the following diseases:

(a) Cancer
(b) Heart disease
(c) Abnormal bleeding tendencies
(d) Unexplained weight loss
(e) Diabetes – controlled on insulin
(f) Hepatitis B infection
(g) Chronic nephritis

(h) HIV/ AIDS
(i) Liver disease
(j) Tuberculosis
(k) Polycythemia Vera
(l) Asthma
(m) Epilepsy
(n) Leprosy
(o) Schizophrenia
(p) Endocrine disorders
(q) Rheumatic fever
(r) Hepatitis C infection

D. Private interview:

Detailed sexual history should be taken. Positive findings should be recorded on confidential notebook.

E. Informed Consent:

Information should be given about following things:

- Need for blood
- Need for voluntary donation
- Regarding transfusion-transmitted infections
- Need for questionnaire & honest answers
- Safety of blood donations
- Processing & use of donated blood
- Screening tests for donated blood

7. DOCUMENTATION

All details should be included in the donor questionnaire form.

STANDARD OPERATION PROCEDURE

BLOOD COLLECTION

Number	Effective Date	Pages	Author	Authorized by
SOP/……		3		
Version	**Review Period**	**No. of Copies**	**Approved by**	**Date**
01	**1 year**			

LOCATION	SUBJECT
Donor Room	Blood Collection
FUNCTION	**DISTRIBUTION**
Assessing suitability of donor for blood collection	– Medical Officer in Charge of Donor Area – Master File

1. Purpose

To assess the blood donor with help of blood donor selection criteria

2. SCOPE & APPLICATION:

This describes a procedure for blood collection from the donor, using an aseptic method. Blood is collected in a sterile closed system bag with a single venepuncture. A correct performance of venepuncture is essential for the quality and safety of the blood donation. Successful venepuncture results not only in safe collection of a full unit of blood suitable for separation of components with good quality yields, but also contributes to the comfort and satisfaction of the donors the donors thus encouraging re-attendance.

3. RESPONSIBILITY:

The Medical Technologist (Lab) or Nurse under supervision of trained registered doctor is responsible for blood collection from the donor after verifying the donor screening details, checking the unit number labels and preparing the phlebotomy site.

4. REFERENCE:

- Technologist Manual of American Association of Blood Banks, 13th edition 1999 Pgs 98, 713-716
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; P 104 –106.

5. Materials :

- Cotton/ Gauze swabs.
- Artery Forceps.
- Pilot Tubes:

i. 1 test tube with anticoagulant for serology
ii. 1 test tube without anticoagulant for TTI

- Tourniquet.
- Oxygen Cylinder with accessories.
- Rubber Gloves.
- First Aid Tray.
- Tubing Stripper.
- Electronic Tube Sealer.
- Needle Destroyer.
- Blood collecting Bags.
- Discard Jar with 10% Sodium Hypochlorite.
- Artery Forceps, Scissors.
- Tapes
- Blood Bag Mixer (Bio mixer)
- Comfortable donor couch or chair
- 70% alcohol swab.

6. PROCEDURE:

- Make the donor lie down with a pillow under the head or recline in a comfortable donor chair. Loosen tight garments.
- Identify the donor by name. Enter the bag and segment numbers on the donor card/form.
- Ask the donor if he/she is in a comfortable position. Give the donor a hand roller/squeezer to hold.
- Select appropriate blood bag for blood collection.
- Clean the venepuncture site with 70% alcohol swab. Disinfect the skin a of venepuncture site about 5 cm diameter from the centre to periphery in a circular manner. Scrub the area for at least 30 sec or till froth forms. Do not touch the area after cleaning is done. Repeat the whole process if the puncture site is touched. Dispose the used swab unit into the waste basket. Dry the skin area with unit the puncture is made.
- Set the bio mixer for the required volume of blood to be collected and place the bag on it.
- Apply the tourniquet on donor arm.
- Clamp the bleed line of the blood bag using plastic forceps to ensure that no air enters the tubing or bag once the needle cover is removed.
- Keep the bevel of the needle facing upward and the shaft at an angle of 150 to the arm.
- Once the needle is beneath the skin, release the clamp.
- Insert the blood bag needle into the vein for about 1 to 1.5 cm by a bold single prick to ensure smooth flow of blood and secure on the arm with adhesive strips.
- Advise the donor to gently squeeze the hand roller to improve blood flow.
- If the venepuncture is unsuccessful do not make further attempt in the same arm. Take the donor's permission for a second attempt. Use a new bag.
- Once blood enters the bag tubing, press the bio mixer 'start' switch to allow the blood to flow into the bag. After the programmed volume of blood is collected, the bio mixer automatically clamps the tubing.
- Clamp the bloodline at 2 sites and cut in the middle. Collect blood in the pilot tubes from the tubing so that blood flows directly into the tubes from the donor arm.
- Release the tourniquet and remove the needle gently from the donor's vein pressing the phlebotomy site. Fasten a cuff around the donor's arm in a flexed position.
- Seal the blood bag tubing with the tube sealer.

- Burn the needle of the bag in the needle incinerator. Discard the tubing with the burnt needle in a container of sodium hypochlorite solution.
- Observe the donor is donor for 30 minutes after the blood collection is over.

7. DOCUMENTATION:

- Make entries in the donor register/computer.
- Make an entry of the failed venepuncutre, as double prick.

STANDARD OPERATING PROCEDURE

POST DONATION CARE

Number	Effective Date	Pages	Author	Authorized by
SOP/…		2		
Version	Review Period	No. of Copies	Approved by	Date
01	1 year			

LOCATION	SUBJECT
Donor Room	Blood Collection
FUNCTION	**DISTRIBUTION**
Post Donation Care	− Medical Officer in Charge of Donor Area − Master File

1. Purpose

To provide utmost care to blood donor after donation

2. SCOPE & APPLICATION:

The donor needs to be observed after blood collection, in order to attend to any adverse reactions in the immediate post-donation period. Time of observation should be minimum 30 minutes.

3. RESPONSIBILITY:

Post donation care should be taken by nurse. In case of adverse reaction, it will be managed by doctor.

4. REFERENCE:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; Page- 106.

5. MATERIAL REQUIRED:

- Sterile Cotton.
- Adhesive tape.
- Leaflet for post donation instructions.

6. PROCEDURE:

- To prevent adverse reactions like giddiness ask the donor not to get up from the chair/cot for 5 minutes even if he feels perfectly all right.
- Observe for another 10 minutes in the refreshment area whilst having light refreshments.

- Inspect the venepuncture site before the donor leaves the donor room. Apply an adhesive tape only after oozing stops. If there is persistent oozing at the site of venepuncute, apply pressure to stop oozing. Then apply adhesive at the site of venepuncture. If oozing still purchase apply pressure with ice. If there is haematoma apply ice gently over the area after 5 minutes. Inform the donor about the expected change in skin colour. If still bleeds consult with doctor.
- Instruct the donor to drink adequate fluid in the day and avoid strenuous activities.
- No smoking for thirty minutes.
- No driving on that day.

7. DOCUMENTATION:

- Give a leaflet of post donation instructions to the donor.
- Record donor profile in the register.
- Note any adverse reaction on the donor record.

If any problem occurs after returning home, inform the Blood Transfusion Center.
Give thanks to the donor.

- Observe the donor is donor for 15 minutes
- Drink more fluids than usual in the next four hours.
- Remove bandage after few hours.

STANDARD OPERATING PROCEDURE

PREPARATION OF RED CELL SUSPENSION

No	Effective Date	Pages	Author	Authorized by
SOP/…..		3		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
0	1 year	6		

LOCATION	SUBJECT
Red Cell Serology Laboratory	Preparation of Red Cell Suspension
FUNCTION	**DISTRIBUTION**
To prepare red cell suspension of 5% concentration	-Red Cell Serology Laboratory -Master file

1. Purpose

To prepare cell suspension for blood ABO blood grouping

2. SCOPE AND APPLICATION

This procedure applies to all testing that requires red cell suspension preparation.

3. RESPONSIBILITY

It is the responsibility of Medical Technologist (Lab) in the red cell serology laboratory performing a given test to prepare the appropriate red cell suspension. Every morning, the technologist must prepare A, B& O red cell suspension for the routine use.

4. REFERENCE:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 727.

5. MATERIALS REQUIRED:

5.1. Equipment:
Calibrated Centrifuge

5.2. Reagent:
0.9% saline

5.3. Specimen:

- Anti-coagulated blood sample of donor
- Donor unit segment

5.4. Glassware:

- Test tubes
- Pasteur pipette

5.5. Miscellaneous:

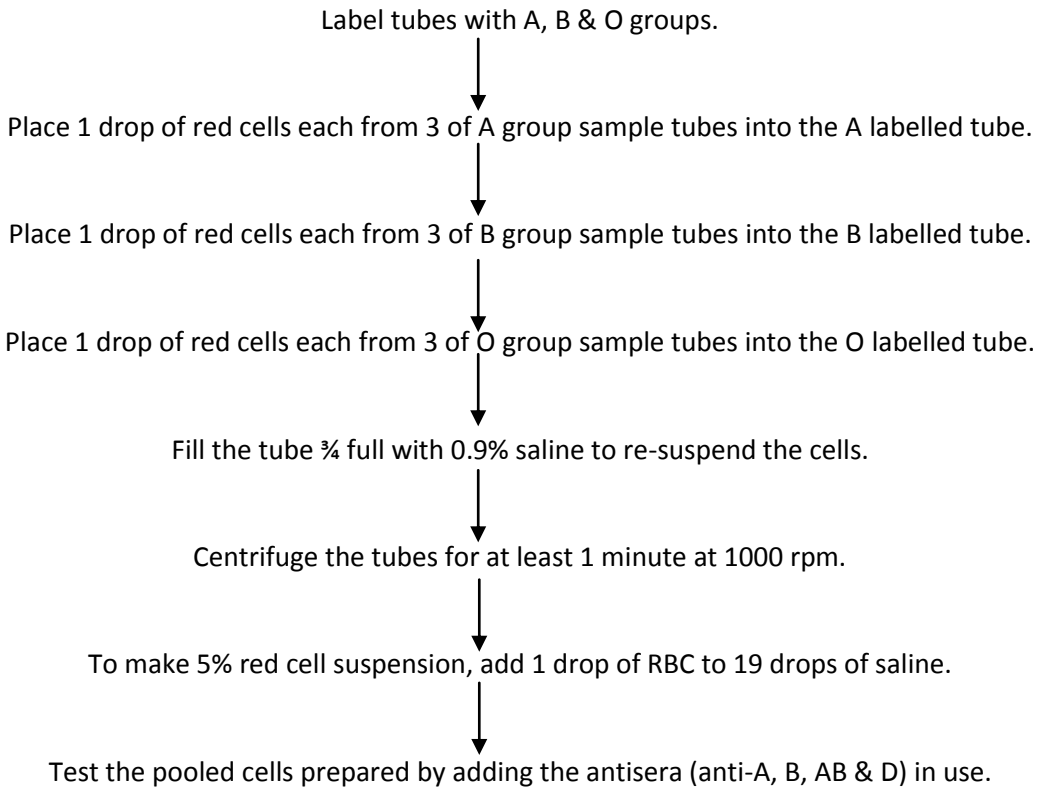
- Disposal box
- 2 plastic beakers
- Racks to hold sample tubes

6. PROCEDURE:

6.1 Principle:

The ratio of serum to red cells may affect the sensitivity of agglutination tests. A 5% red cell suspension is a common reagent in many serological procedures. The suspension need not be exactly 5%; an approximation achieves the appropriate serum-to-cell ratio for most test procedures.

