



Punjab Blood Transfusion  
Authority



Government of the Punjab,  
Health Department

# Standard Operating Procedures For Blood Transfusion Services, Punjab



**PUNJAB BLOOD TRANSFUSION  
AUTHORITY  
HEALTH DEPARTMENT  
GOVERNMENT OF THE PUNJAB**

## **ACKNOWLEDGEMENT**

Blood Transfusion is an essential part of any health care system. Used correctly, it can save life and improve health. The Blood Transfusion Services in Pakistan are fragmented with a wide diversity in the quality and range of services provided. There has been a growing awareness about quality in blood transfusion services with the objective of releasing only those blood products which fulfill the desired standards in terms of efficacy and safety. Keeping in view the vital importance of strict quality control at each stage of vein to vein procedure, Punjab Blood transfusion authority has been established.

The Punjab Blood Transfusion Authority is a regulatory body to ensure good quality blood transfusion services in Punjab. The mandate of Punjab Blood Transfusion Authority is to conduct inspection of all blood banks operating in private as well as public sector. The blood banks meeting the minimum standard are issued a license to operate. One of the prerequisite of such inspections and registration of a blood bank is to develop standard operating procedure and minimum service delivery standard.

The Punjab Health Department constituted a Technical Advisory Committee to advise the authority on the technical aspects of blood transfusion services. The Technical Advisory Committee has reviewed the publications of national blood transfusion services titled, "Standards and Guidelines for Blood Banks and Transfusion Services" and "Standard Operating Procedures for Blood Bank Processes in Pakistan. The Technical Advisory Committee recommended these standards and SOPs to be implemented in Blood Transfusion Services in Punjab.

The Punjab Blood Transfusion Authority is deeply indebted to all members of Technical Advisory Committee for their hard work. Punjab Blood Transfusion Authority is also thankful to GIZ and National Blood Transfusion Services for allowing publication of these books for Punjab. The Punjab Blood Transfusion Authority will welcome any comment/ suggestion to improve the documents.

(Dr. Muhammad Jafar Saleem)

Secretary,  
Punjab Blood Transfusion Authority

## FOREWORD

Standard Operating Procedures are an integral part of a quality system, as they facilitate consistency in the performance of procedures in accordance with standards. SOPs are required for the entire vein to vein transfusion chain. Realizing that most blood banks in Pakistan may not have the capacity to write their own SOPs, the collection presented herewith will provide them with templates to be adopted as such or to be used as a guide to writing and validating their own SOPs.

These SOPs have been generated with a novel methodology. The process started with a field analysis, in which the project collected all available SOPs from a sample of public and private blood banks, listed them in relation to the key domains addressed and documented both availability and gaps. In a second step, an ideal list of SOPs as required by a prototype blood banking and transfusion service was developed, known now as the 'SOP Flyer'. This flyer is used as an educational tool to clearly delineate both the structure of the 'reformed' blood transfusion system and the roles played by the building blocks of this system, i.e. Regional Blood Centers, Hospital Blood Banks, and the 'end users': the Hospital Wards. The overall transfusion chain is broken down into 12 technical areas which represent the entire chain from blood collection to its transfusion in the ward.

The SOP flyer was presented to a wider audience in a national workshop held under the header of 'Information Management and Management Information' (Islamabad, October 2011), in which it was placed into the context of architecture and logic of a comprehensive information system for evidence based decision making. The flyer provided a baseline to identify the documents generated in the different stages of the vein to vein transfusion chain. In the meantime, information management has been formalized in a 'Functional Brief' of MIS, while the recommended reorganization has been described separately in 'Blood Transfusion System Reform'.

The actual development of the of new SOP templates was planned as a joint collaborative effort. The clear breakdown of the transfusion chain domains into consecutive procedures, as outlined in the flyer, served as a reference for the identification of suitable working groups. Members of the working groups represent their respective specialties, but at the same time share the wealth of their regional experience. This bottom up approach is expected to have resulted in SOP templates of high practical value, though both international literature and a previous version of SOPs for Pakistan were consulted in the process.

Six mixed working groups (Haematologists, Blood Bank Incharges, Blood Bank Technologists and Technicians) were established, working on their specific domains of the transfusion chain. The SOP document thus captures local expertise from various well-reputed blood bank establishments all over the country, mostly from secondary or tertiary hospitals, as well as international recommendations and manuals for SOPs (most notably the AABB manual and standards, WHO model SOPs, the European SOP Manual [EuBIS], and the Council of Europe's recommendations for preparation, use and quality assurance of blood components).

The SOPs cover all testing procedures (blood grouping, TTI screening, antibody screening and identification) and working procedures (donor management; collection of donation; component production and storage, quality controls, data processing, record storage and handling of blood requests from wards) for the entire transfusion chain. The design of the procedures is in accordance with international norms, including unique identification numbers, location, scope, responsibilities, material requirements, documentation and interpretation of results.

It is pertinent also to acknowledge the support and encouragement received from our blood programme managers, including Dr. Hasan Abbas Zaheer (ICT), Dr. Muhammad Tahir Khan (KPK), Dr. Naveed Ahmed (AJK), Prof. Mahfooz-ur-Rahman (Punjab), Dr. Zahid Hasan Ansari (Sindh), Prof. Nadeem Samad Sheikh (Balochistan), Dr. Sartaj Khan (FATA), and Dr. Abdul Latif (Gilgit Baltistan), whose advice and recommendations will be sought also for the stages of dissemination and implementation.

We are confident that also our working group members and authors, who have shared their expertise for the development of this collection of SOPs, will contribute to an enhanced understanding and improved quality management in blood transfusion services by stimulating the use of these SOPs in their respective environments.

Islamabad, June 2013

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# Standard Operating Procedures of the Reformed Blood Transfusion System

## Regional Blood Center

<b>Donor Management</b>	<ol style="list-style-type: none"> <li>1 Reception of donor</li> <li>2 Haemoglobin screening</li> <li>3 Pre-donation counseling</li> <li>4 Medical Interview</li> <li>5 Physical examination</li> </ol>	
<b>Collection of Donation</b>	<ol style="list-style-type: none"> <li>6 Inspection of blood bags and labeling</li> <li>7 Preparation of the venipuncture site</li> <li>8 Phlebotomy and collection of whole blood donation</li> <li>9 Collection of blood components through apheresis</li> <li>10 Collection of blood samples</li> <li>11 Post donation care/ refreshments</li> <li>12 Management of adverse reactions</li> <li>13 Documentation of adverse reactions</li> </ol>	
<b>TTI Screening</b>	<ol style="list-style-type: none"> <li>14 Reception of blood samples</li> <li>15 Testing for HBsAg</li> <li>16 Testing for HCV Antibodies</li> <li>17 Testing for HIV Antibodies</li> <li>18 Syphilis Screening</li> <li>19 Malarial Parasite Detection</li> <li>20 Reporting of results</li> </ol>	
<b>Immunohaematology</b>	<ol style="list-style-type: none"> <li>21 ABO grouping</li> <li>22 Identification of weak ABO types</li> <li>23 RhD typing</li> <li>24 Identification of weak Rh types</li> <li>25 Antibody screening</li> <li>26 Antibody Identification</li> </ol>	
<b>Component Preparation</b>	<ol style="list-style-type: none"> <li>27 Red cell concentrates</li> <li>28 FFP/ Cryoprecipitate</li> <li>29 Platelets</li> <li>30 Labeling</li> </ol>	
<b>Storage</b>	<ol style="list-style-type: none"> <li>31 Red cell concentrates</li> <li>32 FFP/ Cryoprecipitate</li> <li>33 Platelets</li> </ol>	
<b>Distribution to HBB</b>	<ol style="list-style-type: none"> <li>34 Receiving requests from HBB &amp; documentation</li> <li>35 Dispatching blood components</li> <li>36 Transporting blood components</li> <li>37 Temperature monitoring of transport boxes</li> </ol>	

## Hospital Blood Bank

<b>Reception of blood components</b>	<ol style="list-style-type: none"> <li>38 Documented reception of blood bags</li> <li>39 Inspection of blood bags</li> </ol>	
<b>Storage</b>	<ol style="list-style-type: none"> <li>40 Red cell concentrates</li> <li>41 Fresh frozen plasma/Cryoprecipitate</li> <li>42 Platelets</li> <li>43 Thawing of FFP</li> </ol>	
<b>Issuance of blood components</b>	<ol style="list-style-type: none"> <li>44 Receiving clinical request forms and blood samples</li> <li>45 Blood grouping</li> <li>46 Cross matching</li> <li>47 Antibody screening</li> <li>48 Antibody identification</li> <li>49 Filtration</li> <li>50 Washing</li> </ol>	
<b>Distribution to ward</b>	<ol style="list-style-type: none"> <li>51 Labeling</li> </ol>	
<b>Discarding of blood components</b>	<ol style="list-style-type: none"> <li>52 Handling returned/expired blood components</li> </ol>	

## Hospital Ward

<b>Transfusion of blood components</b>	<ol style="list-style-type: none"> <li>53 Reception of blood components</li> <li>54 Checking labels on blood bag</li> <li>55 Inspection of blood bags</li> <li>56 Temporary storage(refrigerators)</li> <li>57 Selection of blood infusion sets</li> <li>58 Preparation of transfusion site</li> <li>59 Transfusion of components</li> <li>60 Transfusion reactions</li> <li>61 Management of adverse reactions</li> <li>62 Reporting of transfusion reactions to HTC</li> </ol>	
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# **Donor Management Department**



# Standard Operating Procedures

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# STANDARD OPERATING PROCEDURE

<b>DONOR MANAGEMENT DEPARTMENT</b>		
<b>DONOR RECEPTION</b>		
<b>BTS/SOP/WP/01</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This SOP describes the documentation of the screening process according to the selection criteria for the donor. The main purpose of selecting individuals for blood and component donation is to safeguard the health of both donor and recipient. All donors undergo a screening process to assess their suitability. The major functions are performed by the donor interviewer/Medical Officer (when evaluating the suitability of the potential donor) and the phlebotomist (when preparing the donor and collecting his/her blood).

## 2. RESPONSIBILITY

The Medical Officer is responsible for determining the suitability of the donor for blood donation. After evaluation of the health history questionnaire and medical examination including the results of pre-donation screening tests, s/he should confirm that the criteria are being fulfilled

The Registered Nurse is responsible to make sure all forms are properly filled. She is also responsible to take the vitals and enter the results in the relevant form. The Technician is responsible to check the haemoglobin.

## 3. PRINCIPLE

Accept only voluntary/replacement non-remunerated blood donors.

Donors who are selected must have:

- 3.1 Satisfactorily completed a confidential interview.
- 3.2 Declared about any high-risk behavior, practices and circumstances that prevent them from donating blood.
- 3.3 Satisfactorily completed a health assessment that includes a questionnaire regarding past and present medical conditions.
- 3.4 Satisfied minimum physiological criteria.
- 3.5 Been instructed to contact the Blood Center, even after donation, for any information that may be relevant to their health or which may affect the suitability of their donation.

## 4. MATERIAL

- 4.1 Donor Registration Form (*cf. Annex 1*)
- 4.2 Donor's past blood bank record if any (e.g. Previous Blood Donor Card)
- 4.3 Haemoglobin SOP and related material (*cf. SOP/TP/02a or 02b*)
- 4.4 Pre-donation educational material (*cf. Annex 2 & SOP/WP/03*)

- 4.5 Donor History Questionnaire Form for Medical Review (cf. Annex 3 & SOP/WP/04)
- 4.6 Physical Examination (cf. Annex 4 & SOP/WP/05)

## 5. PROCEDURE

### Donor Interviewer's Responsibilities

- 5.1 To fill the Donor Registration Form starting from data regarding the proper identification of the donor:
  - a. Donor's full name with Father's name
  - b. Donor's Sex: Male/Female
  - c. Donor's date of birth (age)
  - d. Donor's CNIC number
  - e. Donor's home and office address
  - f. Donor's home and work telephone numbers
  - g. Donor's Occupation/Hobby
  - h. Visit date
  - i. Reported ABO/Rh type (this applies only to repeat donors)
  - j. Date of last donation (from most recent previous Donor History Questionnaire Form)
  - k. Next eligibility date (confirm that at least 12 weeks' time has been elapsed since last whole blood donation)
  - l. Total number of donations (keep a running total on each successive DHQF)
- 5.2 Perform donor screening by haemoglobin estimation. (cf. SOP/TP/02a or 02b)
- 5.3 Provide pre-donation educational material to donor. (cf. SOP/WP/03)
- 5.4 Record in donor history questionnaire form whether or not donor appears to be in good health. (cf. SOP/WP/04)
- 5.5 Carry out Physical Examination. (cf. SOP/WP/05)
  - a. Appearance of the donor
  - b. Pulse
  - c. Temperature
  - d. Blood Pressure
- 5.6 Write down your initials in the appropriate box and take the donor's signature as well. This concludes the donor's interviewer role.
- 5.7 Record time of last meal. If time is greater than 4 hours or less than 8 hours then ask donor if he/she wishes to have a snack before giving blood, or give some fruit juice to drink if donor does not wish to eat.

## 6. DOCUMENTATION

Duty nurse or technician enter the donor's data, haemoglobin screening test results and answers of DHQF in the donor record form section of the Blood Transfusion Information System or Donor Record Register. - Take donor's signature on Donor History Questionnaire Form and keep it as a record.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

<b>DONOR MANAGEMENT DEPARTMENT</b>		
<b>HAEMOGLOBIN SCREENING BY CuSO<sub>4</sub> METHOD</b>		
<b>BTS/SOP/TP/02a</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applies to all the steps necessary to perform the pre-donation haemoglobin (Hb) screening. It is the first and foremost test to be done for blood donor selection with the main intention of preventing blood collection from an anemic donor. The primary purpose of haemoglobin screening is donor protection, preventing an anemic individual from exacerbating their condition. The second purpose is to ensure that the patient receives quality product, i.e. the Hb content of the donated blood meets the required criteria. The Hb may be measured by different methods. Most commonly applied and traditional method for Hb estimation for blood donation is copper sulphate (CuSO<sub>4</sub>) method.