6.2 Pooled Cell Suspension:



6.3 Limitations:

Haemolysis of red cells from improper washing may result in false results.

7. DOCUMENTATION:

- Enter the results of donor unit numbers from which pooled cells are produced in the donor register.
- Record the results of testing with the anti-sera in use.
- Enter the manufacturer’s name and batch number of the anti-sera.

STANDARD OPERATING PROCEDURE

ABO BLOOD GROUPING

No	Effective Date	Pages	Author	Authorized by
SOP/…..		4		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
0	1 year	3		

LOCATION	SUBJECT
Blood Group Serology Area	ABO Blood Grouping
FUNCTION	**DISTRIBUTION**
Cell & Serum testing for ABO grouping	- Blood Group Serology Area - In-charge, BTC - Master file

1. Purpose

To determine the determine the correct ABO group of an individual and ensure the reliability of the result

2. SCOPE AND APPLICATION

This Standard Operating Procedure provides the method to be followed to determine the correct ABO group of an individual and ensure the reliability of the result. This procedure describes the method of detection of 'ABO' antigens on the red cell and the reciprocal antibodies in the serum.

3. RESPONSIBILITY

It is the responsibility of Medical Technologist (Lab) in the serology area to perform the ABO grouping of donors and patients. One Medical Technologist (Lab) performs red cell testing and the other serum testing. If a discrepancy is encountered in cell and serum grouping, all tests should be repeated by the same Medical Technologist (Lab) using by new reagent. If the discrepancy persists, the sample should be repeated collection for correct result. It is the responsibility of all staff performing the ABO grouping to ensure that quality controlled reagents and proper cell concentrations are used. It is the responsibility of Medical Officer to sign the report; in case of emergency the Medical Technologist (Lab) can sign the report.

4. REFERENCES:

- Module of safe blood transfusion program, DGHS, Mohakhali, Dhaka.
- Technical Manual of the American Association of Blood Banks, 13 Edition, 1999, pages 150-151, 270, 277-280, 378-379, 285-286, 650-651.
- Introduction to Transfusion Medicine, Zarin Bharucha and D.M. Chouhan, 1 edition,1990. Pages 43-47.
- Procedures in Blood Banking and Immunohaematology - H.M. Bhatia, 1977.

5. MATERIALS REQUIRED:

5.1 Equipment:

- Refrigerator to store samples and reagents at 2 – 8 0 C
- General purpose centrifuge
- Microscope

5.2 Specimen:

- Blood samples of donor/ patient.

5.3 Reagents:

- Anti-A, anti-B, anti-AB anti-sera
- 20% A, B & O pooled cell suspension
- 0.9 % saline

(* All reagents must be used in accordance with the manufacturer's instructions.)

5.4 Glassware:

- Tiles
- Plastic pipette
- Glass slides for microscopic reading

5.5 Miscellaneous:

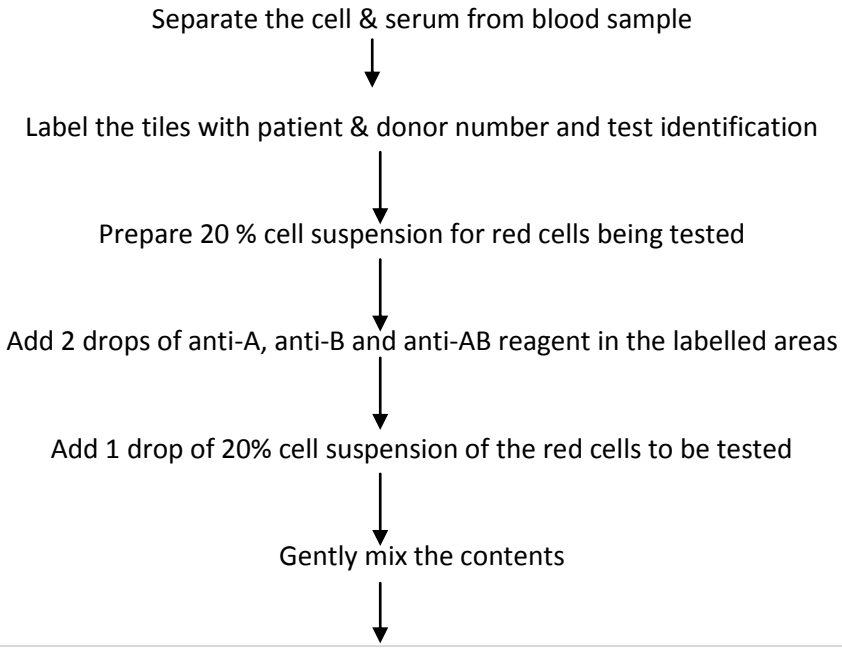
- Disposal bucket with Na hypochlorite
- Plastic beakers - 2
-

6. ROCEDURE:

6.1 Principle:

ABO system is the only system in which there is a reciprocal relationship between the antigens on the red cell and the naturally occurring antibodies in the serum. Routine grouping of donor and patient must therefore include both cell and serum tests, each serving as check on the other. The procedure is based on the principle of agglutination of antigen positive red cells in the presence of antibody directed towards the antigen.

6.2 Cell testing:



Examine for agglutination within 2-4minutes

↓

Interpret & record test results

6.3 Serum testing:

Add 2 drops of test serum in all labelled areas

↓

Add 1 drop of 20% pooled A reagent cells to the area labelled A_c

↓

Add 1 drop of 20% pooled B reagent cells to the area labelled B_c

↓

Add 1 drop of 20% pooled O reagent cells to the area labelled O_c

↓

Mix the contents gently and examine for agglutination within 2-4minutes

↓

Interpret & record test results.

6.4 Results:

- Depending on presence (+) or absence (-) of agglutination, the test is described as positive or negative.
- Observe anti AB as positive control and O cell as negative control.
- Confirm the cell grouping results with those obtained in serum grouping and vice versa.

6.5 Interpretation:

- Agglutination of tested red cells and serum constitute positive test results. Positive reactions characteristically show 3+ to 4+ agglutination.
- Absence of agglutination represents negative test result.
- The interpretation of serum and cell tests for ABO group is as follows:

REACTION FOR ABO GROUP SYSTEM (Forward and Reveres Group).

FORWARD GROUPING (Cell Grouping)			**REVERSE GROUPING (Serum Grouping)**			**Interpretation (ABO Grouping)**
Anti-A + Test Cells	Anti-B + Test Cells	Anti-AB + Test Cells ***(P.C)**	Test Serum + A-Cells	Test Serum + B-Cells	Test Serum + O-Cells ***(N.C)**	
+++	**---**	**++++**	**---**	**+++**	**----**	**A**
---	**+++**	**++++**	**+++**	**---**	**----**	**B**
---	**---**	**---**	**+++**	**+++**	**----**	**O**
+++	**+++**	**++++**	**---**	**---**	**----**	**AB**

*Note:- P.C= Positive Control.
N.C= Negative Control.

- Any discrepancy between cell and serum typing tests should be resolved before an interpretation is recorded for the donor/ patient.

7. DOCUMENTATION:

Enter the results of donor /patient grouping in the grouping register and also record the following details-

- Date on which the test is run.
- Name of the reagents used.
- Lot number of the reagents
- Initials of the technologist who performed the test.
- Initials of the Supervisor who verified the result

STANDARD OPERATING PROCEDURE

CONROL OF ABO & Rh BLOOD GROUP REAENTS

No	Effective Date	Pages	Author	Authorized by
SOP……		2		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION:	SUBJECT
Red Cell Serology Laboratory	To ensure reliability and reproductively of blood group results
Function	**Distribution**
Daily Quality Control of ABO & RhD Blood Group reagents	Medical Officer Supervisor in charge Red Cell Serology Laboratory

1. Purpose

To run daily quality control of ABO and RhD blood group reagents

2. SCOPE & APPLICATION

This Standard Operating Procedure (SOP) provides the daily checks on blood group reagents to ensure reliability and reproducibility of blood group results.

3. RESPONSIBILITY

It is the responsibility of the Medical Technologist (Lab) / supervisor in the red cell serology laboratory to ensure that quality controlled reagents and proper cell concentrations are used for testing for which daily quality control checks and test controls are used with proper documentation. The reagents should be stored and used as per manufacturer's instruction. Any fault in the reagents should be immediately reported to the Quality Assurance Manager.

4. REFERENCES

- Technical Manual of the American Association of Blood Banks 13th edition 1999, Page 22.
- Introduction to Transfusion Medicine, Zarin Bharucha and D.M. Chouhan; 1st Edition, 1990. Pages 225-226.

5. MATERIALS REQUIRED

5.1. Equipment:

- Refrigerator to store samples and reagents at 2-6^0 C.
- Table top Centrifuge.
- Automated Cell Washer/Mcroscope.

5.2. Reagents:

- Anti-A, Anti-B, Anti-AB, Anti D
- (Monoclonal and Bioclone) Antisera.

- Clotted or anticoagulated blood samples of random blood donors.
- Group A,B and O pooled Cells.
- 0.9% saline.

5.3. Glassware:

- Serum tubes.
- Micro tubes.
- Pasteur pipettes.
- Glass slides.

5.4. Miscellaneous:

- Rubber teats.
- Disposal box.
- 2 plastic beakers.
- Wooden block.
- Aluminium racks

6. PROCEDURE

6.1. Principle:

Test for reactivity and specificity is based on the principle of agglutination of antigen positive red cells in the presence of antibody directed towards the antigen.

6.2. Quality Control Checks:

Examine each vial carefully for precipitate, gel formation, turbidity or change in colour.

6.3. Reactivity and Specificity

- Add one drop of 3- 5% suspension of the appropriate red cells to the one drop of antiserum in a micro tube.
- Mix well and incubate
- Note the reactions as under:

	RED CELLS FOR TESTING POSITIVE REACTORS	**RED CELLS FOR TESTING NEGATIVE REACTORS**
Anti-A	Pooled A Cells	Pooled B, Pooled O Cells
Anti-B	Pooled B Cells	Pooled A, Pooled O Cells
Anti-AB	Pooled A, Pooled B Cells	Pooled O Cells
Anti-D Bioclone	RhD- positive cells (any ABO group)	RhD- negative cells (any ABO group)
Anti-D Monoclonal	RhD- positive cells (any ABO group)	RhD- negative cells (any ABO group)

6.4. Results:

Record presence or absence of precipitate, gel formation, turbidity or colour change.

6.5. Reactivity and Specificity:

Centrifuge the Tubes (as per manufacturer's instruction). Resuspend the red cell button and examine for agglutination / haemolysis grade and record test results.

6.6. Interpretation:

The presence of precipitate, gel formation, turbidity, colour change indicates that the reagent is contaminated and should not be used.
The absence of all the above indicates that the reagent is 'clear' and suitable for use.

6.7. Reactivity and Specificity:

Agglutination of specific red cells is a positive reaction and indicates the reactivity of the corresponding antibody in the reagent. The expected agglutination reaction for positive test is +3 to +4. The absence of agglutination / haemolysis is considered to be a negative reaction and indicates the absence of the corresponding antibody specificity in the reagent.
Clear cut negative reactions with the negative reactors rules out the presence of irregular agglutinins and haemolysis in the reagent.

7. DOCUMENTATION

Enter the results in the Blood Group Register in the Red Cell Serology Laboratory. Enter identification number of the individual donor cells used for pooling and the reaction strengths. Sign the results as the individual preparing the pooled cells and testing the reagent.

STANDARD OPERATING PROCEDURE

Rh D TYPING

No	Effective Date	Pages	Author	Authorized by
SOP …….		3		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
0	1 year	3		

LOCATION	SUBJECT
Serology Area	Rh D typing
FUNCTION	**DISTRIBUTION**
Rh D Typing in tiles	-Serology Area -Master file - In charge

1. Purpose

To determine the correct RhD typing of an individual and ensure the reliability of the result

2. SCOPE AND APPLICATION:

This Standard Operating Procedure provides the method to be followed to determine the Rh D type of an individual and ensure the reliability of the result. This procedure describes the method for detection of Rh D antigen on the red cells.

3. RESPONSIBILITY:

It is the responsibility of Medical Technologist (Lab) in the serology area to perform the Rh D typing of an individual using one monoclonal & one blended reagent. If a discrepancy is encountered between the two batches of anti-D, the test should be repeated and confirmed by Indirect Coombs' Test. It is the responsibility of all staff performing the Rh D typing to ensure that quality controlled reagents and proper cell concentration are used.

4. REFERENCES:

- Module of safe blood transfusion program, DGHS, Mohakhali, Dhaka.
- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 739.

5. MATERIAL REQUIRED:

5.1 Equipment:

- Refrigerator to store samples and reagents at 2- 8^0 C
- General purpose centrifuge
- Microscope

5.2 Specimen:

- Blood samples of donors/ patients

5.3 Reagents:

- Anti-D Blended
- Anti-D Monoclonal
- Rh control reagents (positive & negative)
- 0.9 % saline

5.4 Glassware:

- Test tubes
- Plastic pipette
- Glass slides

5.5 Miscellaneous:

- Disposal box
- Glass beakers - 2
- Racks to hold test tubes

6. PROCEDURE:

6.1 Principle:

Testing with anti-D is necessary to determine if red blood cells possess or lack Rh D blood group antigen. Presence of agglutination is a positive test result, which indicates the presence of the D antigen on the red blood cells.

6.2 D Typing:

Label the tiles with patient & donor number and test identification

↓

Prepare 20% cell suspension for cells being tested.

↓

Add 2 drops of anti-D reagents (Monoclonal & Blended) in D1, D2 marked.

↓

Add 2 drops of positive & negative control reagents in the properly labelled areas

↓

Then add 1 drop of 20% patient cell suspension to reagent & control areas

↓

Mix gently & wait for 2-4 minutes.

↓

Read, interpret and record the test and control results immediately.

6.3 Results

- Depending on presence (+) or absence (-) of agglutination, the test is described as a positive or negative.

6.4 Interpretation:

- Agglutination in D1 & D2 indicates that the red cell under investigation is D positive.
- Absence of agglutination in D1 & D2 indicates D negative at this point. All negative results must be verified under microscope.
- In case of any discrepancy, the result is to be confirmed by ICT method.
- The interpretation of Rh D type is as follows:

Rh-D tying				**Result**	**Remarks**
D1 + Pt`s Cells	D2 + Pt`s Cells	Positive Control	Negative Control		
+++	**+++**	**++++**	**----**	D Positive	
--	**---**	**++++**	**----**	D Negative	
+++	**---**	**++++**	**---**	Confirm by ICT	

7. DOCUMENTATION:

Enter the results of donor /patient grouping in the grouping register and also record the following details-

- Date on which the test is run
- Name of the reagents used
- Lot number of the reagents
- Initials of the technologist who performed the test.
- Initials of the Supervisor who verified the result

STANDARD OPERATING PROCEDURE

ANTIBODY SCREENING

No	Effective Date	Pages	Author	Authorized by
SP 00**X**		4		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
0	1 year	6		

LOCATION	SUBJECT
Red Cell Serology Laboratory	Antibody screen
FUNCTION	**DISTRIBUTION**
Detection of unexpected blood group antibody	- Red Cell Serology Laboratory - Master file

1. SCOPE AND APPLICATION:

This procedure applies to all testing that requires antibody screening, including donor units and prenatal specimens.

2. RESPONSIBILITY:

It is the responsibility of MT in the red cell serology laboratory to perform the antibody screen using proper cell concentrations.

3. REFERENCE:

1. WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.

2. Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 815,827.

4. MATERIALS REQUIRED:

4.1 Equipment:

- Refrigerator to store samples & reagents at 2-8 ^{0} C.
- Deep freezer to store enzyme, albumin & AHG reagent.
- Table top centrifuge.
- Automated cell washer.
- Microscope.
- Water bath.

4.2 Reagents:

- Group O pooled cells
- Enzyme
- 22% Bovine albumin
- Antihuman globulin reagent
- IgG sensitized control cells
- 0.9% saline
- Distilled water

4.3 Specimen:

- Clotted blood sample of donor

4.4 Glassware:

- Test tubes
- Pasteur pipette
- Glass slides

4.5 Miscellaneous:

- Disposal box.
- 2 plastic beakers
- Racks to hold sample tubes.

5. PROCEDURE:

5.1 Principle:

The antibody screen test is used to detect unexpected antibodies. In this test, pooled O cells are combined with serum under investigation. The addition enhancer medium enzyme/ albumin helps to promote the interaction of red cells and antibodies allowing antigen-antibody reactions to occur.

5.2. Antibody screen:

Label tubes with donor/ patient and test identification. Test tube (number)

↓

Add 2 drops of serum to each tube.

↓

Add 1 drop of enzyme to the tube labelled ‘enzyme’.

↓

To each of the tube labelled ‘saline’, ‘enzyme’ and ‘ICT’, add 1 drop of 5% pooled O red cell suspension.

↓

Incubate the tube labelled ‘saline’ for 1hr. at room temperature.

↓

Place the tubes labelled ‘enzyme’, ‘albumin’ and ‘ICT’ into the incubator at 37^0 C for 1hr.

e

↓

After 1 hr, examine the tube labelled ‘saline’ for agglutination.

↓

Remove the tubes labelled ‘enzyme’, ‘albumin’ and ‘ICT’ from the incubator and add 1 drop of albumin to the tube labelled ‘albumin’.

↓

Again place tubes labelled ‘albumin’ and ‘ICT’ to the incubator for ½ hr. more.

↓

After ½ hr. incubation, tubes are from the incubator and tube labelled ‘albumin’ are examined for agglutination.

↓

Positive (IGg serum) control and Negative (AB donor serum) control

↓

Add pooled cell

5.3. Results:

1. Gently re-suspend the red cell button and examine for agglutination.
2. Examine all negative results microscopically.
3. Proceed to perform indirect anti-globulin test on tube labelled ‘ICT’.
4. Wash the cells 3 times with saline. Decant completely after last wash.
5. Add 2 drops of AHG reagent to the cell button.
6. After 6-8 minutes, read and record the results.
7. Add 1 drop of IgG sensitized cells to all negative results. This shows a positive result.

5.4. Interpretation:

Haemolysis or agglutination in any test may indicate the presence of an unexpected antibody.

The absence of agglutination in all tests is a negative test

After addition of IgG sensitized cells to a negative test, the presence of agglutination indicates that the AHG added was capable of reacting and that the negative result is valid.

6. DOCUMENTATION:

- Enter the results of donor unit antibody screen in the donor grouping register
- Enter the results of patient unit antibody screen in the patient grouping register.
- All records are initialled by the technologist who performed the test and by the supervisor who verified the result.

STANDARD OPERATING PROCEDURE

DETECTION OF INCOMPATIBILTY BETWEEN PATIENT AND DONOR

Number	Effective Date	Pages	Author	Authorized by
SOP……..		3		
Version	**Review Period**	**No. of Copies**	**Approved by**	**Date**
01	1 Year	3		

LOCATION	SUBJECT
Red Cell Serology Laboratory	Detection of incompatibility between patient and donor
FUNCTION	**DISTRIBUTION**
Saline Cross-match at Room Temperature	− In charge of BTC − Serology Area − Master File

1. Purpose

To detect the ABO incompatibility before transfusion of blood

2. SCOPE & APPLICATION:

This procedure is applied for compatibility testing of all patients requiring transfusion.

3. RESPONSIBILITY:

It is the responsibility of the Medical Technologist (Lab) under Supervision of trained Medical officer in the Red Cell Serology Laboratory to perform compatibility testing and document the results. If any unexpected antibody is detected, the advanced Red Cell Serological Laboratory should be informed.

4. REFERENCES:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 815,827.

5. MATERIAL REQUIRED:

5.1. Equipment:

- Refrigerator to store samples at 2-8^0 C
- General purpose centrifuge
- Microscope

5.2. Specimen:

- Clotted blood sample of donors/patients

- Blood sample with anticoagulant of donor/patients
- 0.9% saline

5.3. Glassware:

- Tiles
- Plastic pipette
- Glass slides

5.4. Miscellaneous:

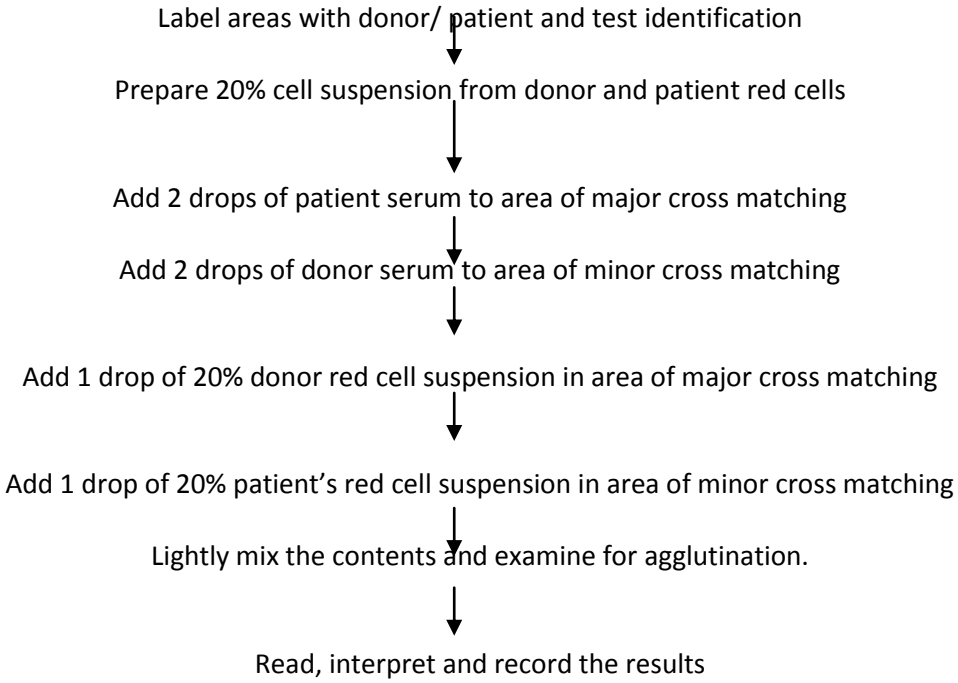
- Disposal bucket with Na hypochlorite
- 2 plastic beakers
- Racks to hold donor and patient sample tubes

6. PROCEDURE:

6.1. Principle:

The cross-match is used to detect unexpected blood group antibodies in patient's serum against antigens on donor cells and to detect antibodies in donor serum against antigens on patient's cells which are complete antibody active at room temperature and cause direct agglutination. Positive reaction in any test indicates incompatibility.