### 2. RESPONSIBILITY

It is the responsibility of the technician working in the donor management department to do the donor's haemoglobin screening test.

### 3. PRINCIPLE

This is a qualitative test based on estimation of haemoglobin content of blood from its specific gravity. A specific gravity of 1.053 corresponds to the haemoglobin concentration of 12.5gm/dl. Hence CuSO<sub>4</sub> solution of specific gravity 1.053 is used. A blood drop when in contact with copper sulphate solution of specific gravity 1.053 becomes encased in a sack of copper proteinate, which prevents any change in the specific gravity for about 15 seconds. Therefore, if the haemoglobin is equal to or more than 12.5 gm/dl (acceptable level for donor) the drop will sink within 15 seconds; if not, the drop will hesitate and remain suspended or rise to the top of the solution.

### 4. MATERIAL

- Copper Sulphate working solution with a specific gravity 1.053
- Sterile gauze/cotton, alcohol swab and sterile disposable lancets
- Heparinized capillaries (dimensions: 75mmx1mm)
- Containers with 1% sodium hypochlorite solution for disposal of sharp lancets
- Capillaries and bio hazardous material
- Coplin jar with lid

## 5. METHODS

- 5.1 Use 30 ml copper sulphate working solution (Sp.gr.1.053) in a clean, dry coplin jar.
- 5.2 Clean the fingertip thoroughly with a spirit swab and allow it to air dry.
- 5.3 Puncture the fingertip firmly with a sterile disposable lancet.
- 5.4 Ensure a good free flow of blood.
- 5.5 Do not squeeze the puncture site repeatedly since it may dilute the drop of blood with excess tissue fluid and gives false low results.
- 5.6 Wipe out the first blood drop and allow the blood sample to fill up to  $\frac{3}{4}$  of the micro capillary by capillary force and avoid any air bubbles to trap in.
- 5.7 Allow one blood drop to fall gently from the capillary at the height of about 1 cm above the surface of the copper sulphate solution into the coplin jar.
- 5.8 Observe the blood drop for 15 seconds.
- 5.9 Dispose of the lancet and capillaries in a container with 1% sodium hypochlorite solution.

## 6. INTERPRETATION OF RESULT

- 6.1 Blood drop sinks or floats.
- 6.2 If the blood drop sinks within 15 seconds (i.e. donor's haemoglobin is more than 12.5 gm/dl), that means donor met one of the pre-requisite for donating blood.
- 6.3 However, if the blood drop sinks midway (i.e. haemoglobin level is less than 12.5 gm/dl), and then comes up, the donor is deferred.
- 6.4 If the drop sinks slowly, hesitates and then goes to the bottom of the jar, confirm the haemoglobin of this donor by some other method, e.g. Automated Cell Counter.
- 6.5 In case the haemoglobin is lower than 12.5 gm/dl, prescribe haematinics and ask the donor to come for a recheck after one month.

## 7. QUALITY CONTROL

- 7.1 Keep the jar covered with a lid when not in use to protect the reagent for evaporation.
- 7.2 Change the working solution after every 25 tests.
- 7.3 Keep the solution at room temperature or mix thoroughly and bring to room temperature before use.
- 7.4 Do not freeze or expose the solution to very high temperature.
- 7.5 Check the specific gravity daily before using.
- 7.6 False-positive reactions are rare.
- 7.7 False-negative reactions occur fairly commonly and may cause inappropriate deferral.

## 8. PREPARATION OF $\text{CuSO}_4$ STOCK SOLUTION

Make the stock solution as follows and keep it in a coplin jar or bottle.

- 8.1 Dissolve 170 gm. crystalline  $\text{CuSO}_4$  in 1000 ml distilled water (Stock Solution).
- 8.2 Every morning prepare the fresh solution.
- 8.3 Add 51 ml stock solution to 49 ml distilled water or add 520ml of stock solution into 480 ml distilled water (Working Solution).
- 8.4 Check Specific Gravity of  $\text{CuSO}_4$  solution which should be 1.053. If not, then adjust it using either stock solution or distilled water.

Method for Quality Control of Copper Sulphate Solution

Measurement of specific gravity directly by hydrometer

- ✓ Add a drop of copper sulphate solution to the hydrometer
- ✓ Observe specific gravity (Specific gravity of  $1.053 \pm 0.0003$  gm/ml is acceptable)

Functional Validity

Observe the behaviour of blood drops (anti-coagulated samples from individuals) with known haemoglobin levels (use 3 samples that are within or 0.5 to 1.0 g/dl above the permissible range and 3 samples that are 0.5 to 1.5 g/dl below 12.5 g/dl), for example:

- |              |              |
|--------------|--------------|
| 1. 13.5 g/dl | 4. 12.0 g/dl |
| 2. 13.0 g/dl | 5. 11.5 g/dl |
| 3. 12.5 g/dl | 6. 11.0 g/dl |

STOCK			WORKING SOLUTION			
Powder	Solution		Prepared on	Specific Gravity	Quantity	Prepared by
Mfr./Batch No.	Batch No	Prep. No				

**9. DOCUMENTATION**

Please enter the donor's haemoglobin result in the Physical Examination Form.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>DONOR MANAGEMENT DEPARTMENT</b>		
<b>HAEMOGLOBIN SCREENING BY HAEMOGLOBINOMETER</b>		
<b>BTS/SOP/TP/02b</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies to all the steps necessary to perform the pre-donation haemoglobin (Hb) screening. It is the first and foremost test to be done for blood donor selection with the main intention of preventing blood collection from an anemic donor. The primary purpose of haemoglobin screening is donor protection, preventing an anemic individual from exacerbating their condition. The second purpose is to ensure the patient receives quality product, i.e. the Hb content of the donated blood meets the required criteria. The Hb may be measured by different methods. Digital haemoglobinometer is applied for Hb estimation for blood donation to reduce the chances of false acceptance as well as false deferral.

## 2. RESPONSIBILITY

It is the responsibility of the technician working in the donor management department to do the donor's haemoglobin screening test.

## 3. PRINCIPLE

This SOP describes the means by which haemoglobin concentration is determined through photometric method. This method uses a portable, battery-operated photometric device based on determination of azide met-hemoglobin. It is one of the easiest and rapid methods for pre-donation hemoglobin estimation.

## 4. MATERIAL

- Haemoglobinometer
- Associated user manual and product inserts
- Disposable lancet
- Alcohol swab
- Adhesive plaster
- Sticking plaster
- Sharp container
- 2" x 2" gauze pad

## 5. PROCEDURE

- 5.1 Prepare one of the donor's fingertips (preferably middle finger) by rubbing it vigorously (but gently) with a 70% isopropyl alcohol pad and allow it to dry.
- 5.2 Puncture the fingertip firmly with a sterile disposable lancet.
- 5.3 Discard first drop of blood by gauze piece and use the next drop(s) as specimen.
- 5.4 Draw correct volume of blood specimen into the microcuvette by capillary action.
- 5.5 After wiping off any excess of the specimen from the sides of the microcuvette, place it in the cuvette holder and insert it into the analyzer.
- 5.6 Dispose of the lancet and capillaries in an appropriate manner or designated sharp container.

## RESULT

The donor's haemoglobin result is displayed automatically on the digital screen of the haemoglobinometer.

## 6. INTERPRETATION OF RESULT

- 7.1 If the haemoglobin is  $> 12.5$  gm/dl in female and  $>13.5$  gm/dl in male then accept the donor.
- 7.2 In case if the haemoglobin is  $< 12.5$  gm/dl in female and  $<13.5$  gm/dl in male then defer the donor, prescribe haematinics and ask him/her to come for a recheck after one month.

## 7. DOCUMENTATION

Record the haemoglobin concentration on Donor's Physical Examination Form (regardless of whether or not the donor passed the haemoglobin test)

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

DONOR MANAGEMENT DEPARTMENT		
PRE-DONATION INFORMATION		
<b>BTS/SOP/WP/03</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

The purpose of this procedure is to provide education to donors about the donation process and high risk behavior necessarily self-exclusion. Provision of pre-donation information is essential for ensuring safe blood supply, because lack of education and awareness aggravate problems of transfusion transmitted infections.

## 2. RESPONSIBILITY

The Medical Officer is responsible for providing pre-donation information to the potential donor. After registration, blood donor education material is provided in printed form as well.

## 3. PRINCIPLE

Provision of pre-donation information is (1) to increase awareness about safe blood and safety of donation process and (2) to discourage the donation if he/she is among high risk group.

## 4. MATERIAL

- Blood Donor Educational Material (*cf. Annex 3*)
- Frequently Asked Questions (FAQs) in the form of booklet
- LCD in waiting room
- Wall posters

## 5. PROCEDURE

Provide information to increase donor awareness, regarding:

- 5.1 Need for blood.
- 5.2 Need of voluntary donation.
- 5.3 Testing of donated blood for screening of transfusion transmissible infections,
- 5.4 Possible route of transmission (TTI) and prevention.
- 5.5 Need of honest answers for questionnaire.
- 5.6 Safety of blood donor and recipient.
- 5.7 Processing and use of donated blood.
- 5.8 Implication and possible consequences of the donation process.
- 5.9 Self-deferral of (suspected) individuals coming only for testing.
- 5.10 Self-exclusion by knowing high risk behavior.
- 5.11 Provision of the educational material in the waiting area.

5.12 Use of wall posters/LCD (electronically) displaying the information.

## 6. DOCUMENTATION

Request the donors to sign the consent form if they feel they are safe donors and willing to donate.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>DONOR MANAGEMENT DEPARTMENT</b>		
<b>DONOR SELECTION BY INTERVIEW/QUESTIONNAIRE</b>		
<b>BTS/SOP/WP/04</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure is intended for the selection of potential donors through interviews and questionnaire by obtaining relevant and reliable information about the donor's medical history and general health.

## 2. RESPONSIBILITY

The Medical Officer is responsible for conducting interviews and getting the questionnaire duly filled. In addition, an interview may be conducted by specifically trained staff who may ask further direct questions to supplement the information in the questionnaire; the final decision for the selection of donor, however, lies with the medical officer in charge.

## 3. PRINCIPLE

The questions asked through questionnaire and interview serve two purposes: (1) to ensure that the donation of blood or blood components will not compromise the health of the potential donor and (2) to make sure that donated blood will not transmit a disease to the recipient. The donor's past and present health status contribute to this determination process. Based on the answers to the questions in the medical history a donor is accepted, temporarily deferred or permanently deferred.

## 4. MATERIAL REQUIRED

Donor History Questionnaire Form (*cf. Annex 3*)

## 5. PROCEDURE

Potential donors are asked a series of questions related to their medical history. Answers to the questions are recorded as "yes" or "no" with details added if required. Based on the answers to specific questions concerning their medical history, general health, relevant risk factors and travel history, the interviewer determines whether or not the potential donor is eligible to donate blood.

5.1 Ask the donor to fill out the questionnaire.

5.2 Follow deferral criteria, in addition to the permanent and temporary deferral criteria, given below.

a) *The interval between blood donations should not be less than three months for males and four months for females.*

- b) *The donor shall be free from any acute respiratory disease.*  
 c) *The donor shall be free from any skin disease at the site of phlebotomy.*

5.3 Defer the donor permanently in the following conditions.

- Malignant disease/cancer including Polycythemia Vera, Leukemia etc
- Heart and blood vessel, especially coronary disease, angina pectoris, severe cardiac arrhythmia, cerebrovascular disease, arterial thrombosis or recurrent venous thrombosis
- Diabetes, if requiring insulin therapy
- Any history of I/V drug use
- Carriers of HIV,HBV,HCV
- Leishmaniasis
- Hepatitis
- Tuberculosis
- Leprosy
- Schizophrenia
- Epilepsy, convulsions or fainting spells
- Endocrine disorders
- Abnormal bleeding tendencies
- Unexplained weight loss

5.4 Defer the donor temporarily for the period mentioned below.

Conditions	Temporary Deferral
Endoscopy with biopsy by using flexible instruments, inoculation injury, acupuncture, tattooing, or body piercing, mucosal splash with blood, tissue, or cell splash with blood, tissue or cell transplant.	12 months.
Transfusion of blood components.	12 months.
Epilepsy	3 years off treatment and without an attack.
Fever above 38°C, flu like illness.	2 weeks following session of symptoms.
Kidney disease	Acute Glomerulonephritis : 5 years deferral period following complete recovery.
Medication (antibiotics, aspirin and other as mentioned in medication deferral list).	For the period as defined by the medical officer per annex attached to Questionnaire.
Pregnancy	6 months after delivery or termination.
Rheumatic Fever.	2 years following attack with no evidence of chronic heart disease. The latter complication is a cause for permanent deferral.
Surgery	Until fully recovered and fit to be donors, typically about 12 months.
Tooth extraction.	If no complications, one week.
Tropical Diseases	6 months following return from tropical areas.
Malaria.	3 months (endemic). 3 years (non-endemic).
Dengue	6 months
Typhoid Fever	12 months after recovery
Nursing mothers	12 months after delivery

<b>Prophylactic Immunization</b>	
Immunization: Cholera, Typhoid, Diphtheria, Tetanus, Measles, Mumps, Plague, Gammaglobin	2 weeks
Rabies vaccination	1 year after vaccination
Chicken pox vaccination	4 weeks
Hepatitis B Immune globulin	12 months
Hepatitis in family or close contact	12 months
Treated case of syphilis/gonorrhoea	12 months

### 5.5 Interview.

- Conduct a confidential interview to enquire about donors engagement in any risk behavior
- Ask further direct questions to supplement the information in the questionnaire
- Try and identify result seeking donors and refer them to Reference Diagnostic Laboratory
- Reassure the donor for keeping the strict confidentiality of all information received
- Certify the inclusiveness of the relevant questions of verbal assessment on part of the interviewer

## 6. DOCUMENTATION

6.1 Make sure the presence of all the information required in the donor history questionnaire form and donors signature.

6.2 Keep the signed form for record.

6.3 Enter all answers and conclusion (donor accepted/deferred) in BT IS as well.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>DONOR MANAGEMENT DEPARTMENT</b>		
<b>PHYSICAL EXAMINATION</b>		
<b>BTS/SOP/WP/05</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies to all activities that are required to perform a physical examination of the donor for confirming fulfillment of the criteria which ensure safety of the donor as well as the recipient. This SOP also describes the means by which vital signs (e.g. temperature, pulse, and blood pressure) are taken and provides specific guidance on what clinical and physical evidence to look for when screening a donor during the health assessment.

## 2. RESPONSIBILITY

The Medical Officer is responsible to perform the physical examination of the donor. Duty nurse is responsible to record the vitals, i.e. temperature, pulse and blood pressure in addition to measure the weight of the donor

## 3. PRINCIPLE

If a potential donor passes the interview and clears the questionnaire, then he/she undergoes a very limited physical examination. The physical examination is performed and documented to assess a donor for signs suggestive of any risk factor for a relevant communicable disease. Based on the results, it is determined if the donor is eligible for blood donation.

## 4. MATERIAL

- Donor Physical Examination Form (*cf. Annex 2*)
- Donor Card
- Weighing scale
- Sphygmomanometer
- Stethoscope
- Clinical thermometer
- Wrist watch

## 5. PROCEDURE

### Physical Examination:

- Take special note on 'General Appearance' in case of plethora, poor physique, debilitation, under nutrition, anemia, jaundice, cyanosis, dyspnoea, mental instability, intoxication from alcohol or drugs.

- Defer a donor who appears ill, under the influence of drugs/alcohol or do not appear to be providing reliable answers to medical history.
- Examine the skin near venipuncture site on both arms.
- Note down signs of needle marks and sclerotic veins on arms indicative of intravenous drug use.
- Defer a donor with boils, purulent wounds or severe skin infections anywhere on the body.

Check and enter donor's weight:

- The weight should be >50 kg to collect 450 ml and between 45 and 50 kg to collect 350 ml blood.

Check the Temperature:

- Place an oral thermometer (that has been disinfected in isopropyl alcohol, covered with a “barrier” wrap, and then shaken so as to prevent a falsely elevated reading) under the donor’s tongue for a minimum of 120 seconds.
- Remove and then read thermometer.
- *Acceptable* = < 98.6° F or < 37.5° C.

Record the pulse rate

- Place the fingertips of your index, middle, and ring fingers against the radial artery over the donor’s wrist, making certain that you can easily feel the pulse
- Count the number of beats over a 30 seconds period. Multiply the beats with “2” to determine the number of beats per minute.
- If any skipped beats are appreciated, feel pulse for an additional 60 seconds and count the number of skipped beats that occur over this duration.
- *Acceptable* = < 100 or > 60 regular beats/minute.

Record the blood pressure

- Using a stethoscope and blood pressure cuff positioned over the donor’s brachial artery, take his/her blood pressure.
- *Acceptable* = between 100 - 140 mm Hg (systolic) and between 70 - 90 mm Hg (diastolic).

**6. DOCUMENTATION**

Enter the details of all parameters in the donor physical examination form/donor record register in BT IS.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

# ANNEXES

## Annex 1

### DONOR REGISTRATION FORM

Donor Registration No: \_\_\_\_\_ Visit Date: \_\_\_\_\_

Donor's Full Name: \_\_\_\_\_ S/O, D/O \_\_\_\_\_

Donor's Sex: \_\_\_\_\_ M/F

Age/DOB: \_\_\_\_\_ (18 yrs - 60 yrs)

CNIC No. 

					--							--	
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Home Address: \_\_\_\_\_

\_\_\_\_\_ Home Tel. No: \_\_\_\_\_

Office Address: \_\_\_\_\_

\_\_\_\_\_ Office Tel. No: \_\_\_\_\_

Occupation/Hobby: \_\_\_\_\_

Email Address: \_\_\_\_\_

Blood Group if known: \_\_\_\_\_

Have you donated before Yes/ No.: \_\_\_\_\_ When: \_\_\_\_\_ Where: \_\_\_\_\_

No. of donations: \_\_\_\_\_

Date of next eligibility of donation: (After 12 weeks) \_\_\_\_\_

Attending Nurse Signature: \_\_\_\_\_

**Annex 2**

**DONOR PHYSICAL EXAMINATION FORM**

**Donor Appearance and Inspection**

**YES NO**

Take special note in case of;

Plethora \_\_\_\_\_

Poor Physique \_\_\_\_\_

Debilitation \_\_\_\_\_

Under-nutrition \_\_\_\_\_

Anemia \_\_\_\_\_

Jaundice \_\_\_\_\_

Cyanosis \_\_\_\_\_

Dyspnoea \_\_\_\_\_

Mental instability \_\_\_\_\_

Intoxication from alcohol or drugs \_\_\_\_\_


**Skin Examination:**

Any signs of needle marks and sclerotic veins on arms indicative of intravenous drug use. \_\_\_\_\_

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Presence of boils, purulent wounds or severe skin infections anywhere on the body. \_\_\_\_\_

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**Vital Signs:**

Pulse: \_\_\_\_\_ (Regular between 60-100 beats/minute)

Body Temperature: \_\_\_\_\_ (< 37.5° C or <98.6° F)

Blood Pressure: \_\_\_\_\_ (Systolic 100 -140 mm of Hg, Diastolic 70 - 90 mm of Hg)

Haemoglobin: \_\_\_\_\_ (>12.5gm/dl Female, >13.5gm/dl Male)

Weight: \_\_\_\_\_ (>50 Kg)

Attending Medical Officer's Signature: \_\_\_\_\_

## Annex 3

### BLOOD DONOR EDUCATIONAL MATERIAL

#### Making Your Blood Donation Safe

Thank you for coming today! This information sheet explains how YOU can help us make the donation process safe for yourself and patients who might receive your blood.

#### **PLEASE READ THIS INFORMATION**

**BEFORE YOU DONATE!** If you have any questions now or anytime during the screening process, please ask blood center staff.

#### **ACCURACY AND HONESTY ARE ESSENTIAL!**

Your **complete honesty** in answering all questions is very important for the safety of patients who receive your blood.

**All information you provide will be confidential.**

#### **DONATION PROCESS: To determine if you are eligible to donate we will:**

- Ask questions about health, travel, and medicines
- Ask questions to see if you might be at risk for hepatitis, HIV, or AIDS
- Take your blood pressure, temperature and pulse
- Take a small blood sample to make sure you are not anemic

#### **If you are able to donate we will:**

- Cleanse your arm with an antiseptic. **(If you are allergic to iodine, please tell us!)**
- Use a new, sterile, disposable needle to collect your blood

#### **SPECIFIC INFORMATION ABOUT HIGH RISK BEHAVIORS**

##### **Why we ask questions about sexual contact:**

Sexual contact may cause contagious diseases like HIV and Hepatitis to get into the bloodstream and be spread through transfusions to someone else.

AIDS is caused by HIV. HIV is spread mainly through sexual contact with an infected person OR by sharing needles or syringes used for injecting drugs.

**DO NOT DONATE IF YOU:**

- Ever have had AIDS or positive HIV test
- Have ever used needles to take drugs, steroids, or anything not prescribed by your doctor
- You are male and are homosexual.
- Have ever taken money, drugs or other payment for sex.
- Have had sexual contact in the past 12 months with anyone described above
- Have had syphilis or gonorrhea in the past 12 months
- In the last 12 months have been in juvenile detention, lockup, jail or imprison for more than 72 hours
- Have any of the following conditions that can be signs or symptoms of HIV/AIDS:
  - Unexplained weight loss or night sweats
  - Blue or purple spots in your mouth or skin
  - Swollen lymph nodes for more than one month
  - White spots or unusual sores in your mouth
  - Cough that won't go away or shortness of breath
  - Diarrhea that won't go away
  - Fever of more than 100.5°F for more than 10 days

Remember that you CAN give HIV to someone else through blood transfusions even if you feel well and have a negative HIV test. This is because tests cannot detect infections for a period of time after a person is exposed to HIV. **If you think you may be at risk for HIV/AIDS or want an HIV/AIDS test, please ask for information about other testing facilities.**

***PLEASE DO NOT DONATE FOR HIV TESTING!***

**Travel to or birth in other countries**

Blood donor tests may not be available for some contagious diseases that are found only in certain countries. If you were born in, have lived in, or visited certain countries, you may not be eligible to donate.

**What happens after your donation:**

To protect patients, your blood is tested for hepatitis B and C, HIV, Malaria, and Syphilis. If your blood tests positive it will not be given to a patient. You will be notified about test results that may disqualify you from donating in the future.

**Thank you for donating blood today!** (Regional Blood Center's Name)

Donor's Name: \_\_\_\_\_ Donor's Signature: \_\_\_\_\_

## Annex 4

### DONOR HISTORY QUESTIONNAIRE FORM For medical history and general health

	YES	NO
<b>Are you</b>		
1. Feeling healthy and well today?		
2. Currently taking an antibiotic or any other medication for an infection?		
3. Have you taken aspirin or anything that has aspirin in it?		
4. Currently taking or have you ever taken any medications mentioned on the Medication Deferral List? ( <i>Cf. annex</i> )		
5. Have you read and understood the educational material on donation process and information on AIDS (HIV infection) and Hepatitis?		
<b>In the past 6 weeks</b>		
6. Female donors: Have you been pregnant or are you pregnant now?		
<b>In the past 8 weeks H/O</b>		
7. Donated blood, platelets or plasma?		
8. Vaccinations or other shots?		
9. Dental treatment or tooth extraction?		
10. Allergies or rashes		
<b>In the past 16 weeks</b>		
11. Have you donated a double unit of red cells using an aphaeresis machine?		
12. Unexplained weight loss		
<b>In the past 12 months have you had</b>		
13. Blood Transfusion?		
14. Serious illness or an operation		
15. Transplant such as organ, tissue, or bone marrow?		
16. Graft such as bone or skin?		
17. An accidental needle-stick?		
18. Ear or body piercing?		
19. A tattoo?		
<b>High risk behavior/unusual sexual practices</b>		
20. Sexual contact with someone who		
• Is HIV positive or has hepatitis?		
• Receives or has received payment for sex in money or drugs?		
• Has injected drugs?		
21. For women: has any man with whom you have had sex in the past 12 months had sex with a man?		

22. For men: have you ever had sex with another man?		
23. Have you ever treated for syphilis or gonorrhoea?		
<b>Have you ever had</b>		
24. A positive test for the HIV/AIDS virus?		
25. Used needles to take drugs, steroid, or anything not prescribed by your doctor?		
26. Hepatitis/Jaundice?		
27. Sexually transmitted disease e.g. Syphilis		
28. Tuberculosis?		
29. Typhoid fever?		
30. Rheumatic fever?		
31. Any heart disease/hypertension?		
32. Asthma?		
33. Persistent cough and chest pain?		
34. Any type of cancer, including Leukemia?		
35. Bleeding condition or a blood disease?		
36. Epilepsy?		
37. Diabetes?		
38. Malaria/Dengue?		
39. Leishmaniasis?		
40. Any foreign visit in recent past?		
41. Any history of imprisonment?		

<b>MEDICATION DEFERRAL LIST</b>			
<b>Generic Names</b>	<b>Example</b>	<b>Usually given for</b>	<b>Deferral Period</b>
1 Isotretinoin	Accutane R	Severe acne	1 month
2 Etreinate	Tegison R	Severe psoriasis	permanently
3 Acitretin	Neotigason R	Severe psoriasis	3 years
4 Finasteride	Proscar R, Propecia R	Prostate gland enlargement	1 month
5 Dutasteride	Avodart R	Prostate enlargement	6 months

## Donor Consent Form

*"I have been made to understand that I should not donate blood if I am involved in altered sexual behaviour or I/V drug use.*

*I am donating blood by my own will for use by Blood Center XXX. I provided the information and it is correct to the best of my knowledge. I am aware of the fact that my blood will be tested for Hepatitis B, C, Human Immunodeficiency Virus (HIV/AIDS), Malarial Parasite (MP), Syphilis and also know about any risk/side effects of blood donation like vasovagal syndrome, convulsions, vomiting, haematoma, muscular spasm, local allergic reactions, etc. I will not be entitled to claim any exchange for my donation."*

Donor's Name:

Donor's Signature:

Date:

Attending MO's Signature:

**Annex 5**

**DONOR RECORD REGISTER**

Blood Bag No.	Donor's Name	Donor's Unique ID	Donor's Blood Group	Donor's Age/Sex	Donor's Hb/B.P	Date and Time of donation	Donor's address & Tel. No.	Donor's Screening			Post donation Adverse reaction	Remarks/ Signature
								HBs Ag	HCV Ab	HIV Ab		

# **Collection of Donation**

# Standard Operating Procedures

## COLLECTION OF DONATION

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# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
INSPECTION OF BLOOD BAGS AND LABELING		
BTS/SOP/WP/06	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Robust labels of bags are required to allow full traceability or tracking of blood from the donor to the final recipient. This SOP focuses on the first step for labeling that need to survive multiple processing, testing, and storage steps through challenging environmental conditions. All labeling steps must be performed with precise attention to details. Mixing up two or more units with one another potentially can lead to serious consequences like ABO incompatible transfusion reaction to the recipient.