6.2. Cross-match:



6.3. Interpretation:

- Hemolysis or agglutination in any test indicates incompatibility.
- Absence of hemolysis/ agglutination in all tests indicates compatibility.
- Examine all visually negative reactions under microscope.

7. DOCUMENTATION:

- Enter results in cross-match register and compatibility report form.
- All records are initialled by Medical Technologist (Lab) who performed and checked the results and signed by Medical officer.

STANDARD OPERATING PROCEDURE

ANTIGLOBULIN CROSS-MATCH

Number	Effective Date	Pages	Author	Authorized by
SOP 018		3		
Version	**Review Period**	**No. of Copies**	**Approved by**	**Date**
01	1 Year			

LOCATION	SUBJECT
Red Cell Serology Laboratory	Anti-globulin Cross-Match
FUNCTION	**DISTRIBUTION**
Detection of incompatibilities caused by antibodies active at 37^0C	– Supervisor in charge of Red Cell Serology Laboratory – Master File

1. Purpose

To detect incompatibility between donor and patient at 37^0C

2. SCOPE & APPLICATION:

This procedure applies to compatibility testing of all Multi-transfused patients and transfusion recipients who currently demonstrative or have a history of clinically significant antibodies.

3. RESPONSIBILITY:

It is the responsibility of the Medical Technologist (Lab) in the cross match facility of the red cell serology laboratory to perform the anti-globulin cross match using quality controlled reagents and proper dell concentrations. One MT performs the tests and another checks it. If any unexpected blood group antibody is detected, inform the staff of Advanced Red Cell Serology to carry out further investigations.

4. REFERENCES:

1. WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.

2. Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 815,827.

5. MATERIAL REQUIRED:

5.1. Equipment:

- Refrigerator to store samples & reagents at 2-6^0C.
- Table top centrifuge.
- Microscope.

5.2. Specimen:

- Clotted blood sample of patient.
- Segment from donor unit.
- Donor red cells suspended in saline.

5.3. Reagents:

- Antihuman globulin reagent (anti-lgG+anti-C3d).
- lgG sensitised control cells.
- 0.9% Saline.
- Distilled water.

5.4. Glassware:

- Serum tubes.
- Pasteur pipettes.
- Glass slides.

5.5. Miscellaneous:

- Rubber teats.
- Disposal box.
- 2 plastic beakers.
- Aluminum racks to hold test tubes.

6. PROCEDURE:

6.1. Principle:

The cross match through the anti-globulin phase permits detection of clinically significant incompatibilities caused by incomplete antibodies that sensitized cells at 37^0C, but do not directly cause agglutination.

6.2. Cross-match:

i. Label tube with patient/ unit and test identification.
ii. Add two drops of patient serum to each tube.
iii. Prepare a 5% cell suspension in saline from each donor unit segment.
iv. Add 1 drop of donor's 5% red cell suspension to the tube.
v. Incubate tubes at 37^0C for minimum 15 minutes. (Follow manufacturer's directions when using commercial reagents).
vi. Wash the cells a minimum of 3 times with saline. Decant completely after last wash (washing can be done manually or in automated cell washer).
vii. Add two drops of antihuman globulin reagent to the dry cell button.
viii. Mix well and centrifuge at 1000 rpm for 1 minute.
ix. Re-suspend and read for agglutination. Grade and record test results immediately.
x. To all negative anti-globulin tests add 1 drop of lgG-sensitized control cells. Centrifuge, resuspend and read for agglutination. Grade and record test results. After the addition

of lgG-sensitised control cells to a negative test, the presence of agglutination indicates that the AHG serum added was capable of reacting and that the negative anti-globulin test is valid.

6.3. Interpretation:

xi. Hemolysis or agglutination indicates the presence of a serologically incompatible cross-match. This result is interpreted as incompatible.
xii. Absence of agglutination and hemolysis is a negative test result and indicates a serologically compatible cross-match. This result is interpreted as Compatible.

7. DOCUMENTATION:

Enter all results on the transfusion record card and OT/Ward transfusion register. Enter only the results of compatible units in the blood compatibility form. The Medical Technologist (Lab) who performed the test and the one who checked the results sign all records.

STANDARD OPERATING PROCEDURE

RED CELL CONCENTRATE PREPARATION

No	Effective Date	Pages	Author	Authorized by
SOP/……		3		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION	SUBJECT
Component Laboratory	Blood Component Preparation
FUNCTION	**DISTRIBUTION**
Preparation of Red Cell Concentration from whole blood	- In charge of Component Laboratory - Master file

1. Purpose

To prepare blood component from whole blood through blood donation

2. SCOPE AND APPLICATION

For judicious use of blood it is necessary to use the components as per the need than use of whole blood. From the whole blood Red cell concentrate (RCC), FFP and Platelet Concentrate (PC) are separated.

3. RESPONSIBILITY

It is the responsibility of Medical Technologist (Lab) to separate component from the whole blood collected in multiple bags. The Medical Technologist (Lab) will work under the direct supervision of blood transfusion expert.

4. REFERENCE:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 197-199,804.

5. EQUIPMENT AND MATERIALS REQUIRED:

- Freshly collected whole blood
- Refrigerated centrifuge machine
- Metal clips and tube sealer
- Plasma extractor
- Double pan weighing balance
- Double bags / triple bags

- Manuals of all equipment for reference regarding use and maintenance of each equipment.

6. PROCEDURE:

6.1. Principle:

Red Cell Concentrate (RCC) is obtained by removal of supernatant plasma from centrifuged whole blood. The volume of plasma removed determines the hematocrit of the component. A hematocrit of 80% or lowers ensures the presence of adequate glucose for red cell metabolism for specified days of storage.

6.2. Preparation of packed red cells:

- Process the blood collected within 6 hours.
- Keep the bags vertical for 10–15 minutes.
- Note the weight of the primary bags and record in the register.
- Balance the bags in the buckets using dry rubber or unused bags.
- Keep equally balanced bucket diagonally opposite each other in refrigerated centrifuge.
- Position the bags in buckets parallel to the direction of the spin.
- Centrifuge the bags at 1500 rpm for 10 minutes at 1 to 6^0 C unless also preparing platelets. If platelet concentrate is to be prepared then set the temperature of refrigerated centrifuge machine at 20 to 24 ^{0}C (Standardize the speed of the centrifuge according to the machine used).
- Break the seal of the tubing connecting to the satellite bag and express the Platelet Rich Plasma (PRP) into the satellite bag leaving 50-60 mL plasma along with red cells.
- Mix the contents thoroughly and seal the tubing and detach the bags.
- Keep the primary bag containing packed cells in quarantine storage in blood bank refrigerator kept in the component room.
- Label the bag and take it on the inventory after the testing for serology and TTI is completed.

6.3. QUALITY CONTROL OF RCC:

The contents of the final product should be periodically assessed to make sure that they meet expected result. RCC prepared must have satisfy the following criteria –

1. Volume: 150-200 mL
2. Hematocrit: 55 -75% (< 80%)

This is to be established during process validation and periodically confirmed.
At least 95% of units sampled meet this specification.

7. DOCUMENTATION:

Enter the following details in the component register –

- Date and time of separation.
- Unit number.
- Type of bag used, with batch number and manufacturer's name.
- Weight of the whole blood and RCC.
- Date of expiry of RCC.
- Type of centrifuge and speed used.
- Blood group and serology code.

Enter in stock register of red cell concentrates after the testing is completed and the units are labelled.
Incident reporting: If there are any problems encountered during the component processing, inform the Supervisor / In-charge.

STANDARD OPERATING PROCEDURE

FRESH FROZEN PLASMA PREPARATION

No	Effective Date	Pages	Author	Authorized by
SOP…….		3		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION	SUBJECT
Component Laboratory	Fresh Frozen Plasma Preparation
FUNCTION	**DISTRIBUTION**
PRP method for separation of blood component	- In charge of Component Laboratory - Master file

1. Purpose

To produce FFP by refrigerated centrifuge machine

2. SCOPE AND APPLICATION

For judicious use of blood it is necessary to use the components as per the need than use of whole blood. From the whole blood Red cell concentrate (RCC), Fresh-frozen-plasma (FFP) and Platelet Concentrate (PC) are separated.

3. RESPONSIBILITY

It is the responsibility of Medical Technologist (Lab) to separate component from whole blood collected in multiple bags.

4. REFERENCE:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 199, 813.

5. EQUIPMENT AND MATERIALS REQUIRED:

- Freshly collected whole blood
- Refrigerated centrifuge
- Metal clips and tube sealer
- Plasma extractor
- Double pan weighing balance
- Double bags / triple bags
- Freezing apparatus
- Manuals of all equipment for reference regarding use and maintenance of each equipment.

5. PROCEDURE:

5.1. Principle:

Plasma is separated from cellular blood elements and frozen to preserve the activity of labile coagulation factors. Plasma must be placed in the freezer within 8 hour.

5.2. Preparation of fresh frozen plasma:

- Centrifuge blood soon after collection, using 2000 x g for 3 minutes at 1- 6^0 C unless also preparing platelets. If platelet concentrate is to be prepared then set the temperature of refrigerated centrifuge machine at 20-24 ^{0}C.
- Place the primary bag containing centrifuged blood on a plasma extractor and place the attached satellite bag on a scale adjusted to zero. Express the plasma into the satellite bag and weigh the plasma.
- Seal the transfer tubing with a dielectric sealer or metal clips but do not obliterate the segment numbers of the tubing. Place another seal nearer the transfer bag.
- Label the transfer bag with the unit number before it is separated from the original container. Record the volume of plasma on the label.
- Cut the tubing between the two seals.
- Label the bag and take it on the inventory after the testing is over.
- Place the plasma at –18^{0}C or colder within the time frame required for the anticoagulant or collection process.
- Thaw fresh-frozen-plasma rapidly at 30-37^{0}C in a water bath with shaker prior to infusion. Once thawed use it within 6 hr.

6. **QUALITY CONTROL OF FFP:**

FFP prepared must have satisfy the following criteria –

Test item	Requirement	Method
Label	Clear, corrected, completed	
Volume	200 -300 mL	
Factor VIII	≤ 80 IU	
Sterility	Sterilized	

This is to be established during process of validation and periodically confirmed. 1% of total platelets or 4 units per month shall be tested. At least 95% of units sampled meet this specification.