## 2. RESPONSIBILITY

It is the responsibility of the phlebotomist or technician from the donor management area to label the blood bags with initial data.

## 3. PRINCIPLE

Labeling of blood units is a three-step process. The first step, performed by the phlebotomist, involves the appropriate labeling of the primary collection bag. The second step, performed by the laboratory staff of Immunohaematology Department, Component Preparation Department and TTI Screening Lab, occurs after the blood is tested, and which involves (among other processes) demonstrating the ABO/Rh type of the donor, the expiration dates of the components and TTI results. Finally, the third step, also performed by the laboratory staff, is done when the blood is ordered for transfusion. It involves compatibility testing results and recipient ID..

## 4. MATERIAL

- Triple blood bag with tubing having unique printed segment number for each unit
- Donor base label
- The name of manufacturer and unique identification number of bag with lot number
- Name, composition and volume of the anticoagulant
- The required temperature for storage
- Adhesive barcode labels for unique identification of donor, and production facility, i.e. RBC-ICT, ABO Blood Group, Rh Type printed as per regulatory requirement
- Labels for one red-top and one EDTA test tube—each 7 ml

## 5. PROCEDURE

- 5.1 Select triple bag with Donor base labels as “CPDA-1 WHOLE BLOOD”.
- 5.2 Inspect the empty bag and contents visually for any signs of deterioration or damage. (In case of puncture or dis-colouration, do not use it).
- 5.3 Check the expiry date of the bag.
- 5.4 Check *Donor base label already in place* onto the primary collection bag, prior to collection of blood.
- 5.5 Attach adhesive barcode label or manual labeling for identification of donor on top of the donor base label. (Care must be taken to ensure that this barcode number has never been used before by the blood center).
- 5.6 Write down the collection date onto the donor base label.
- 5.7 Finally, check the unique identification number of bag on to the collection tubes, which are attached to the secondary collection bags.
- 5.8 At this time, the blood bags are ready for the blood collection process.
- 5.9 Write down date and time of donation on its completion.
- 5.10 Label sampling test tubes which are used for blood grouping and TTI screening.

## 6. DOCUMENTATION

Make sure the bag labeling on first step according to following checklist:

- a) *Type of bag*
- b) *Manufacturer's name*
- c) *Bag lot No*
- d) *Donor's Unique ID*
- e) *Donor's blood group (after confirming result from immunohaematology)*
- f) *Date and time of donation*
- g) *Donation number*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
PREPARATION OF VENIPUNCTURE SITE		
<b>BTS/SOP/WP/07</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Cases of transmission of bacterial infection in blood are fortunately rare, but when they do occur can be fatal. This SOP describes the steps required for careful preparation of the skin at the phlebotomy site before venipuncture.

## 2. RESPONSIBILITY

The phlebotomist collecting the blood from the donor is responsible for preparation of phlebotomy site.

## 3. PRINCIPLE

Although it is impossible to guarantee 100% sterility of the skin surface for phlebotomy, a strict, standardized procedure for the preparation of the phlebotomy area must exist. Iodophor compounds, or other sterilizing compounds, are used to sterilize the venipuncture site before blood collection.

## 4. MATERIAL

- Sterilising tray
- Demethylated Spirit
- 10% Povidone- Iodine
- Cotton/swabs
- Blood pressure cuff
- Sterile gauze

## 5. PROCEDURE

- 5.1 Make the donor lie down with a pillow under the head or recline in a comfortable donor chair. Ask the donor if he/she is in a comfortable position.
- 5.2 Identify the donor by name.
- 5.3 Enter the bag number on the Donor Record Register.
- 5.4 Identify venipuncture site free from scar or skin lesions.
- 5.5 Apply blood pressure cuff to arm, immediately above the ante-cubital fossa and inflate up to a pressure of between 40 and 60mm of Hg to enlarge the vein; ask donor to open and close hand several times in order to make the vein more prominent.
- 5.6 Select and palpate the vein in the ante-cubital fossa for venipuncture; then release the cuff. Scrub area at least 4 cm (1.5 inches) in all directions from the intended site of

venipuncture (i.e. 8 cm or 3 inches in diameter) for a minimum of 30 seconds with povidine-iodine compound.

- 5.7 Starting at the intended site of venipuncture and moving outward in a concentric spiral, apply spirit swab; let stand for 30 seconds.
- 5.8 Allow the disinfected venipuncture site to air dry completely. Do not blow on it.
- 5.9 Do not touch the prepared area before the needle has been inserted.
- 5.10 Do not repalpate the vein at the intended venipuncture site.
- 5.11 Cover the area with dry, sterile gauze until the time of venipuncture.
- 5.12 Dispose of used swab(s) into a waste bin meant for bio-hazardous material.
- 5.13 Notes.
- 5.14 For donors sensitive to iodine (tincture or povidone preparations), another method (e.g, Chlora Prep 2% chlorhexidine and 70% isopropyl alcohol) should be designated by the blood bank physician.
- 5.15 For donors sensitive to both iodine and chlorhexidine, a method using only isopropyl alcohol could be considered.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
PHLEBOTOMY AND COLLECTION OF WHOLE BLOOD DONATION		
BTS/SOP/WP/08	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This describes a procedure for blood collection from the donor that has been proven eligible to donate, using an aseptic method. Blood is collected in a sterile closed system bag with a single venipuncture. A correct performance of venipuncture is essential for the quality and safety of the blood donation. Successful venipuncture 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 thus encouraging re-attendance.

## 2. RESPONSIBILITY

The phlebotomist is responsible for blood collection from the donor after verifying the donor screening details, checking the unit number labels and preparing the phlebotomy site. The Medical Officer Incharge is responsible to supervise the whole procedure.

## 3. PRINCIPLE

The collection of whole blood is the key, first step that enables us to produce a variety of life-saving components, such as red blood cells, fresh frozen plasma, and platelet concentrates.

## 4. MATERIAL

- Povidine-iodine solution
- Blood collecting triple bag
- Hand sealers
- Blood Mixer with Automatic Balance system to monitor volume of blood drawn
- Sterile gauze and haemostats, and forceps
- One (1) red-top and one (1) EDTA- test tube (7 ml each)
- Blood tubing stripping device
- Adhesive Tape
- Sharp container
- Blood pressure cuff or Tourniquet
- Comfortable donor couch or chair
- Tennis ball
- SOP for preparation of venipuncture site
- SOP for Inspection and labeling of bag.

## 5. PROCEDURE

- 5.1 Make the donor lie down with a pillow under the head or recline in a comfortable donor chair. Ask the donor if he/she is in a comfortable position.
- 5.2 Give the donor a hand roller/squeezer to hold.
- 5.3 Prepare the venipuncture site. (**SOP/WP/07**)
- 5.4 Cover the area with dry, sterile gauze until the time of venipuncture (*after the skin has been prepared, it must not be touched again*)
- 5.5 Inspect and label the bag. (**SOP/WP/06**)
- 5.6 Position bag below level of donor's arm.
- 5.7 Adjust the balance for the required volume of blood to be drawn (350/450ml) in addition to combined weight of bag and anticoagulant and place the bag on it.
- 5.8 Apply a haemostat to tubing before needle is uncapped to prevent air from entering line.
- 5.9 Reapply tourniquet or inflate blood pressure cuff. Have donor open and close hand until previously selected vein is again prominent.
- 5.10 Uncover sterile needle and Keep the bevel of the needle facing upward and the shaft at an angle of 15° to the arm.
- 5.11 Once the needle is in the vein beneath the skin, release the haemostat. Insert the needle into the vein for about 1 to 1.5 cm by a bold single prick to ensure smooth flow of blood.
- 5.12 Secure the needle in place by applying adhesive tape on the tubing to the donor's arm and cover site with sterile gauze.
- 5.13 Advise the donor open and close hand or squeeze the tennis ball every 10-12 seconds during collection to improve the blood flow.
- 5.14 Once blood enters the bag tubing, press the blood mixer 'start' switch to allow the blood to flow into the bag.
- 5.15 Make certain that the automatic mixer/balance is working properly, and be sure that blood flow is relatively brisk (collection should be complete within 10 minutes)
- 5.16 When appropriate/programmed amount of blood has been collected, the balance/mixer automatically interrupts blood flow by clamping of tubing. (nevertheless, carefully monitor the collection to be certain that donor is not overdrawn)
- 5.17 Keep the donor under observation throughout the donation process. The donor never should be left unattended during or immediately after the donation.
- 5.18 When blood draw is complete, clamp tubing near venipuncture site using a haemostat or other temporary clamp.
- 5.19 Deflate cuff; remove tourniquet; and remove needle gently from the donor's arm, pressing the phlebotomy site with a sterilize guaze.
- 5.20 Apply pressure over gauze and, with one hand, help donor raise arm straight up, holding gauze firmly over phlebotomy site with other hand.
- 5.21 Take the test samples directly from the bleed line or from sample Pouch (deviation bag) of the collecting system.
- 5.22 Discard needle assembly into biohazard container designed to prevent accidental needle-sticks.
- 5.23 Seal the blood bag tubing with the tube sealer.
- 5.24 Invert bag several times to mix thoroughly.
- 5.25 Allow blood collecting tubing to refill with anticoagulated blood from the bag.
- 5.26 Using the hand sealer, seal tubing attached to collection bag into segments, leaving each segment number clearly and completely readable.
- 5.27 Recheck donor identification number, donation number on bag, processing laboratory sample tubes, donation record, and retention segment—Make certain they all match.
- 5.28 Place the blood bags into controlled temperature storage and transport to the processing site under temperature conditions appropriate for the component that will be prepared (**SOP/WP/31-32-33**).

5.29 For instructions on attending the donor (cf. *Post-donation Care SOP-11*).

## 6. DOCUMENTATION

Record the following on the Bag labeling form/ Donor Record Register/BT IS

- a) Blood bag type
- b) Blood bag supplier
- c) Blood bag lot
- d) Whether or not arm prep was done
- e) Time at start of venipuncture
- f) Time at stop of venipuncture
- g) Gross weight of unit
- h) Initials of person doing venipuncture in the appropriate box

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
COLLECTION OF BLOOD COMPONENTS THROUGH APHRAESIS		
BTS/SOP/WP/09	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This describes a procedure for collection of individual blood components.

## 2. RESPONSIBILITY

Trained clinician/Medical Technologist is responsible for performing the aphaeresis procedure on the donor under the supervision of a consultant.

## 3. PRINCIPLE

Depending on the substance that is being removed, different processes are employed in aphaeresis. Centrifugation is the most common method.

### **Continuous flow centrifugation (CFC):**

Historically required two venipuncture as the "continuous" means the blood is collected, spun, and returned simultaneously. Newer systems can use a single venipuncture.

### **Intermittent flow centrifugation:**

Intermittent flow centrifugation works in cycles, taking blood, spinning/processing it and then giving back the necessary parts to the donor in a bolus. The main advantage is a single venipuncture site. To stop the blood from coagulating, anticoagulant is automatically mixed with the blood as it is pumped from the body into the aphaeresis machine.

The main categories of component collections are:

Plasmapheresis is useful in collecting FFP (fresh frozen plasma)

Erythrocytapheresis is the separation of erythrocytes from whole blood

Plateletapheresis is the collection of platelets by aphaeresis

Leukapheresis is the removal of neutrophils, basophils, eosinophils for transfusion

The blood flows through a needle into a machine that contains a sterile, disposable plastic kit specifically designed for this purpose. The platelets are isolated and channeled out into a special bag, and red blood cells and other parts of the blood are returned to donor through a needle in the opposite arm. It is thus an extracorporeal therapy.

## 4. MATERIAL

- Aphaeresis test kit
- ACD-A (anticoagulant)
- Normal saline

- Spirit Swabs
- Adhesive bandage

## 5. PROCEDURE

- 5.1 Adjust the aphaeresis set and install the kit in the machine.
- 5.2 Programme the equipment and enter the following values before separation starts:
- 5.3 Weight, Height, Sex, PCV (Pre), Platelet Count (Pre)
- 5.4 Place a needle in each arm. The blood will flow through the needle into the chamber of the equipment at a low speed (1000 rpm). The centrifuge speed is increased when the interface detector detects the blood in the separation chamber.
- 5.5 The machine stops the separation process once the target volumes (pre-selected) have been achieved.
- 5.6 Disconnect the inlet line and add saline and ACD-A to displace the blood from the line set and to return the blood to the donor via the return line (Reinfusion).
- 5.7 De-airate the concentrate bags by pressing excessive air into the PC sample collection bag.
- 5.8 Thoroughly mix the concentrate in the open concentrate bag.
- 5.9 Disconnect the concentrate bag from the set and keep the concentrate on an agitator for one hour.
- 5.10 Place the blood bags into controlled temperature storage and transport to the processing site under temperature conditions appropriate for the component that will be prepared (*cf. SOP/WP/31-32-33*).
- 5.11 For instructions on attending the donor (*cf. Post-donation Care SOP-11*).

## 6. DOCUMENTATION

Record the following on the Bag labeling form/ Donor Record Register/BT IS

- a) Blood bag type
- b) Blood bag supplier
- c) Blood bag lot
- d) Whether or not arm prep was done
- e) Time at start of venipuncture
- f) Time at stop of venipuncture
- g) Gross weight of unit
- h) Initials of person doing venipuncture in the appropriate box

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>COLLECTION OF DONATION</b>		
<b>COLLECTION OF BLOOD SAMPLES</b>		
<b>BTS/SOP/WP/10</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This describes a procedure for blood sample collection from the blood bag for the screening of Transfusion Transmitted Infections and blood grouping.