7. DOCUMENTATION:

Enter the following details in the component register –

- Date and time of separation.
- Unit number.
- Type of bag used, with batch number and manufacturer's name.
- Weight of whole blood and different components.
- Date of expiry of different components.
- Type of centrifuge and speed used.
- Blood group and serology code.

Enter in stock register of FFP after the testing is completed and the units are labelled. Summary report for daily freezing. Incident reporting: If there are any problems encountered during the component processing, inform the Supervisor / In-charge.

STANDARD OPERATING PROCEDURE

PLATELET CONCENTRATE PREPARATION

No	Effective Date	Pages	Author	Authorized by
SOP……		2		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION	SUBJECT
Component Room	Platelet Concentrate Preparation
FUNCTION	**DISTRIBUTION**
Preparation of Platelet Concentrates from whole blood	- In charge of Component Laboratory - Master file

1. Purpose

To prepare Platelet concentrate from whole blood by Refrigerated centrifuge machine

2. SCOPE AND APPLICATION:

For judicious use of blood it is necessary to use the components as per the need than use of whole blood. From the whole blood red cell concentrates, FFP and platelet concentrates are separated.

3. RESPONSIBILITY:

It is the responsibility of Medical Technologist (Lab) to separate components from whole blood collected in triple bags. The Medical Technologist (Lab) will work under the direct supervision of blood transfusion expert.

4. REFERENCE:

- WHO (2002).Model standard operating procedures for blood transfusion service; New Delhi.
- Mark E. Brecher (eds). Technical Manual of the American Association of Blood Banks; 15th Edition; Bethesda, Maryland; AABB 2005; p 815,827.

5. MATERIALS AND EQUIPMENT REQUIRED:

- Freshly collected whole blood
- Tube sealer
- Refrigerated centrifuge
- Plasma expresser
- Double pan weighing balance
- Triple bags

- Platelet incubator with shaker
- Manuals of all equipment for reference regarding use and maintenance of each equipment.

6. PROCEDURE:

6.1 Principle:

Platelet-rich-plasma is separated from whole blood by 1500x g for 10 minutes centrifugation and the platelets are concentrated by 4000 x g for 15 minutes centrifugation, with subsequent removal of supernatant plasma.

6.2 Preparation of Platelet concentrate:

- Process the blood collected within 6 hrs.
- Keep the bags vertical position for 10–15 minutes.
- Note the weight of the primary bag and record in the register.
- Balance the bags in the buckets using dry rubber or unused bags. Keep equally balanced bucket diagonally opposite each other in refrigerated centrifuge machine.
- Position the bags in buckets parallel to the direction of the spin.
- Centrifuge the bags at 1500 x g for 10 minutes at 22^0 C (Standardize the speed of the centrifuge according to the machine used).
- Break the seal of the tubing connecting to the satellite bag and express the PRP into satellite bag. Seal the tubing twice between the primary bag and Y connector of the two satellite bags. Place the red cells at 1 to 6^0 C.
- Centrifuge the platelet-rich-plasma by 4000 x g for 10 minutes at 22 ^{0}C.
- Express the platelet-poor–plasma into the second satellite bag and seal the tubing. Some plasma should remain on the platelet button to maintain the pH at 6.2 or higher for the entire storage period. This usually requires a minimum of 35 mL of plasma, but 50-70 mL is preferable.
- Left the platelet concentrate bag stationary, with the label side down, at room temperature for 1 hour.
- Re-suspend the platelet concentrate by placing the bag on a rotator with slow gentle agitation for 1 hour at room temperature.
- Inspect the platelet concentrate to ensure that no platelet aggregates are visible.
- After the required test results are available place the platelet concentrates in the incubator at 20 to 24 ^{0}C with continuous gentle agitation.

6.3 QUALITY CONTROL OF PLATELET CONCENTRATES:

The contents of the final product should be periodically assessed to make sure that they meet expected result. RCC prepared must have satisfy the following criteria –

- Volume: 50-70 mL
- Platelet count: At least 5.5 x 10^9/bag
- Platelet function: Swirling phenomenon
- pH: 6.2 or more
- Red cells: <1.2 x 10^9/bag
- Leucocyte count: <0.12 x 10^9 /bag
- Sterility: pyrogen free

This is to be established during process validation and periodically confirmed.

At least 95% of units sampled meet this specification.

7. DOCUMENTATION:

Enter the following details in the component register –

- Date and time of separation.
- Unit number.
- Type of bag used, with batch number and manufacturer's name.
- Weight of whole blood and different components.
- Date of expiry of different components.
- Type of centrifuge and speed used.
- Blood group and serology code.

Enter in stock register of red cell concentrates after the testing is completed and the units are labelled.

Incident reporting: If there is any problem encountered during the component processing, inform the Supervisor / In-charge.

STANDARD OPERATING PROCEDURE

PREPARATION OF HYPOCHLORITE SOLUTION

Number	Effective Date	Pages	Author	Authorized by
SOP/RL/DR/002/01		2		
Version	**Review Period**	**No. of Copies**	**Approved by**	**Date**
01	Biennial			

LOCATION	SUBJECT
Donor Room	Hypochlorite Solution
FUNCTION	**DISTRIBUTION**
Preparation of Hypochlorite Solution	− Donor Area − Medical Officer In-charge − Master File

1. Purpose

To prepare hypochlorite Solution

2. SCOPE & APPLICATION:

To define the procedure for the preparation of sodium hypochlorite solution used for disinfection decontamination of infections agent that may present in blood or other body fluids. It should the available for clearing working surface, equipment that cannot be autoclaved and non-disposable items & for any spillages of infections material.

3. RESPONSIBILITY:

i. The Medical Technologist (Lab)/ nurse in respected area.

4. REFERENCE:

i. Introductory module guidelines and principals for Safe Blood Transfusion Programme, WHO, 2002, page- 123.
ii. Model SOP for Blood Transfusion service by WHO 2002.

5. MATERIALS REQUIRED:

- Stock hypochlorite solution
- Good quality designed/ distilled water
- Pre-printed self-adhesive labels with, reagent name, batch, date of preparation, date of expiry.
- Documentation in sodium hypochlorite solution preparation work sheet

6. PROCEDURE:

- According to the initial concentration of sodium hypochlorite (HaHocl) solution, necessary concentration (0.1% & 1%) should be prepared.
- Following volumes of sodium hypochlorite (NaHocl) and water should be added:

Initial concentration of hypochlorite	To preparation 0.1% solution		To preparation 1% solution	
	NaHocl	Water	NaHocl	Water
04%	25 ml	975 ml	250 ml	750 ml
05%	20 ml	980 ml	200 ml	800 ml
06%	10 ml	990 ml	100 ml	900 ml

- Time of preparation should be written & should be used within 24 hours and also keep away from direct sunlight.
- Use of these two concentrations should be specified-
    - 0.1% - Table/ Work top cleaning
    - 1.0% - Tubes/ tips/ slides/ container/ spillages
- Complete the preparation record & dispense for use label sufficient dispense bottles with the reagent name, concentration, batch number, date of preparation, date of expiry. Dispense 200 ml of solution in each bottle
- Quarantine the bottles until the release cheeks/ tests have been performed.

STANDARD OPERATING PROCEDURE

STORAGE OF CONSUMABLES, REAGENTS AND KITS.

Number	Effective Date	Pages	Author	Authorized by
SOP		3		
Version	Review Period	No. of Copies	Approved by	Date
01	1 year			

LOCATION	SUBJECT
Quality Control Laboratory	Optimum Quality Assurance
FUNCTION	**DISTRIBUTION**
Optimum Storage of Consumables, Reagents and Kits	– Supervisor in charge of Donor Room – Supervisor- Red Cell Serology Laboratory – Supervisor- TTI Testing Laboratory – Supervisor- Quality Control Laboratory – Supervisor- HLA Laboratory – Master File

1. SCOPE & APPLICATION:

The quality assurance system requires that all the regents used for various test procedures are stored according to the manufacturer's instructions. Any lacunae in the storage conditions, reduces the affectivity of the reagents.

2. RESPONSIBILITY:

It is the responsibility of all the staff members of different laboratories to store all the reagents and kits as per manufacturer's instructions.

3. REFERENCES:

- Indian Pharmacopoeia, Volume II, Annexure 29 (A-29, 1996.
- Reagent manufacturer's instructions.

4. MATERIALS REQUIRED:

- Domestic refrigerator
- Blood bank refrigerator
- Deep Freezer
- A. C. Store room
- Stock register or stock cards

5. PROCEDURE:

a. Donor Room :

i. Store disinfectants for preparation of phlebotomy sites at room temperature (22^0 C-25^0 C).

ii. Store blood collection bags and aphaeresis sets in air-conditioned room (22^0 C-25^0 C).

b. Red Cell Serology Laboratory (RCS):

i. Store ABO reagents Anti-A, Anti-B, Anti-AB, Anti-D, bovine albumin, antihuman globulin, A, B, and O red blood cells, papain and cystein powder in the cold room maintained at 4^0 - 6^0 C or as per manufacturer's instructions.
ii. Store 10ml aliquots of papain-cystein in the Deep Freezer at 70^0 C in RCS laboratory.

c. Transfusion Transmissible Infections Testing (TTI):

Store Kits for HBsAg, HIV, HCV and VDRL at 4^0C - 6^0C in the blood bank refrigerator in the TTI Laboratory or as per manufacturer's instructions.

d. Quality Control Laboratory (QC):

i. Store Kits for Factor VIII assay at (4^0C - 6^0C in the QC laboratory or as per manufacturer's instructions.
ii. Store Copper sulphate stock and working solutions, 0.9% normal saline, and distilled water at RT ((22^0C - 25^0C) in the QC laboratory.
iii. Store chemicals like copper sulphate, sodium chloride and calcium chloride powders at RT (22^0C - 25^0C). in the QC laboratory.

6. DOCUMENTATION:

- Maintain a stock register for all reagents.
- On receipt, make entries of number of vials/kits received, name of manufacture, batch number and expiry date in this register.
- Issue the reagents for use, only after a QC check is performed.
- Enter all issue records in the stock register.
- Order all reagents/kits, no sooner the critical level is reached.

N.B.:

Critical level for all reagents/kits is normally adjusted as per the requirement of reagents, as well as the time taken by the procurement procedure to ensure that reagents are received before the stock in use is exhausted. The new batch received should be tasted against the batch in use.

STANDARD OPERATING PROCEDURE

EQUIPMENT MAINTENANCE

Number	Effective Date	Pages	Author	Authorized by
SOP….		5		
Version	Review Period	No. of Copies	Approved by	Date
01	1 year			

LOCATION	SUBJECT
Quality Assurance Laboratory	Equipment Maintenance
FUNCTION	**DISTRIBUTION**
Calibration	− Quality Assurance Manager − Master File

1. Purpose, SCOPE & APPLICATION:

This procedure covers those measures taken to ensure the integrity, accuracy and reliability of measurement data for equipment and instruments used in the collection, testing and storage of blood products. The procedure is applicable to all equipment used to control or evaluate suitability of starting materials, in process products and finished products.

2. RESPONSIBILITY:

It is the responsibility of all the supervisor of the section to which the equipment belongs to:

- Plan, schedule, organise and maintain records of the calibration programmes fro various equipment under their control.
- Ensure that equipment and instruments are continuously calibrated or are removed from use.
- The Supervisor should train staff for performing calibration/performance checks.