## 2. RESPONSIBILITY

The phlebotomist is responsible for blood sample collection from the blood bag. The Medical Officer In charge is responsible to supervise the whole procedure.

## 3. PRINCIPLE

The blood sample collection from the donor's blood bag helps to ascertain the TTI screening status and the blood group type of the donor.

## 4. MATERIAL

- One (1) red-top and one (1) EDTA- test tube (7 ml each)
- Spirit Swab

## 5. PROCEDURE

- 5.1 During the phlebotomy procedure, take the test samples directly from the bleed line or from sample pouch (deviation bag) of the collecting system.
- 5.2 Centrifuge the sample and send to TTI screening laboratory and Immunohaematology Department or place at refrigeration temperature.

## 6. DOCUMENTATION

Record the following on the sample tube

- Blood bag type*
- Blood bag Number*
- Donor's unique ID*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
POST-DONATION CARE/REFRESHMENT		
BTS/SOP/WP/11	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This SOP includes all the steps necessary for ensuring the safety of donor post-collection, immediately recognizing any post-donation adverse effects/events and making the donation experience a positive one for the donor.

## 2. RESPONSIBILITY

The medical officer in donor management area attends to the donor.

## 3. PRINCIPLE

The donor needs to be observed after blood collection.

This is necessary in order to:

- Recognize any adverse effects in the immediate post-donation period.
- Make the donation experience enjoyable enough for the donor to return for donation

## 4. MATERIAL

- Sterile swabs
- Adhesive tap
- Post-Donation Instructions Leaflet
- Refreshments (e.g. Tea, Juice, Coffee etc.)

## 5. PROCEDURE

- 5.1 After completing the phlebotomy apply firm pressure with sterile gauze over the entry point of the needle into the vein for several seconds (continuing to hold the donor's arm straight up into the air).
- 5.2 Next, instruct the donor to continue holding his arm straight up while applying pressure over the veni-puncture site (for at least 60 seconds).
- 5.3 To prevent adverse reactions like giddiness ask the donor remain reclining in the donor chair for a few (5) minutes under close observation of the staff, even he feels perfectly all right.
- 5.4 When the donor's condition appears satisfactory, have him sit up, under observation, and follow him to the observation/refreshment area.

- 5.5 Instruct donor that he/she is to remain in this area for at least 10-15 minutes during which let him to enjoy any drink and food like tea, coffee, juice, or any other refreshments that he can drink/eat.
- 5.6 Inspect the venipuncture site before the donor leaves the donation room and give donor the following written and verbal instructions about post-donation care.
- 5.7 Eat/drink something before leaving.
- 5.8 Do not leave until released by a staff member.
- 5.9 Drink extra fluids over the next 24 hours.
- 5.10 Avoid alcohol until the following day.
- 5.11 Do not drive a car for 2 hours.
- 5.12 Do not operate heavy machine and avoid strenuous activities at least on the day of donation.
- 5.13 Do not smoke for at least half an hour.
- 5.14 If there is bleeding from phlebotomy site, raise arm and apply pressure.
- 5.15 If fainting or dizziness occurs, either lie down or sit with head between knees.
- 5.16 If any symptoms persist, either telephone or return to the Blood Center to see a doctor.
- 5.17 Remove bandage after 2 hours.
- 5.18 Thank donor for his important contribution and encourage repeat donation (after 8 weeks, of course).

## 6. DOCUMENTATION

Give a leaflet of post-donation instructions to the donor.

Record if any adverse reaction is noticed.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION		
MANAGEMENT OF ADVERSE EFFECTS		
BTS/SOP/WP/12	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Any adverse reaction in the immediate post-donation period requires to be attended. The source of the adverse reaction is identified and corrective and preventive measures considered.

## 2. PRINCIPLE

Some untoward reactions occur in less than 1 percent donations. The most frequent type of reaction is simple faint. There is usually a feeling of dizziness and light headedness, often accompanied by tingling of fingers, and a cold and clammy feeling in the palms. there may be grayish pallor of the donors face with beads of sweat appearing on the upper lip. The then may lose consciousness and his/her B.P may fall to 50-60 mm of Hg systolic and pulse rate slow down to 40-60 /min. In about one third of severe reactions, there may be vomiting, increased neuromuscular excitability (fits/convulsions), and hyperventilation leading to tetany.

## 3. RESPONSIBILITY

The medical officer in attendance is responsible for managing all the adverse effects in the donor.

The Phlebotomist is responsible to follow these instructions:

- Give prior information to the prospective donors about the possible adverse reactions of blood donation and their prevention.
- Be attentive for early signs of an adverse reaction.
- Reassure and keep the donor relax and busy in pleasant conversation.
- Respond immediately with the appropriate action.
- Observe the donor until full recovery.
- Inform medical officer about serious adverse reactions.

## 4. MATERIAL

The following material are required for the attention of any emergency arising in the post donation period.

- Epinephrine (Adrenaline)
- Atropine sulphate

- Pheniramine maleate
- Glucocorticosteroid
- Glucose (Dextrose 25%)
- Calcium gluconate
- Sodium bicarbonate
- Injectable Antiemetic
- I/V Infusion 500/1000ml: 5% Dextrose Saline or 0.9% Normal Saline
- Plasma expander

#### Antiseptics

- Savlon
- Pyodine
- 70% alcohol

#### Miscellaneous

- Bandages/Dressing kit
- paper tape
- Band-aids
- Anti-histaminic Cream.
- Analgesic balm
- Tongue depressor and airway tube
- Disposable syringes
- I/V cannulas 18-20G with heparin lock
- Oxygen cylinder with flow meter and oxygen mask
- Infusion set
- Paper bag/polythene bags
- Ice Packs

## **5. PROCEDURE**

### **5.1 MANAGEMENT OF ADVERSE REACTIONS**

#### Vasovagal Reaction (Immediate/Delayed type)

- Make the donor lie down in the bed in a relaxing position
- Raise feet and lower head end
- Loosen tight clothing (belt, tie etc.)
- Ensure adequate airway
- Check vitals e.g. pulse and blood pressure
- Inform the donor about the risk of delayed fainting
- Inform such donor not to drive a vehicle or resume work or any hazardous occupation hobby in the ensuing 12 hours

#### Convulsions

- Keep the head tilted to the side
- Prevent the tongue bite
- Keep the airway patent by inserting a tongue depressor or gauze between the teeth.
- Medical management

#### Vomiting

- Usually subsides on its own
- If vomiting is severe, inject appropriate antiemetic

#### Tetany muscular spasm/twitching

- These are usually due to hyperventilation in an apprehensive donor

- Ask the donor to breath in and out into a paper bag, which provides prompt relief

Haematoma

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

Local Allergic reactions:

Apply steroid ointment.

Severe Reaction

- The donor may need oxygen inhalation and various injectables like anti-emetics, barbiturates, vasopressor agents, coronary vasodilators, bronchodilators or anti-histamines
- If there is bradycardia and hypotension
- Administer inj. Atropine 1 ml IM, if bradycardia continues for more than 20 minutes
- Administer IV Infusion of 0.9% Normal Saline or 5% Dextrose Saline infusions if hypotension is prolonged
- In a very severe reaction the donor may be shifted to close by hospital for further management

**6. DOCUMENTATION**

Record the treatment and outcome according to *BTS/SOP/WP/13*.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>COLLECTION OF DONATION</b>		
<b>DOCUMENTATION OF ADVERSE EFFECTS</b>		
<b>BTS/SOP/WP/13</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Any adverse reaction in the immediate post-donation period requires to be documented.

## 2. RESPONSIBILITY

The medical officer in attendance is responsible for documenting the adverse reaction in the donor record register.

## 3. MATERIAL

BT Information System with relevant reporting system

## 4. PROCEDURE

- 4.1 Note the type of reaction along with the donor unique identification number.
- 4.2 Record the treatment and outcome of all adverse reactions related to blood donation at any stage of the procedure in a register and BT IS.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

## **ANNEXES**

### **POST- DONATION CARE INSTRUCTIONS**

- a) *Eat/drink something before leaving.*
- b) *Do not leave until released by a staff member.*
- c) *Drink extra fluids over the next 24 hours.*
- d) *Avoid alcohol beverages until the following day.*
- e) *Do not operate a heavy machine and avoid strenuous activities at least on the day of donation.*
- f) *Remove bandage after 2 hours.*
- g) *If there is bleeding from the phlebotomy site, raise your arm and apply pressure.*
- h) *If fainting or dizziness occurs, either lie down or sit with your head between your knees.*
- i) *If any symptoms persist, either telephone or return to the Blood Center to see a doctor.*

# **TTI Screening Laboratory**

# Standard Operating Procedures

## TTI SCREENING

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# STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>RECEPTION OF BLOOD SAMPLES</b>		
<b>BTS/SOP/WP/14</b>	<b>REGIONAL BLOOD CENTRE</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure ensures that the donor blood samples received are correct/properly labeled, documented and visually inspected for signs of haemolysis.

## 2. RESPONSIBILITY

It is the responsibility of technician working in the TTI screening laboratory on sample receiving desk to ensure correct/properly labeled samples with proper ID are received from donation collection area.

## 3. MATERIAL

- Blood sample with donor ID
- Sample record entry register

## 4. PROCEDURE

IMPORTANT: Check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 1 Check the label on the blood samples for the donor identification number.
- 2 Inspect the blood sample for any sign of haemolysis.
- 3 Any sample showing improper labeling or any sign of deterioration is not accepted.
- 4 Record the sample with ID in a separate entry register.
- 5 Centrifuge the sample and place it in the refrigerator till processing

## 5. DOCUMENTATION

Maintain the record of every sample received in TTI Screening lab in a separate entry register and initial with date and time on the form at receiver's column.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

## STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>HBs Ag TESTING BY ELISA</b>		
<b>BTS/SOP/TP/15a</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	<b>Effective Date:</b>	<b>Review Period:1 Year</b>

### 1. SCOPE AND APPLICATION

HBsAg is a mandatory test for blood unit screening before it is transfused. This is carried out on all donor units' samples. The enzyme-linked immunosorbent assay (EIA or ELISA) is the test of choice for screening donor blood for HBsAg.

### 2. RESPONSIBILITY

It is the responsibility of technician from TTI screening lab to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

### 3. PRINCIPLE

In this procedure microtest plate wells are coated with monoclonal antibody to Hepatitis B Surface Antigen (Anti-HBs) are incubated with serum or plasma and Anti-HBs peroxidase (Horse radish) conjugate in one step assay. During the incubation period HBs Ag, if present, is bound to the conjugate (Anti-HBs-HRPO).

Unbound material is aspirated and washed away. On the addition of substrate colour develops in proportion to the amount of HBsAg which is bound. The enzyme reaction is stopped by the addition of stopping solution.

### 4. MATERIAL

- HBs Ag Test Kit
- Elisa Washer
- Micro-shaker
- Elisa Reader
- Micropipettes and disposable tips
- Disposable gloves
- Disposal container with Na Hypochlorite
- Timer
- Incubator
- Distilled water
- N/10 Sulphuric Acid (H<sub>2</sub>SO<sub>4</sub>)

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 The provided micro plate has 12 columns (strips) and 8 rows (A-H).
- 5.2 Bring reagents and samples to room temperature 15 minutes before the testing.
- 5.3 Arrange all donor unit test tube samples, apharesis samples, serially in ascending order in a test tube rack.
- 5.4 Make a summary sheet of the micro plate.
- 5.5 A1 is for Blank, B1, C1, D1 are for Negative Controls, E1, F1 are for Positive Controls.
- 5.6 G1 onwards are test samples.
- 5.7 Add 100 µl sample & control (positive & negative) using micropipette and fresh disposable tip for every sample.
- 5.8 Seal plate with the adhesive tape (provided with kit).
- 5.9 Incubate at 37<sup>o</sup>C for 60 minutes.(1hr)
- 5.10 Wash plate 5 times with washing buffer solution.(to be diluted 1/10 in distilled water, i.e. 100 ml washing solution in 1000 ml distilled water)
- 5.11 Concentrate Conjugate to be diluted 1/50 with conjugate diluents, i.e. 20 µl concentrate conjugate in 1 ml conjugate diluents conjugate.(For 1 strip -----1 ml diluents conjugate, similarly in 6 ml diluents conjugate +120 µconcentrate conjugate).
- 5.12 Add 100µl of diluted Conjugate in each well, except the well for Blank, and seal.
- 5.13 Incubate at 37<sup>o</sup>C for 30 minutes.
- 5.14 Wash plate 5 times with washing solution.
- 5.15 Make substrate reagent (1ml/strip, add 20µl/ml conc. substrate with diluting solution).
- 5.16 Add 100µl of above in each well including well for blank.
- 5.17 Incubate at 22<sup>o</sup>C for 30 minutes (in dark at the bench)
- 5.18 Colour develops. Add 100 µl stopping solution (N/10 H<sub>2</sub>SO<sub>4</sub>) to each well mix gently on the microwell shaker and wait for 3-4 minutes.
- 5.19 Interpret the results using semiautomatic plate reader.
- 5.20 Handle the microtest plates as infectious material and dispose them accordingly.  
Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

Final results are displayed on the screen

The samples below the cut off value are considered NON-REACTIVE

Above cut off value are considered REACTIVE

Equal to cut off value/showing grey zone are considered BORDERLINE

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000rpm for 10 minute. Do retesting with the same and another technique. (use RCF, that's equipment independent)

## 7. DOCUMENTATION

Enter the results in the donor screening test results area in the Blood Transfusion Information System.