3. REFERENCES:

Blood Programme Quality Manual IFRCRCS Page 23.

4. DEFINITIONS:

Calibration:
A set of operations which establish under special conditions the relationship between values indicated by measuring instruments and standards.

Performance checks:
The routine checking of an instrument to verify that it has remained within specified range of accuracy and precision.

Accuracy:
The closeness of agreement between the result of a measure and the true value of measurement. Calibration is used to determine the accuracy of an instrument.

Precision (Repeatability)**:**
The closeness of agreement between the results of successive measurement of a defined procedure several times under prescribed conditions.

Measurement Standard:
A measuring instrument or material which physically defines a unit of measurement or value of a quantity. Measurement standards used for calibration should be traceable to the SI units of standard measurements.

5. PROCEDURES:

a. Calibration Schedules:

- Purchase each new piece of equipment or instrument according to specifications.
- Place new equipment on an Asset register prior to use.
- Ask the supplier prior to delivery or after installation to calibrate new equipment and provide a certificate of calibration.
- Maintain Calibration/ Maintenance schedules for all equipment.

The schedules of calibration of performance checks should be based on:

- Manufacturers recommendations.
- The history of the item as per reliability.
- Reference standards.
- Recalibrate the measuring devices based on time intervals.

b. Reference standards, Traceability and Calibration Limits:

1. Reference Standards and Trace ability:

All measurement standards used to calibrate measuring devices should be traceable to a national standard of measurement either:

- Directly through purchase of pre-calibrated certified standards. These shall be supported by calibration documents or certification from the supplier stating the date, accuracy (assigned value and units of measure), trace ability and conditions under which the results were obtained. These standards shall be re-calibrated at pre-determined intervals.
- Indirectly by preparation of an internal working standard calibrated against a certified standard. These shall be supported by internal test reposts and any other supporting documentation.
- Where no recognised external standard exists an internal standard may be prepared and calibrated provided a written procedure is prepared and a rational for assigning values, accuracy and units is established. These standards shall be supported by suitable records of calibration as above.
-

2. Calibration Limits:

Calibration is concerned with the measurement of values and their comparison with acceptable limits of standards resulting in adjustment or correction, if necessary.

Compare calibration results with established limits for accuracy for the measuring device. If the device being calibrated does not fall within the limits then re-adjust and re-calibrate until it falls within pre-established limits. If not, remove from use.

The establishment of limits should be based on a combination of:

- Those specified at the time of purchase.
- Recommendations for the manufacturer.
- Limits established in reference standards.

The acceptable limits required for satisfactory calibration of each instrument should be identified or referenced in the relevant procedure.

3. Calibration and Performance Check Procedures:

Prepare documented procedures based on the instrument manufacturer's written instructions and use for the calibration and performance checks for all measuring instruments and measurement standards.

Calibration procedure should include:

- A list of equipment to which the procedure is applicable.
- Calibration points, environmental requirements and special conditions.
- Limits for accuracy.
- Sequence of calibration steps.
- Instructions for recording data with reference to the relevant Standard Form.

Performance check procedures should follow a similar format.

4. Labeling:

Label all calibrated equipment with a label that has the following information:

- Date of last calibration.
- Signature of person who performed the calibration.
- Date next calibration due.

Label the equipment that has passed its calibration due date until it is re-calibrated.

5. DOCUMENTATION:

Maintain complete records for the calibration and performance checks of all equipment and instruments.

Calibration and Performance Check test records should include (where appropriate):

- Asset Register Number.
- Instrument Serial Number.
- Limits for calibration (refer 4.4.2).
- Date of calibration/ performance check.
- Due date for next calibration.

- Any details of adjustment* or repair.
- Results of the calibration*/ performance check.
- Statement of compliance, or details of non-compliance and action taken.
- Signature/ initials of the person performing the calibration/ performance check.

*** It is important that the results of calibration before and after any adjustment are recorded.**

Maintain calibration and performance check records for five years.

7. CORRECTIVE ACTION:

Conduct a review if any measuring device is found to be out of calibration and requires adjustment. Take corrective action where appropriate.

If the item can be adjusted back into calibration, it many continue to be used. If the item cannot be adjusted back into calibration, it must not be used until the situation is corrected. Under these circumstances attach an identifying label stating that the item is under repair and is not to be used.

The Supervisor must assess the likely impact of the inaccuracy of the affected measurement on the quality of current product and product produced since the previous satisfactory calibration. Factors influencing the degree of risk include:

- Critical nature of the measurement.
- Sensitivity of quality control testing to the consequences of the inaccuracy.
- History of production records and performance checks.

Additional quality control testing many be instituted to determine whether quality has been compromised. Where it is likely that quality has been compromised this shall be communicated to senior management and document reports.

8. RELOCATION OF INSTRUMENTS:

Recalibrate the equipment (especially non-portable) when relocated. The manufacturer's recommendations on the need for re-calibration shall be sought when relocating non-portable instruments.

9. EXTERNAL CALIBRATION CONTRACTORS:

Make an agreement with the contractors to supply written reports of calibrations which should include:

- Use of standards and references traceable to national standards.
- Certification/ licensing by the equipment manufacturer, if available.
- Check all certificates or reports supplied by approved external laboratories on receipt. Certificates and reports should contain the same information as required in 5.0 above.

Note: SOPs for TTI screening is to be developed as per kits and Assay (Rapid /EIS/CLIA) used in the blood transfusion centre. Here example are given for development of respective SOPs as per need.

STANDARD OPERATING PROCEDURE

INVENTRY OF BLOOD BAGS AND BLOOD COMPOENTS

No	Effective Date	Pages	Author	Authorized by
SOP……		2		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION:	**SUBJECT**
Storage Area	Inventory of Blood Bags and Blood Components
Function	**Distribution**
Availability of Blood for Transfusion	Medical Officer Supervisor in charge of Storage and Distribution

1. Purpose

To make an inventory of blood bags and component for smooth utilization

2. SCOPE & APPLICATION

In order to avoid outdating and make optimum use of available blood, it is important to maintain a day to day inventory of tested blood which helps selection of blood to be cross matched for patients requiring transfusion.

3. RESPONSIBILITY

The Medical Technologist (Lab) from the red cell laboratory checks the records and transfers all the units which are serologically negative and labelled to inventory.

4. REFERENCES

- Technical Manual of American Association of blood banks 13th Edition, 1999. Pages 83-84, 86.

5. MATERIALS REQUIRED

Inventory Register

6. ROCEDURE

- Keep Inventory on a day to day basis.
- Label the units, enter the numbers of whole blood or packed cells numbers group wise on the right hand page of the inventory register kept in the main red cell laboratory.
- In case of packed cells units, write the alphabet “PC” above the unit number. PC denotes packed cells without additive solutions. PCS denotes packed cells with additive solution. The inventory bears columns for A group, B group, AB group, O group as well as negative groups of these four groups.
- Enter the units group wise and according to the date of collection in the inventory register (daily stock).

- The Medical Technologist (Lab) on night duty is responsible for physical checking of the printed number tag with the hand written number on the label and enters in the inventory.
- Labelling the FFP, enter the donor units numbers group wise in the stock register of FFP similar to blood units.
- Enter FVIII Deficient Plasma units labelled group wise in the stock register similar to plasma register.
- Enter the labelled cryoprecipitate unit numbers in the register.
- Clearly mark the inventory of bags that have less volume of blood collected or are reserved for specific patients with specific instructions.

7. DOCUMENTATION

All unit numbers are entered group wise and expiry date wise in the inventory register.

STANDARD OPERATING PROCEDURE

INVESTIGATION OF TRANSFUSION RECTION

No	Effective Date	Pages	Author	Authorized by
SOP……		2		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION:	**SUBJECT**
Advanced Red Cell Serology	Investigation of Transfusion Reaction
Function	**Distribution**
To identify cause of transfusion reaction	Supervisor of Advanced Red Cell Serology Laboratory Supervisor of Red Cell Serelogy Laboratory Medical Officer

1. Purpose

To investigate the transfusion reaction through laboratory aids

2. SCOPE & APPLICATION

This Standard Operating Procedure (SOP provides the protocol to be followed to identify the cause of an adverse transfusion reaction and prevent its reoccurrence).

3. RESPONSIBILITY

It is responsibility of the Medical Technologist (Lab) in the Red Cell Serology Laboratory to accept the blood/component implicated in the transfusion reaction which is returned from the ward/OT. It is the duty of the same Medical Technologist (Lab) to ensure that there is documented evidence of the nature of reaction either on the transfusion request form or on a separate letter addressed to blood bank, along with the post-transfusion blood sample (both EDTA and clotted) and urine specimen, if necessary. The direct antiglobulin test (DAT) should be performed on the post-transfusion EDTA sample immediately on receipt before refrigeration. The unit and samples should be preserved properly and handed over to the advanced red cell serology Medical Technologist (Lab) who is responsible for detail investigation.

4. REFERENCES

1. Introduction to Transfusion Medicine: Zarin Bharucha and D.M. Chouhan
1st Edition, 1990. Pages 216-219.

5. MATERIALS REQUIRED

5.1. Equipment:

- Refrigerator to store samples and reagents at 2- 6^0 C.

- Deep Freezer to store enzyme papain-cystein in frozen state.
- Table Top Centrifuge.
- Automated Cell washer.
- Microscope.
- Dri bath / Incubator.

5.2. Specimen:

- Blood/component bag returned room ward/OT.
- Patient's pre-transfusion blood sample (clotted).
- Patient's post-transfusion blood sample (EDTA and clotted).
- Patient's post-transfusion urine sample.

5.3. Reagents:

- ANTI-A, Anti-B, Anti-AB Antisera.
- Group A,B &O pooled cells.
- Papain-cystein/22% Bovine albumin.
- Antihuman globulin reagent (anti-IgG anti-C3d).
- IgG Sensitised Control Cells.
- 0.9% Saline.
- Distilled water.
- 30g/l sulfosalicylic acid solution.
- Ammonium Sulphate {NH (so) 2}.

5.4. Glassware:

- Serum tubes.
- Coombs' tubes(for patient grouping only).
- Micro tubes.
- Pasteur pipettes.
- Glass slides.
- Small funnel.
- 20ml test tubes.
- 5ml pipette.

5.5. Miscellaneous:

- Rubber teats.
- Disposal box.
- 2 plastic beakers.
- Wooden block to hold micro tubes.
- Aluminium racks to hold serum and coombs' tubes.
- Whatmen No.1 filter paper.
- 5ml plastic vial with screw cap.

6. PROCEDURE

6.1. Principle:

Red Cell Serological tests are based on the principle of agglutination and help to identify haemolytic transfusion reactions caused either by ABO incompatible transfusion or irregular red cell antibodies in patient's blood. Leuco-agglutinations, if present are detected by agglutination of random donor leucocytes in cases of febrile transfusion reaction. Serum bilirubin total and indirect are raised in case of

haemolysis. The sulfosalicylic acid test helps to differentiate between haemoglobin and non-protein pigment, probably porphyrin in the urine. The ammonium sulphate precipitation test is based on the fact that haemoglobin and myoglobin are precipitated in urine at different degrees of ammonium sulphate saturation.

6.2. Serological Tests

- Perform a direct antiglobulin test (DAT) on post-transfusion EDTA sample before refrigeration immediately on receipt. If test is positive, perform DAT on pre-transfusion sample to verify whether sensitisation is due to transfusion or it pre-existed.
- Repeat grouping and antibody screening of patient's pre-transfusion sample.
- Repeat grouping and antibody screening of patient's post-transfusion sample.
- Repeat grouping and antibody screening of donor sample.
- Repeat grouping of unit from bag. In case of packed cell unit, do only cell grouping. In case of FFP, do only serum grouping.
- Repeat cross matching of donor with patient's pre and post transfusion samples using saline / enzyme / IAT. Use donor cells from blood bag and not the pilot tube.

6.3. Leucocyte Antibody Test:

In case of febrile transfusion reaction and hypotension, look for leukocyte antibodies.

6.4. Biochemical Tests:

- Note colour of plasma. Plasma is pink, if haemoglobin is present and icteric if bilirubin is present.
- Separate the patient's pre and post transfusion serum and send to biochemistry department in a 5 ml screw cap plastic vial bearing the date, patient and test identification for estimation of serum bilirubin total, direct and indirect and estimation of plasma hemoglobin.
- Send the biochemistry request form with proper entries along with the sample.
- Collect the report from biochemistry lab.

6.5. Tests on post-transfusion urine sample.

- Red colour indicates haematuria or haemaglobinuria.
- Add 3ml of 30g/l solution of sulfosalicylic
- acid to 1 ml urine.
- Mix well and filter.
- No precipitate Filter retains Colour
- Precipitate Formed
- Pigment is a protein
- Non protein pigment is probably porphyrin
- Add 2- 8 g NG4 (SO4) 2 to 5 ml urine
- (=80% saturation)
- Shake and mix to dissolve NH4 (SO4) 2
- Filter
- Filter is clear/ Precipitate is coloured--------→ Haemoglobin
- Filter retains colour----→ Myoglobin

6.6. Microbiology:

- Send the donor unit for smear and culture (at 370C, room temperature and 40C) to bacteriology department.

- Make proper entries in the bacteriology dispatch book and bacteriology request form and send along with the unit.
- Collect the report from bacteriology lab.
- If donor unit reveals bacteremia, then request the attending doctor to get the patient's blood culture done and report the findings to the blood bank officer.

6.7. Interpretation:

- Any red cell incompatibility found during the investigation explains a haemolytic transfusion reaction.
- The DAT will be positive and a mixed field reaction will be seen if in vivo sensitisation of transfused red cell has occurred.
- The DAT may be negative even in cases of haemolytic transfusion reaction, if the cell destruction is severe.
- If any antibody is detected in patient's serum, the donor cells should be positive for the corresponding antigen.
- Detection of leucoagglutination explains a febrile reaction or hypotension.
- Serum bilirubin total and indirect are raised in case of haemolysis.
- Haemoglobinemic and haemoglobinuric are highly suggestive of red cell destruction, but are not necessarily caused by antigen-antibody reaction, unless confirmed.

6.8. Limitations:

The non-serologic possibilities of haemoglobinemia and haemoglobinuria are:-

- Haemolysis of blood before transfusion.
- Poor technique of collecting post transfusion sample.
- Myoglobinuria following major surgery.
- Infusion of distilled water during prostatectomy.
- Hemolysis due to artificial valve.
- Patient's clinical condition; Autoimmune haemolytic anaemia or paroxysmal nocturnal haemoglobinuria.
- Use of glucose or dextrose through the same line before starting blood.
- Addition of certain drugs to blood such as ethacrynic acid, hydrocortisone or diphenyl hydantoin.

7. DOCUMENTATION

- Enter the transfusion reaction in blood issue register, showing date and time of return of the unit and nature of reaction.
- Enter the DAT/IAT results in the Antiglobulin test book in the red cell serology laboratory.
- Document the results of the entire investigations in the Transfusion Reaction work up form.
- Keep record in the Transfusion Reaction Record Register in advanced red cell serology laboratory.

STANDARD OPERATING PROCEDURE

MECHANISM FOR CORRECTION AND PREVENTION OF ERROR AND INCIDENT

No	Effective Date	Pages	Author	Authorized by
SOP……		2		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 year	6		

LOCATION	Quality Assurance Laboratory
SUBJECT	Incident Report
FUNCTION	Mechanism for correction and Prevention of error and incidents
DISTRIBUTION	- Quality Assurance Manager - Supervisor in charge of Donor Area - Supervisor- Red Cell Serology Laboratory - Supervisor- TTI Testing Laboratory - Supervisor- Quality Control Laboratory - Supervisor- Component Laboratory - Master File

1. Purpose

To take measures against errors and incident

2. SCOPE & APPLICATION

The procedure covers all incidents that would affect the quality of blood products & services. The procedure applies to all incidents, adverse reactions, equipment used in collection, testing & storage of blood products. The incident reporting process should be clearly defined so that information is tracked and acted on and feedback provided.

3. RESPONSIBILITY

- It is the responsibility of all the technical staff to report any incident/accident to the section supervisor who will submit the report to the Quality Assurance Supervisor/Manager.
- The Quality Assurance Supervisor/Manager is responsible to review the completed report and report to the Director for further investigation and implementation of remedial measures if any.

4. REFERENCES

Technical Manual of American Association of Blood banks 13th Edition, 1999, Pages 3, 14-15.

4.1. DEFINITIONS

Incident Reporting:
Is a process improvement tool that is used to identify problems, analyse the cause, develop solutions, execute the solution and track the effectiveness.

Corrective Action:
Is required for error and accident reports and is usually connected to a process improvement activity. It is an immediate remedial action taken to correct the effect of a defined event.

Preventive Action:
Follow up action taken to prevent a defined event from re-occurring.

Incident:
An Event that results from a deviation from a system, process or procedure that may affect the:

- Safety, purity, potency or effectiveness of the product.
- Health or safety of a donor, product recipient, member of staff/public.
- Trace ability of records. This event may have been identified either prior to or after distribution of a product or service.

5. PROCEDURE

- Document all incidents on the standard form (Incident Report Form).
- Forward the incident summary report to the section supervisor for evaluation and completion.
- Initiate incident tracking.
- Develop corrective/preventive Action in consultation with Section Supervisor, QA Manager and the Director.
- Forward original documents to the QA Manager within 3 working days of the event.
- The QA Manager reviews the report for completeness and appropriateness of corrective action.
- The status of an event remains active until effective action is taken and closed out. Record the details, date of action and close out and get the reports form signed by the Director.
- (viii)Notify the Director immediately in case of critical incidents such as those that could result in loss of life, product recall, failure to operate or adverse publicity
- Provide monthly summary reports to the Director.

6. FLOW CHART FOR

INCIDENT REPORTING PROCESS

- Medical Technologist (Lab) Reports to Section Supervisor…..>
- Section Supervisor completes report and evaluates…..>
- Report to QA Manager……..>
- Initiate incident tracking……..>
- Corrective/ Preventive Action……>
- Submission of documents to QA Manager…….>
- Review by Director & QA Manager ……>Close Out.

7. DOCUMENTATION

Record all incidents on an incident report form. File all record

Example SOP-1

STANDARD OPERATING PROCEDURE

ONE STEP, RAPID HBsAg TESTING

No	Effective Date	Pages	Author	Authorized by
SOP				
Version	**Review period**	**No of copies**	**Approved by**	**Date**
0	One year	06		

LOCATION	SUBJECT
TTI testing Laboratory	One step, Rapid HBsAg Testing
FUNCTION	**DISTRIBUTION**
Donor Samples Tested for HBsAg by GENEDIA HBsAg Rapid Device	Supervisor in charge of TTI Testing Laboratory Master file

1. Purpose

To test the HBsAg marker in blood donor

2. SCOPE AND APPLICATION:

HBsAg is a mandatory test for blood unit screening before it is transfused. This is carried out on all donor unit samples.

3. RESPONSIBILITY:

It is the responsibility of technologist from TTI testing lab to carry out the test and report as required. It is the responsibility of the supervisor of TTI Lab to analyse & sign the report before release.

4. REFERENCE:

- Kit package insert (GENEDIA HBsAg one step Rapid device).
- Model Standard Operative Procedure for Blood Transfusion Service (WHO)

5. MATERIALS REQUIRED:

- GENEDIA HBsAg Rapid Device (Kit)
- Timer
- Disposable gloves
- Disposable Container with Na Hypochlorite (1%) Absorbent tissue
- Serum – Donor serum. If not tested immediately should be refrigerated at 2-8^{0}C with 0.1% sodium azide, NaN_3 for up to 3 days following collection. Store at -20^{0}C, if testing is not possible within 3 days.

6. PROCEDURE

6.1. Principle:

- The GENEDIA HBsAg Rapid Device is made up of test strip and plastic case.
- The test strip is composed of nitrocellulose membrance. Dried gold particle pad, absorbent pad and sample pad.
- The nitrocellulose membrance is immobilized with goat anti HBs on the test band region and goat anti mouse IgG on the control band region.
- During the assay, the serum flows laterally through an absorbent pad and a gold conjugate pad where it mixes with the colour reagent.
- If the serum contains HBsAg, the colloidal gold antibody conjugate binds to the antigens forming an antigen antibody colloidal gold complex.
- The complex then migrates chromatographically through the nitrocellulose strip by the capillary action.
- The serum and anti HBs Colloidal gold complex move through the immobilized goat anti HBs capture band region and then on the control band region.
- For a positive result, a colour band (pink or red) with the complexes will form in the test band region on the membrance.
- Absence of this colour band in the test band region suggests a negative result.
- To serve as a procedural control, a colour band (pink or red) at control region appears in the control band region.

6.2. Kit Composition:

Each kit contains 100 device (10 in each pauch)

- Mouse Monoclonal Anti HBs Gold colloid
- goat anti HBs
- Goat anti mouse /IgG
- Nitrocellulose membrane
- Conjugate pad
- Absorbent pad
- Sticker indicating the top

6.3. Kit Storage temperature. 2-30^{0}C

6.3.1. **Kit Expiry**- 12 months from the manufacturing date.

6.3.2. Appearance

The test strips are assembled into device. The strip is composed of nitrocellulose membrance, dried gold particle pad, absorbent pad, sample pad and sticker indicating the top. There is horizontal line in the middle of device. The character “C” as indicated “Control line” & “T” as “Test line” are shown around the horizontal line. The sticker is indicated by “HBsAg C>” adheres on to membrane of the upper end.

Method:

Preparation of specimens:

- Centrifuge the serum to remove all hematocytes and blood coagulating components
- Allow all specimen to reach room temperatures prior to testing

Storage and expiry date of the opened kits

- Storage Temp. 2 – 30^0C
- Expiry date 4 weeks

Assay procedure

- Bring all test device and test specimens to room temp for 15 – 30 min before removal from the pouch and prior to use.