In case of equipment interfacing, results are transferred automatically.

Record the following details in HBs Ag testing section of the TTI Laboratory Register.

- a) *The date on which the test is run.*
- b) *The name of the kit used.*
- c) *Lot No. and expiry date of the kit.*
- d) *Initials of the Technician who performed the test.*
- e) *Initials of the Technologist who verifies the result.*
- f) *Reactive units are marked in red.*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>HBs Ag TESTING BY RAPID KIT METHOD</b>		
<b>BTS/SOP/TP/15b</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This document describes the step-by-step method for rapid chromatographic immunoassay for the qualitative detection of surface antigen of Hepatitis B Virus (HBV). This rapid test kit is used only for urgent donor screening in emergency and life threatening cases.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

The membrane is coated with anti-HBsAg on the test line region of the device. The serum or plasma specimen reacts with the particle coated with anti-HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colour line which indicates a positive reaction or presence of HBsAg.

## 4. MATERIAL

- Rapid Test Kit
- Blood sample (plasma/serum)
- Sample racks
- Disposable gloves
- Stop watch
- Micropipette 10-100 ul with micropipette stand
- Na Hypochlorite

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 Bring all the specimens and kit contents at room temperature (18-25°C) before performing the test.
- 5.2 Remove the protective foil cover.
- 5.3 Label the test strip with donor identification number.
- 5.4 Place approximately 100 ul of the sample to the sample well and start the stop watch.

- 5.5 Examine macroscopically (after 15 minutes) for the appearance of colour line in the test region.
- 5.6 Handle the device as infectious material and dispose them accordingly.
- 5.7 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

**POSITIVE:** A pink colour line should appear in the control region (C) and another in the test region (T).

**NEGATIVE:** No line appears in the test region (T) but that of control region (C) appears.

**INVALID:** Absence of line on control region (C); retest these samples

**Note:** Retest all samples showing grey zone after ultra-centrifugation at 10,000 rpm for 10 minute. Do retesting with same and another technique. (use rcf, this is equipment independent)

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY		
HCV ANTIBODY TESTING BY ELISA METHOD		
BTS/SOP/TP/16a	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Anti HCV is a mandatory test for blood unit screening before it is transfused. This is carried out on all donor units' samples and pre-donation samples of aphaeresis donors. The enzyme-linked immunosorbent assay (EIA or ELISA) is the test of choice for screening donor blood for HCV Antibody.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

In HCV ELISA, the micro well is coated with recombinant Hepatitis C Virus encoded antigens as the solid phase. If the HCV antibody is present, it becomes bound to the solid phase and can be detected by a complementary anti-human IgG conjugated to an enzyme (capable of acting on a chromogenic substrate). When substrate is added to the bound complex, the presence of antibody can be detected by development of a coloured end product.

## 4. MATERIAL

- HCV Reagent Test Kit
- Elisa Washer
- Micro-shaker
- Elisa Reader
- Micropipettes and disposable pipette tips
- Disposable gloves
- Disposal container with Na Hypochlorite
- Timer
- Incubator
- Distilled water
- N /10 Sulphuric Acid (H<sub>2</sub>SO<sub>4</sub>)

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 The provided micro plate has 12 columns (strips) and 8 rows (A-H).
- 5.2 Make a summary sheet of the micro plate.
- 5.3 A1 is for Blank, B1, C1, D1 are for Negative Controls, E1, F1 are for Positive Controls.
- 5.4 G1 onwards are test samples.
- 5.5 Put 200µl Sample Diluent in each samples well (and not in the control wells) .
- 5.6 Add 10µl of donor test samples in respective wells. How much in control cells?
- 5.7 Shake the microtest plate gently on the microwell shaker.
- 5.8 Seal plate with the adhesive tape (provided with kit).
- 5.9 Incubate at 37°C for 60 minutes in Incubator.
- 5.10 Wash plate 5 times with Washing Solution.(to be diluted 1/10 in distilled water, i.e. 100ml washing solution in 1000 ml distilled water).
- 5.11 Concentrate Conjugate to be diluted 1/50 with conjugate diluents, i.e. 20 µl concentrate conjugate in 1ml conjugate diluents conjugate.(For 1 strip -----1 ml diluents conjugate similarly in 6 ml diluents conjugate +120 µconcentrate conjugate).
- 5.12 Add 100µl of above diluted conjugate in each well except the well for Blank.
- 5.13 Seal the plate with adhesive tape and incubate at 37°C for 30 minutes in the Incubator.
- 5.14 Wash plate 5 times with Washing Solution.
- 5.15 Make Substrate Solution also called TMB or Chromogen. (1ml/strip, add 20µl/ml conc. substrate with diluting solution).
- 5.16 Add 100 µl of above in each well including well for blank.
- 5.17 Incubate the plate at 22°C for 30 minutes (in dark at the bench).
- 5.18 Colour develops. Add 100µl Stopping Solution (N/10 H<sub>2</sub>SO<sub>4</sub>) to each well and wait for 3-4 minutes.
- 5.19 Interpret the results using semiautomatic plate reader.
- 5.20 Handle the microtest plates as infectious material and dispose them accordingly.
- 5.21 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

Final results are displayed on the [=]'10screen.

The samples below the cut off are considered NON-REACTIVE.

Above cut off are considered REACTIVE.

Equal to cut off value/showing grey zone are considered BORDERLINE cases

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000 rpm for 10 minute. Do retesting with same and another technique. (use RCF, this is equipment independent)

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

In case of equipment interfacing, results are transferred automatically.

Record the following details in HCV Ab testing section of the TTI Laboratory Register.

- a) *The date on which the test is run*
- b) *The name of the kit used*
- c) *Lot No. and expiry date of the kit*
- d) *Initials of the Technician who performed the test*
- e) *Initials of the Technologist who verifies the result*
- f) *Reactive results are marked in red and are encircled*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

# STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>HCV ANTIBODY TESTING BY RAPID KIT METHOD</b>		
<b>BTS/SOP/TP/16b</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This document describes the step-by-step method for rapid chromatographic immunoassay for the qualitative detection of antibodies to Hepatitis C Virus (HCV). This rapid test kit is used for urgent donor screening in emergency and life threatening cases.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

The membrane is coated with recombinant antigens (highly immuno-reactive regions of HCV) on the test line region of the device. The HCV antigen -colloidal gold conjugate embedded in the sample pad reacts with the HCV antibody present in blood, serum or plasma sample forming conjugate/HCV antibody complex. As the mixture is allowed to migrate along the test strip, the conjugate/HCV antibody complex is captured by an antibody-binding protein A immobilized on a membrane forming a coloured test band in the test region.

## 4. MATERIAL

- Rapid Test Kit
- Blood sample (plasma/serum)
- Sample racks
- Sample buffer (diluent)
- Disposable gloves
- Stop watch
- Micropipette 10-100 ul with micropipette stand

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 Bring all the specimens and kit contents at room temperature (18-25°C) before performing the test.
- 5.2 Remove the protective foil cover.
- 5.3 Label the test strip with client identification number.

- 5.4 Place 10 ul of the sample to the sample well.
- 5.5 Add 2 drops of buffer and start the stop watch.
- 5.6 Examine macroscopically (after 15 minutes) for the appearance of colour line in the test region.
- 5.7 Handle the device as infectious material and dispose them accordingly.
- 5.8 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

POSITIVE: A pink colour line should appear in the control region (C) and another in the test region (T)

NEGATIVE: No line appears in the test region (T) but that of control region (C) appears

INVALID: Absence of line on control region (C); retest these samples

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000rpm for 10 minute. Do retesting with same and another technique. (use rcf, this is equipment independent)

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY		
HIV ANTIBODY TESTING BY ELISA METHOD		
BTS/SOP/TP/17a	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Anti HIV antibodies testing is carried out on all bag samples before these are released for transfusion. Pre-donation samples of aphaeresis donors are also tested. The enzyme-linked immunosorbent assay (EIA or ELISA) is the test of choice for screening donor blood for HIV Antibody.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

Human serum or plasma diluted in specimen diluent and incubated with the proteins of HIV 1 HIV 2, coated auto micro plate wells and incubated. If the HIV antibodies are present in the samples that are tested, it will bind with the proteins coated on the micro well. After washing off the unbound analyte, horse radish peroxidase conjugated with anti-human IgG antibodies is added. Enzyme conjugate binds through the antigen antibody complex if present. Unbound analyte is washed and substrate solution is added. Colour will develop in proportion to the amount of HIV antibodies present in the specimen. Stopping solution is added at the end of the incubation to stop the reaction. The reaction is read by EIA reader.

## 4. MATERIAL

- Elisa Washer
- Micro-shaker
- Elisa Reader
- Micropipettes and disposable pipette tips
- Disposable gloves
- Disposal container with Na Hypochlorite
- Timer
- Incubator
- Distilled water
- N /10 Sulphuric Acid (H<sub>2</sub>SO<sub>4</sub>)
- HIV Reagent Test Kit

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 The provided micro plate has 12 columns (strips) and 8 rows (A-H).
- 5.2 Make a summary sheet of the micro plate.
- 5.3 A1 is for Blank, B1, C1, D1 are for Negative Controls, E1, F1 are for Positive Controls.
- 5.4 G1 onwards are test samples.
- 5.5 Put 200µl Sample Diluent in each samples well (and not in the control wells).
- 5.6 Add 10µl of donor test samples in respective wells.
- 5.7 Seal plate with the adhesive tape (provided with kit).
- 5.8 Incubate at 37°C for 60 minutes in Incubator Shaker.
- 5.9 Wash plate 5 times with Washing Solution.(to be diluted 1/10 in distilled water, i.e. 100ml washing solution in 1000 ml distilled water).
- 5.10 Concentrate Conjugate to be diluted 1/50 with conjugate diluents, i.e. 20 µl concentrate conjugate in 1ml conjugate diluents conjugate.(For 1 strip -----1 ml diluents conjugate, similarly in 6 ml diluents conjugate +120 µconcentrate conjugate).
- 5.11 Add 100µl of above diluted conjugate in each well except the well for Blank.
- 5.12 Seal the plate with adhesive tape and incubate at 37°C for 30 minutes in the Incubator Shaker.
- 5.13 Wash plate 5 times with Washing Solution.
- 5.14 Make Substrate Solution also called TMB or Chromogen. (1ml/strip, add 20µl/ml conc. substrate with diluting solution).
- 5.15 Add 100 µl of above in each well including well for blank.
- 5.16 Incubate the plate at 22°C for 30 minutes (in dark at the bench).
- 5.17 Colour develops. Add 100µl Stopping Solution (N/10 H<sub>2</sub>SO<sub>4</sub>) to each well and wait for 3-4 minutes.
- 5.18 Interpret the results using semiautomatic plate reader.
- 5.19 Handle the microtest plates as infectious material and dispose them accordingly.
- 5.20 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

Final results are displayed on the screen.

The samples below the cut off are considered NON-REACTIVE.

Above cut off are considered REACTIVE.

Equal to cut off value/showing grey zone are considered BORDERLINE cases.

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000rpm for 10 minute. Do retesting with same and another technique

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

In case of equipment interfacing RS232, results are transferred automatically.

Record the following details in HIV Ab testing section of the TTI Laboratory Register.

- a) *The date on which the test is run.*

- b) *The name of the kit used.*
- c) *Lot No. and expiry date of the kit.*
- d) *Initials of the Technician who performed the test.*
- e) *Initials of the Technologist who verifies the result.*
- f) *Reactive results are marked in red and encircled.*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY		
HIV ANTIBODY TESTING BY RAPID KIT METHOD		
BTS/SOP/TP/17b	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This document describes the step-by-step method for rapid chromatographic immunoassay with a double antigen system for the qualitative detection of antibodies to Human Immunodeficiency Virus (HIV). This rapid test kit method is used for urgent donor screening in emergency and life threatening cases.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

The membrane is coated with recombinant HIV antigens on the test line region of the device. When a specimen is applied at one end of the membrane, it reacts with HIV antigen coated gold conjugate in the test strip. The mixture then migrates chromatographically by capillary action and reacts with the recombinant HIV antigens on the membrane in the test line region. A coloured line appears in the test region if the sample contains HIV antibodies.

## 4. MATERIAL

- Rapid Test Kit
- Blood sample (plasma/serum)
- Sample racks
- Disposable gloves
- Sample buffer (diluent)
- Stop watch
- Micropipette 10-100 ul with micropipette stand

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 Bring all the specimens and kit contents at room temperature (18-25°C) before performing the test.
- 5.2 Remove the protective foil cover.
- 5.3 Label the test strip with client identification number.
- 5.4 Place 25 ul of the sample to the sample well.
- 5.5 Add 1 full drop (40 ul) of buffer and start the stop watch.
- 5.6 Examine macroscopically (after 15 minutes) for the appearance of colour line in the test region.
- 5.7 Handle the device as infectious material and dispose them accordingly.
- 5.8 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

POSITIVE: A pink colour line should appear in the control region (C) and another in the test region (T)

NEGATIVE: No line appears in the test region (T) but that of control region (C) appears

INVALID: Absence of line on control region (C); retest these samples

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000rpm for 10 minute. Do retesting with same and another technique. (use rcf, this is equipment independent)

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY		
SYPHILIS TESTING BY RPR		
BTS/SOP/TP/18a	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

Screening for syphilis antibodies is carried out on all donor samples before issuing blood bags. The Rapid Plasma Reagin (RPR) slide agglutination test is the most commonly used conventional method for detection of antibodies to *Treponema pallidum* in serum or plasma.

## 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report the result as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

This test is a non-treponemal slide agglutination test used for the diagnosis of syphilis which is a venereal disease. Serum or plasma is allowed to react with the carbon particles coated with a lipid complex extracted from *Treponema pallidum*. Agglutination will be observed if antibodies are present in serum or plasma.

## 4. MATERIAL

- Carbon coated antigen
- Serum/Plasma
- Positive and negative controls
- Disposable card with three circles
- Sterile wooden/plastic sticks for mixing
- Mechanical rotator
- Timer

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 Allow the samples and reagents to reach room temperature.
- 5.2 Place 50 ul of the sample and one drop of each positive and negative controls into the separate circles on the disposable card.

- 5.3 Swirl the carbon coated antigen bottle and place one drop (20 ul) on each of the three circles
- 5.4 Mix the drops with a sterile wooden/plastic stick, spreading them over the entire surface of the circle.
- 5.5 Place the card on mechanical rotator for 8 minutes (80-100 rpm).
- 5.6 Examine macroscopically for the appearance of visible agglutination after 8 minutes. Rotate the slide twice by hand before reading.
- 5.7 Handle the card as infectious material and dispose them accordingly.
- 5.8 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

Medium or large clumps of agglutination are reported as 'Reactive'.

Small clumps are reported as 'Weakly Reactive'.

No clumping or very slight roughness is reported as 'Non-Reactive'.

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System.

Record the following details in syphilis screening section of the TTI Laboratory Register.

- a) *The date on which the test is run*
- b) *The name of the kit used*
- c) *Lot No. and expiry date of the kit*
- d) *Initials of the Technician who performed the test*
- e) *Initials of the Technologist who verifies the result*
- f) *Reactive units are marked in red*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY		
SYPHILIS TESTING BY ICT-TP		
BTS/SOP/WP/18b	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

Syphilis TP Antibodies testing is carried out on all bag samples before the issuance of blood bags. Pre-donation samples of aphaeresis donors are also tested. A rapid Immuno-Chromatographic Test for the qualitative detection of antibodies to *Treponema pallidum* (TP) is also widely used for screening of blood donor for Syphilis.

### 2. RESPONSIBILITY

Technician from TTI screening lab: Responsible to carry out the test and report the result as required.

Technologist from TTI screening lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

### 3. PRINCIPLE

In this test procedure, recombinant syphilis antigen is immobilized in the test line region of the test. After specimen is added to specimen's well of the device, it reacts with Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized Syphilis antigen. If the specimen contains TP antibodies, a coloured line will appear in the test line region, indicating a positive result. If the specimen does not contains TP antibodies, a coloured line will not appear in the test line region, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

### 4. MATERIAL

- Test devices
- Droppers
- Buffer (For whole blood specimen, if needed)
- Micropipettes and disposable pipette tips
- Disposable gloves
- Disposal container with Na Hypochlorite
- Timer

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ in each kit!

- 5.1 Allow the test devices, specimen and other material to reach room temperature prior to testing.
- 5.2 Place the test device on a clean and level surface.
- 5.3 Add 3 drops of serum or plasma in the specimen well on device.
- 5.4 Wait for 10 minutes.
- 5.5 Read the result immediately. Do not read the results after 30 minutes.
- 5.6 Handle the device as infectious material and dispose them accordingly.
- 5.7 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

- 6.1 The result will be positive if two coloured lines appear, one in control line region and the other in the test line region. The intensity of colour line in test region will vary depending on concentration of TP antibodies in the specimen. Therefore any shade of colour in the test line region should be considered as positive.
- 6.2 The result will be Negative if only one coloured line appears only in the control line region and no line in test line region.
- 6.3 The test will be invalid if the control line does appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test.

## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System. Record the following details in Syphilis TP Abs testing section of the TTI Laboratory Register.

- a) *The date on which the test is run.*
- b) *The name of the kit used.*
- c) *Lot No. and expiry date of the kit.*
- d) *Initials of the Technician who performed the test.*
- e) *Initials of the Technologist who verifies the result.*
- f) *Reactive units are marked in red.*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

## STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>SYPHILIS TESTING BY TPHA</b>		
<b>BTS/SOP/WP/18c</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

Screening for syphilis antibodies is carried out on all blood bags before issuance. TPHA is a specific, sensitive passive haemagglutination test for the detection of antibodies to syphilis.

### 2. RESPONSIBILITY

Technician from TTI Testing lab: Responsible to carry out the test and report the result as required.

Technologist from TTI Testing lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

### 3. PRINCIPLE

The test is a treponemal test for the serological detection of antibodies to *T. pallidum*. The test is a passive haemagglutination assay based on the flocculation of avian erythrocytes sensitized with *T. pallidum* antigen by antibodies found in the donor's plasma.

### 4. MATERIAL

- Micro titration plates
- Cell droppers
- Positive and negative controls (pre-diluted)
- Sample diluent
- Test cells (preserved avian erythrocytes sensitized with *T. pallidum* antigen)
- Control cells (preserved avian erythrocyte.)

### 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

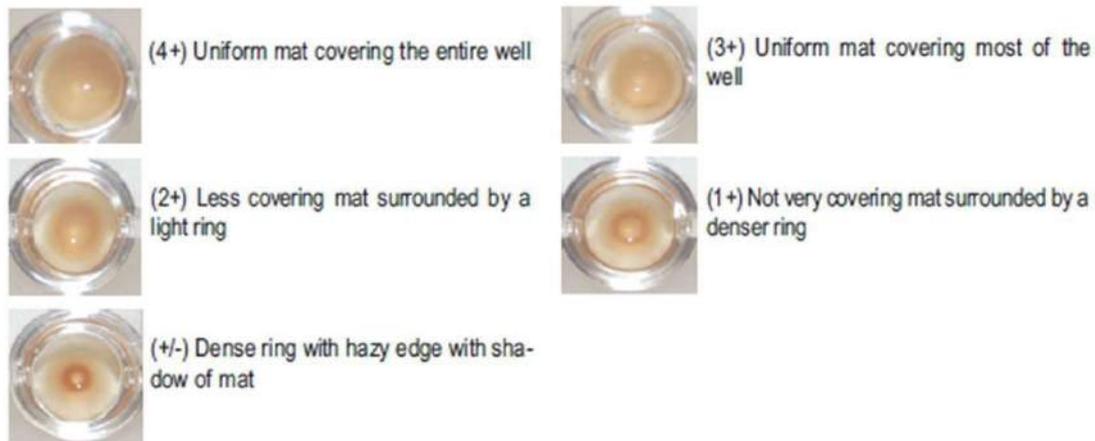
- 5.1 Allow the samples and reagents to reach room temperature.
- 5.2 Each test requires 4 wells of a microtest plate.
- 5.3 Place 25 ul of the sample diluent in well no. 1, 3 and 4 while 100 ul in well no. 2.
- 5.4 Place 25 ul of sample in to well 1 and mix.
- 5.5 Transfer 25 ul from well 1 to well 2.
- 5.6 Transfer 25 ul from well 2 to well 3 and discard 25 ul from well 3.
- 5.7 Transfer 25 ul from well 2 to well 4 and discard 25 ul from well 4.

- 5.8 Place 75 ul of Control Cells to well 3 (dilution is 1/80).
- 5.9 Place 75 ul of Test Cells to well 4 (dilution is 1/80).
- 5.10 Cover the plate and leave for 1 hour at room temperature.
- 5.11 Examine well 3 and 4 macroscopically for the appearance of visible agglutination.
- 5.12 Handle the microtest plates as infectious material and dispose them accordingly.
- 5.13 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

Agglutinated cells form an even layer over the bottom of the well and reported “REACTIVE”. Non-agglutinated cells form a compact button in the centre of the well and reported as “NON-REACTIVE”. (Not all samples that have a “not an even layer” in the well are negative. Normally a negative has a solid point of cells in the middle of the well; all others are positive.)

INVALID: if agglutinated cells in well 3 are observed the test is invalid. Repeat the sample in duplicate.



## 7. DOCUMENTATION

Enter the results in the donor test results area in the Blood Transfusion Information System. Record the following details in syphilis screening section of the TTI Laboratory Register.

- a) *The date on which the test is run*
- b) *The name of the kit used*
- c) *Lot No. and expiry date of the kit*
- d) *Initials of the Technician who performed the test*
- e) *Initials of the Technologist who verifies the result*
- f) *Reactive units are marked in red*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

## STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>SLIDE METHOD FOR MALARIAL PARASITE DETECTION</b>		
<b>BTS/SOP/TP/19a</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

The samples from donors are tested for Transfusion Transmitted Infection. It includes testing for Malarial Parasite as well, if the parasite is present in the blood and left untreated it will increase rapidly in the blood stream and can lead to the sudden deterioration in the health of the individual receiving blood.

### 2. RESPONSIBILITY

The Technician is responsible to prepare the slides. Trained Technologist and Duty Medical officer are responsible for microscopy.

### 3. PRINCIPLE

Malaria microscopy is the key to the diagnosis of malarial parasite. Two types of smears are prepared from the peripheral blood, one thin smear and the other thick smear which is more sensitive in detection of malaria parasite. The recognition of parasite species is also carried out through the microscopy as well.

### 4. MATERIAL/REAGENTS REQUIRED

- Glass slides
- Coverslips
- Methanol
- M.P. buffer
- Giemsa stain
- Dryer
- Microscope

### 5. PROCEDURE

#### 5.1 Thin Smear Preparation

- 1 Make a thin blood smear and dry it.
- 2 After 5-10 minutes fix the smear in absolute methanol for 5 minutes.
- 3 Allow the smear to dry.
- 4 Prepare 1:20 dilution of Giemsa stain with M.P. buffer.
- 5 Flood the stain on smear.
- 6 Allow it to stand for 20 minutes.

- 7 Rinse the smear with running tap water.
- 8 Dry and examine under microscope 100x oil immersion lens.
- 9 Observe blood film for at least 100 fields to determine whether the blood film is positive or negative for malaria.
- 10 Handle the slides infectious material and dispose them accordingly.
- 11 Clean the work bench with hypochlorite

## 5.2 Thick smear Preparation

- 1 Make thick smear and dry it for 8-10 hours or overnight in an incubator at 37°C.
- 2 Do not fix the smear.
- 3 Prepare 1:50 dilution of Giemsa stain with buffered water.
- 4 Flood the stain on smear
- 5 Allow to stand for 20 minutes
- 6 Rinse the smear under running tap water.
- 7 Dry and examine under microscope
- 8 Handle the slides as infectious material and dispose them accordingly.
- 9 Clean the work bench with hypochlorite

## 6. INTERPRETATION OF RESULT

Observe blood film for at least 100 fields to determine whether the blood film is positive or negative for malarial parasite and its species.

## 7. DOCUMENTATION

Enter the results in the donor screening test result area in the Blood Transfusion Information system.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

# STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>IMMUNO-CHROMATOGRAPHIC TEST FOR MALARIAL PARASITE</b>		
<b>BTS/SOP/TP/19b</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

The samples from donors are tested for Transfusion Transmitted Infection. It includes testing for Malarial Parasite as well, if the parasite is present in the blood and left untreated it will increase rapidly in the blood stream and can lead to the sudden deterioration in the health of the individual receiving blood.

## 2. RESPONSIBILITY

Technician from TTI Testing lab: Responsible to carry out the test and report the result as required.

Technologist from TTI Testing lab: Responsible to verify the results. Responsible to check possible changes in kit insert at every new lot number received.

## 3. PRINCIPLE

ICT-MP is a rapid, sensitive test for malaria that differentiates between *P. falciparum* and other malaria parasites. The test is highly accurate and is capable of detecting as few as 50-100 parasites per  $\mu\text{L}$  of blood; results are achieved within 20 minutes. In the presence of plasmodium species, in the blood sample, the pLDH (Lactate dehydrogenase from the parasite) captured by the conjugate in the test device reacts with specific antibodies against plasmodium falciparum and / or plasmodium species.

## 4. MATERIAL

- The Test Package contains
- Test device with dipstick, conjugate well, wash well/1 well cover
- One dropper ampoule with buffer
- One micropipette with 10 $\mu\text{l}$  mark
- Donor's whole blood sample

## 5. PROCEDURE

IMPORTANT: check the actual volumes and procedure steps provided with the test kit; this can differ from lot to lot number!

- 5.1 Tear one test package and label the device with the donor's Identification number.
- 5.2 Tear open the ampoule of buffer, add 1 drop to the well 1, add 4 drops to the well 2 and wait for 1 minute.
- 5.3 Take the micropipette provided and fill donor's sample up to the mark (10 $\mu\text{l}$ ).
- 5.4 Add this blood in well 1 of device and stir gently.
- 5.5 Take dipstick out of device and place it in the well.

- 5.6 After 10 minutes transfer dipstick in well 2.
- 5.7 When the background becomes clear after 10 minutes, take dipstick out and read result.
- 5.8 Place well cover over wells 1 and 2.
- 5.9 Snap wells off and dispose them safely. Snap the feet off to leave the cassette.
- 5.10 Keep the cassette as record.
- 5.11 Handle the devices as infectious material and dispose them accordingly.
- 5.12 Clean the work bench with hypochlorite.

## 6. INTERPRETATION OF RESULT

The results are valid if

- *The control band is clearly visible.*
- *The reaction field is clear.*

The results are not valid if:

- The dipstick is not sufficiently cleared (reaction fields remain red),
- The control band is not present,
- The control band is not visible even if one or both diagnostic bands are present and the control band and Pf.

### NEGATIVE REACTION

No detectable pLDH in the sample.