↓

- Take the required number of device out of the moisture proof pouch

↓

- Dispense 100 µl of specimen into the well of the test device

↓

- Read the results after 30 minutes

↓

- Evaluate the results. In all testing results a colour band (pink and red) at control region always appears in the test area

Validation:
Cloudy specimen, hyperlipidemic specimens, specimens by heavy haemolysis and heat treatment may give inconsistent test result.

INTERPRETATION OF RESULTS:

Negative: A pink or red band appears only in the control region.

Positive: Two pink or red bands appear in the control and test region.

RETEST

- No colour band appears in both regions
- If the specimen is shown positive in the 1^{st} that, repeat the test. If retest result is positive, the sample can be regarded as positive.
- In case of strong colour development in the test region only, retest after diluting the specimen 10 fold with buffered saline.

7. DOCUMENTATION:

Record the results in the HBsAg Rapid test register and also record the following details

- Date on which the test is run
- Name of the kit used
- Lot number and expiring date of the kit
- Initials of the technologist who performed the test.
- Initials of the Supervisor who verified the result
- Reactive units are marked in red in the result sheet.
- Transfer the result in donor grouping register.

Example SOP-2

STANDARD OPERATING PROCEDURE

ANTI HIV(1+2) TESTING

No	Effective Date	Pages	Author	Authorized by
SOP…..		6		
Version	**Review period**	**No of copies**	**Approved by**	**Date**
01	1 Year	6		

LOCATION	SUBJECT
TTI testing Laboratory of Reference Lab	Anti HIV(1+2) testing
FUNCTION	**DISTRIBUTION**
Donor sample tested for Anti HIV(1+2) antibodies by ANI Lab system 3rd generation Anti-HIV(1+2) EIA kit	- Supervisor in charge of TTI - Testing Laboratory - Master file

1. Purpose, SCOPE AND APPLICATION:

Anti HIV 1 & 2 antibodies testing is carried out on all donor bag samples before these are released for transfusion.

2. RESPONSIBILITY:

It is the responsibility of technologist from TTI testing lab to carry out the test and report as required. It is the responsibility of the supervisor of TTI Lab to analyse & sign the report before release.

3. REFERENCE:

1. ANI Lab system 3rd generation Anti-HIV (1+2) EIA Kit package insert.
2. Model standard operative procedures for blood transfusion service (WHO).

4. MATERIALS REQUIRED:

- ANI Lab system 3rd generation Anti-HIV (1+2) EIA Kit
- Micropipette (e.g.: one channel 20- 200 µl, 40- 200µl, and multi-channel 50-300µl).
- Disposable pipette tips
- Distilled or de ionized water, preferably sterile.
- Graduated cylinders, up to 50 ml, for reagent dilutions.
- Vials to store the diluted reagents.
- Timer, 60 min. Range.
- Paper towels or absorbent paper.
- Micro plate incubator 37^0 C
- Micro plate photometer (plate or Micro strip® reader), 450 nm.
- Micro plate washer.
- Sodium hypochlorite solution, free available chlorine 20-500 mg/1 (18).
- Disposable gloves.

- Stopping solutions (2 M H_2SO_4).Available from Ani Labsystems (2 M Sulphuric acid, 7.5ml- cat. No. 61 11 016).

ANI Lab system 3rd generation Anti-HIV (1+2) EIA Kit contains

1. MICROSTRIPS®: 12 × **8** wells (10 x 12 x 8 wells)
Coated Microstrips®

2. SAMPLE DILUENT: 25 ml (200 ml)
Tris buffered saline with proprietary additives, a blue colouring reagent, and 0.05 % Bronidox® as preservative.

3a. NEGATIVE CONTROL, 1.0 ml (2.5 ml)
HIV antibody negative human serum, a blue colouring reagent, and 0.05% Bronidox® as preservative.

3b. POSITIVE CONTROL 1, 1.0 ml (3.5ml)
Heat inactivated HIV-2 antibody positive human serum, a red colouring reagent, and 0.05% Bronidox® as preservative.

4. CONJUGATE: 30 ml (250 ml)
Buffered salt solution with proprietary additives, are colouring reagent, horseradish peroxidase conjugated anti-human lgG (sheep) with 0.01% Thiomersal® as preservative.

5. SUBSTRATE BUFFER: 50 ml (250 ml)
Citrate-acetate buffer containing 0.005% hydrogen peroxide and 0.05% bromo-Nitro-Dioxane as preservative.

6. TMB-CHROMOGEN in DMSO, 1. ml (5.5 ml)
3,3`,5,5`- Tetramethylbenzidine dissolved in dimethy-sulfoxide (DMSO).

7. WASHING SOLUTION: 100 ml concentrate (500 ml concentrate)
Concentrated citrate buffered saline, with proprietary additives, and 0.05% Bronidox® as preservative.

Kit storage temp.: 2 – 8^0 C All reagents and Micro strips must be warmed up to +20^0C to +25^0Cbefore use. Return to 2–8^0 C storage immediately after use.

REAGENT PREPARATION

Reagent	Preparation	Stability of opened/diluted reagents (+2^0C to +8^0C)
1 Coated Micro strips®	Ready for use	6 months *)
2 Sample diluents	Ready for use	6 months *)
3a Negative control	Ready for use	6 months *)
3b Positive control 1	Ready for use	6 months *)
3c Positive control 2	Ready for use	6 months *)
4 Conjugate	Ready for use	6 months *)
5 Substrate buffer	Ready for use for the substrate solution	6 months *)
6 TMB- Chromogen in		6 months *)

DMSO Substrate solution 7 Washing solution concentrate (10x) Washing solution Stopping solution **)	Ready for use for the substrate solution Dilute TMB (vial 6) 1 + 49 (1:50) with substrate buffer (vial 5) just prior to use Dilute the concentrate (vial 7) 1+9 (1:10) with distilled water. Read for use	Discard unused reagent. A deep blue colour present in the substrate solution indicates that the solution has been contaminated and must be discarded. 7 months *) 1 month at + 4^0c or 1 week at room temperature.

* The stability of the opened reagents is the maximum 6 months only if they are stored properly at +2^0C to +8^0C. However, high environmental temperature and contamination may decrease the stability.

** Not included in kit composition.

Preliminary Preparations

- Wear disposable glove throughout the procedure.
- Bring all reagents and Microstrips® to room temperature (+20^0C to +25^0C) before starting the assay.
- Pre warm the incubator to +37^0C.

Pipette first the samples, and then the controls. Mix the sample diluents While pipetting to avoid gradient.

NOTE: Samples must not be diluted or polled!

blank	sample 1									
blank	sample 2									
3a	Etc.									
3c										
3c										
3b										
3b										
3b										

TEST PROCEDURE

Outline of the Procedure

STEP 1 Add 150 sample diluents (vial 2) into each Microstrip® well.
Add 50 sample diluent (vial 2) to wells A1 and B1 for blank.
↓
Pipette 50 serum or plasma specimens into Microstrip® wells starting from well A2
Pipette 50 of negative control (vial 3a) into Microstrip® wells C1.
Pipette 50 of positive control 2 (vial 3c) into two Microstrip® wells: D1 and E1
Pipette 50 of positive control 1 (vial 3b) into three F1, G1 and H1.
↓
Mix carefully while pipetting
↓
Cover the Microstrip® wells

↓

Incubate for 30 min., at + 37^0C

↓

Wash(diluted vial 7) 5 times (400µl/ well, soak at least 10 seconds.)

↓

STEP II Add 200µl of conjugate (vial 4) into each well.

↓

Cover the Microstrip® wells with Microstrip® cover.

↓

Incubate 30 min., at +37^0C

↓

Wash 5 times (400µl/ well, soak at least 10 seconds).

↓

STEP III Add 200 of the substrate solution (vial 5 and 6) into each well

↓

Incubate 15 min. at RT in dark

↓

STEP IV Add 50µl 2M H_2SO_4 solution into each well

↓

Read the absorbance's immediately at 450 nm

Blank the photometer at 450 against air. Most microplate readers can be blanked against the reagent blank. This means that the absorbance of the reagent blank is automatically subtracted from the absorbance of the samples. However, the absorbance of the reagent blank has to be measured first to check that it falls within the quality control values.

RESULTS

Quality Control Values

Before calculating the results, make sure that the absorbance values obtained for the reagent blank and controls fall within the quality control guidelines.

HIV positive 1 and 2 negative controls are provided in each kit. The negative control should be included in single, the positive control 2 in duplicate and the positive control 1 in triplicate in each test urn. Expected absorbance values for these controls are shown in Table 1. If the blanks and the controls do not give expected values, the results are invalid and the specimens should be **retested.**

Two values of positive control 1 must fall within the expected values for the assay to be valid.

Table 1. Quality Control Values

Quality Control Sample	Expected value at 450 nm (in absorbance units)
Reagent blank	$\leq$ 0.10
Negative control (3a)	$\leq$ 0.15 x)
Positive control 1(3b)	0.70 $\leq$ Apc1 < 2.00 x)
Positive control 2 (3c)	> 0.50 x)

The absorbance of the reagent blank has already been subtracted from these values.

Calculation of the Results

Abbreviations:

A = Absorbance
Arb = Mean absorbance of the reagent blank.
Apc1 = Mean absorbance of the positive control 1 (3b).
CO = The cut-off value in absorbance units.

Example

QC Sample	Mean of the measured absorbance's at 450 nm
Reagent blank	0.04
Positive control 1	1.37

CO =0.3 x (1.37—0.04)+ 0.04 = 0.44

Interpretation of the Results

Result	Interpretation
<CO	A negative result means that the sample tested either contains no antibodies to HIV or the antibody level is below the detection limit of the test kit. With negative test results, when infection is suspected, it is advised to repeat the test with a new serum sample taken 2-4 weeks later.
≥CO	An initially reactive test result has to be retested. Only after receiving a repeatedly reactive result, the sample may be presumed to contain antibodies to HIV. The result should be verified with a recognized confirmatory test. As with other immunoassays, occasional false positive results may occur, which are in most instances non-repeatable. It is therefore recommended to retest all samples giving an initially positive result.

Precautions for users:

1. Do not use the kit after the expiration date.
2. Do not substitute reagents from one kit lot to another.
3. Do not pipette by mouth.
4. Use only reagent grade quality de-ionized or distilled water to dilute reagents.
5. Do not expose substrate and TMB to strong light.
6. Avoid contact of TMB and sulphuric acid with any oxidizing agent or metal.
7. Avoid repeatedly opening and closing the incubator during incubation steps.
8. Safety precautions
   - Handle assay specimens, positive and negative controls as potentially infectious agents. Wear laboratory coats and disposable gloves while performing the assay. Discard gloves in biohazard waste bags. Wash hands thoroughly afterwards
   - Autoclave all used and contaminated materials at 121^0 C, for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solutions for 30 – 60 minutes.
   - Wipe any spills promptly with 1% sodium hypochlorite solution.

- The stopping solution is strong acid. Wipe spills immediately. Flush the area of the spill with water. If the stopping solution contacts the skin or eyes, flush with copious amounts of water and seek medical attention.

Documentation

Record the test result in the register book and also record the following details

- The date on which the test is run
- The name of the kit used.
- Lot number and expiring date of the kit
- Initials of the technologist who performed the test and the supervisor who verified the results.
- The reactive units are marked in red in the result sheet.
- Transfer the result to donor records.