P Band	Pf Band	Control Band	Result Interpretation
Absent	Absent	Present	Negative for all types of malaria spp.
Present	Present	Present	Positive for Plasmodium falciparum +/- (P.vivax/P.ovale/P.malariae)
Present	Absent	Present	Positive for Plasmodium sp. P.vivax/P.ovale/P.malariae)

### POSITIVE REACTION

The pLDH present in the sample reacts with the anti pLDH conjugate and rises up the dipstick where it is captured by one or both specific pLDH antibodies. The test is positive only when live parasites are present in the blood so the test is suitable for verifying effectiveness of therapy.

## 7. DOCUMENTATION

Enter the results in the donor screening test result area in the Blood Transfusion Information system.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>TTI SCREENING LABORATORY</b>		
<b>REPORTING OF RESULTS</b>		
<b>BTS/SOP/WP/20</b>	<b>REGIONAL BLOOD CENTRE</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This SOP is related to the reporting of TTI screening results. Blood and blood components are used as per need of the patients.

## 2. RESPONSIBILITY

It is the responsibility of technician working in the TTI screening laboratory to report the results after being verified by the Technologist. The results are entered in screening register or BTIS.

## 3. MATERIAL

- TTI screening register
- Blood Transfusion Information System

## 4. PROCEDURE

- 1 Note down the results of TTI screening according to interpretation given in respective SOPs.
- 2 Enter the results in screening register or BTIS.
- 3 Add standardized comments to each report.
- 4 Take out all positive units from refrigerator and sent for incineration.
- 5 Make note in the BTIS that the units and related products are discarded.

## 5. DOCUMENTATION

Provide your initial with date and time on the screening register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

# **Immunohaematology**

# Standard Operating Procedures

## IMMUNOHAEMATOLOGY

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## STANDARD OPERATING PROCEDURE

<b>IMMUNOHAEMATOLOGY LABORATORY</b>		
<b>ABO GROUPING AND Rh D TYPING BY TUBE TEST</b>		
<b>BTS/SOP/TP/21a-23a</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applies to all those activities that are performed to determine the correct ABO group and Rh D type of a donor and ensuring the reliability of the results. This procedure describes the method of detection of the presence or absence of A, B & D antigens on red cells by using Anti-A, Anti-B and anti D antisera (antibodies) against the corresponding antigens. The Anti A & Anti B are monoclonal IgM antibodies specific against A & B red cell antigen. Anti D is also monoclonal which may be purely IgM or a blend of IgG and IgM (blend preferably). Reverse blood grouping should always be run in parallel with forward ABO typing for group confirmation Mismatch transfusion of ABO/D blood group can cause fatal transfusion reactions and sensitization against transfused D positive antigens in Rh D negative individuals especially in child bearing age females where it may cause haemolytic disease on the new born.

### 2. RESPONSIBILITY

In the Immunohaematology Laboratory following staffs are responsible for this procedure:

Trained Technician is responsible to perform the ABO grouping and RhD typing of donors

Technologist is responsible to verify the results

Medical Officer is responsible to supervise the procedure and to rule out any blood group discrepancy by further workup

It is the responsibility of all staff performing the ABO grouping and D typing to ensure that quality controlled reagents, proper cell concentrations and calibrated centrifuges are used.

### 3. PRINCIPLE

ABO system is the only system in which there is a reciprocal relationship between the antigen on the red cells and the naturally occurring antibodies in the serum. Routine grouping of donors must therefore include both red blood cells and serum tests, each serving as check on the other.

#### **Forward Blood Grouping / Cell Grouping / Front Type Grouping**

Known antibodies (commercially prepared anti A and anti B) are reacted with unknown antigens on the red blood cells of a patient or donor is called Forward Blood Grouping/Cell Grouping/Front Type Grouping.

Direct agglutination of unknown antigens on red cells (of patient/donor) with a particular reagent (known anti A or anti B) indicates the presence of corresponding antigen, and the blood group is termed as “A”/“B” or “AB”. No agglutination indicates the absence of A, B or AB antigens and the blood group is termed as “O”.

### **Reverse Blood Grouping / Serum Grouping / Back Type Grouping**

Unknown antibodies (anti A/anti B or both) present in the donor/patient serum or plasma are reacted with known red cell antigens (A, B and O red cells) is called reverse/serum/back typing grouping. All normal individuals have naturally occurring antibodies opposite to their antigens present on the red cells. For example; agglutination of the donor or patient serum / plasma with A cells indicates that the blood group is B, agglutination with B cells indicates A group, agglutination with both A and B cells indicates O group and no agglutination with A or B cells indicates that the blood group is AB. Donor or patient serum / plasma should not show any agglutination with O red cell of reverse blood grouping. If agglutination is seen with O cells than blood group should be considered as a discrepant and further workup is necessary (for confirmation of Bombay or allo/auto antibodies).

After ABO Blood Group System Rh D is the most immunogenic. The expression of Rh “D” positive or Rh “D” negative is based on the agglutination with anti D antisera.

For all donors who are typed Rh-D-negative, a weak D test must always being performed (cf. BTS/SOP/TP/24a).

## **4. MATERIAL**

### **4.1 EQUIPMENT**

- Refrigerator to store samples and reagents at 2- 6°C
- Calibrated table top centrifuge
- Lighted agglutination viewer

### **4.2 SPECIMEN**

Two blood samples; properly labeled, 2-3 cc, one EDTA (purple), one clotted (red)

Freshly drawn blood sample is preferred but it should not be older than 14 days

Blood sample should not be haemolysed

### **4.3 REAGENTS**

Commercially available monoclonal antisera: Anti-A, Anti-B, and Anti D<sup>1</sup>

- Rh control: 6% Bovine Albumin OR commercially available Rh control (*cf. Annex 4*)
- Fresh 0.9% normal saline in washing bottle

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<sup>1</sup> Follow the manufacturer's instructions for the use of the reagent. Anti-D reagents that detect Dvi is selected for donor's sample)

- Prepare 3%-5% red cell suspension of donor red cells (60 microliters or one drop of washed packed red cells in 2 ml 0.9% normal saline) OR Prepare 5% red cell suspension of donor cells (100 microliters OR 2 drops of washed packed red cells in 2 ml 0.9% normal saline) for reverse grouping A<sub>1</sub>, B & O cells. All A/B/O reverse blood grouping cells should be Rh “D” negative to avoid agglutination with anti D formed in sensitized individuals like pregnant women. Otherwise the anti D will react/agglutinate with all A/B/O positive with reverse grouping cells

#### 4.4 MISCELLANEOUS

- Adjustable pipette, 50 -100 microliters, OR plastic dropper
- Tips
- Test tubes (12 x 75mm)
- Test tube rack
- Permanent Markers
- Timer
- 2 plastic beakers

### 5. PROCEDURE

#### 5.1 RED BLOOD CELLS TESTING / FORWARD GROUP TESTING

IMPORTANT: check the actual volumes/drops and procedure steps in the inserts provided with the antibodies; these can differ from lot to lot number!

- 1 Label 4 clean test tubes with A, B, auto and D along donor ID (auto=autocontrol).
- 2 Arrange the test tubes in a row.
- 3 Prepare cell suspension for cells being tested. (*cf. Annex-1*)
- 4 Dispense one drop of anti-A, anti-B, and anti-D in the appropriately labeled tubes A, B and D respectively.
- 5 Dispense two drops of donor plasma or serum to the tube marked “auto”.
- 6 Add to each test tube one drop of a 3-5% red cell suspension to tubes labeled as A, B, auto and D.
- 7 Mix the contents of the tubes gently and centrifuge immediately after balancing at 3400rpm for 15 seconds.
- 8 Gently take out the tubes and re-suspend the red cell button.
- 9 Examine individually each tube macroscopically for agglutination. (*cf. Annex 2*)
- 10 Grade and record test results. (*cf. Annex-3*)

#### 5.2 SERUM TESTING / REVERSE GROUP TESTING

- 1 Label 3 clean test tubes with A<sub>1</sub>, B, and O along with unique donor ID.
- 2 Arrange the test tubes in a row.
- 3 Centrifuge donor blood specimen to get clear serum / plasma for reverse grouping.
- 4 Add 2 drops of donor serum in all tubes in the corresponding tube.
- 5 Add one drop of known 3% or 5% reverse grouping red cell suspension of A, B & O cells to tubes labeled as A<sub>1</sub>, B and O.
- 6 Mix the contents and centrifuge all tubes immediately after balancing, at 3400 rpm for 15 seconds.
- 7 Gently take out the tubes and re-suspend the red cell button.

- 8 Examine individually each tube macroscopically for haemolysis and agglutination (cf. Annex 2&3).
- 9 Grade and record test results.

### 5.3 CONTROLS FOR Rh D GROUPING

Rh Control should be tested in parallel with “Rh-D-Positive” cases for true identification of Rh D blood grouping and not due to auto antibodies.

- 1 Take a clean labeled test tube.
- 2 Dispense one drop of 6% Bovine Albumin or commercially available Rh control.
- 3 Add one drop of 3-5% donor specimen.
- 4 Mix and centrifuge at 3400 rpm for 15 seconds.
- 5 Take out the tube gently, read macroscopically Document result.

Result Interpretation:

Results must be negative because 6% Bovine Albumin does not contain any antibodies. If the result is Positive than the case is referred to the shift incharge/head of the blood bank to solve Rh D discrepancy

### 6. RESULTS OF ABO/RhD BLOOD GROUPING

POSITIVE: Agglutination / Mixed Field/ Haemolysis

NEGATIVE: No agglutination/No Mixed Field/No Haemolysis

Confirm the ABO cell grouping results with those obtained in serum/reverse grouping and vice versa.

### 7. INTERPRETATION OF RESULT

- 7.1 Agglutination/Mixed Field in any tube of Red Blood Cells tests and agglutination, mixed field or haemolysis in serum test constitutes a positive test result. The expected agglutination reactions for positive tests are 3+ to 4+ (cf. Annex2&3).
- 7.2 A smooth suspension of Red Blood Cells after re-suspension of Red Blood Cells button is a negative test result. The interpretation of ABO group is as follows:

Reaction of Red Cells with Antisera /Forward Group Typing				Reaction of Serum with reagent Red Cells/Reverse Group Typing			Interpretation of Group	
Anti-A	Anti-B	Rh D	auto	A1 Cells	B Cells	O Cells	ABO	Rh D
+++	-	+++	-	-	+++	-	A	Pos
-	+++	+++	-	+++	-	-	B	Pos
+++	+++	+++	-	-	-	-	AB	Pos
-	-	+++	-	+++	+++	-	O	Pos
+++	-	-	-	-	+++	-	A	Neg*
-	+++	-	-	+++	-	-	B	Neg*
+++	+++	-	-	-	-	-	AB	Neg*
-	-	-	-	+++	+++	-	O	Neg*

Positive(+++)= Agglutination/Lysis/Mixed Field
Negative(-)= No Agglutination/Lysis/Mixed Field
* Proceed with weak D (Du) Typing using indirect anti-globulin technique in case of donor blood sample.(Refer to SOP of weak D Test; BTS/SOP/TP/24a)

7.3 If any of the following discrepancies occur, the sample should be handed over to the Medical Officer Incharge: (**cf. SOP- 22**)

*There is a positive reaction in the reverse grouping with O cells*

*D- control is positive*

*Auto- control is positive*

*There is a discrepancy between the forward and reversed ABO blood grouping*

*There is a discrepancy between the results of the two tubes for Rh D grouping*

7.4 Any discrepancy between results on cell and serum or plasma tests should be resolved before an interpretation is recorded for the donor's ABO group.

## 8. DOCUMENTATION

Enter the results of donor grouping in the donor record register and BTIS. Enter the results of patient's grouping in the patient record register, blood group requisition form, serial case number register and BTIS.

## SOURCES OF ERRORS

	False Negative	False Positive
1	Centrifugation time too short	Over centrifugation
2	Reagent or Serum not added	Incorrect Interpretation
3	Inappropriate ratio of serum / reagent to cells	Used dirty glass ware
4	Wrong technique, not following manufacturer advice	Used contaminated reagents, cells, normal saline
5	Haemolysis not identified as positive reaction	Cells contaminated with Wharton's jelly
6	In correct interpretation	Incorrect interpretation
7	Weak D Test not performed	False Positive weak D test, due to positive DAT

### NOTE:

1. All reagents should be used according to the manufacture's advice within expiry date.
2. Do not run large batches, each batch should not be more than five samples.
3. Perform both Forward and Reverse Blood Grouping.
4. All reagents / antisera should be stored at 2 – 8 °C when not in use.
5. Quality control of all reagents cells / antisera should be performed on daily basis.
6. Use high tittered antisera; titration of Anti A should be 1:256, Anti B 1:256 and Anti D 1:128. The expected agglutination reaction for positive tests with undiluted antisera is 3+ to 4+.
7. Confirm possible Bombay blood group with Anti-H.

- 8.
9. After centrifugation, all tubes should be read immediately as delay may cause dissociation of antigen antibody complex leading to false negative or weak positive results.
10. Discrepant results should be informed to the shift in charge or head of the blood bank.
11. All steps should be done immediately one after the other.

Presence of "Weak D" in blood bank: As a donor he/she should be considered as Rh-D-Positive (*cf. BTS/SOP/TP/24a*).

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

IMMUNOHAEMATOLOGY LABORATORY		
ABO GROUPING AND Rh D TYPING BY MICROTEST PLATE TEST		
BTS/SOP/TP/21b-23b	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applies to all those activities that are performed to determine the correct ABO group and Rh D type of a donor and ensuring the reliability of the results. This procedure describes the method of detection of the presence or absence of A, B & D antigens on red cells by using Anti-A, Anti-B and anti D antisera (antibodies) against the corresponding antigens. The Anti A & Anti B are monoclonal IgM antibodies specific against A & B red cell antigen. Anti D is also monoclonal which may be purely IgM or a blend of IgG and IgM (preferably). Reverse blood grouping should always be run in parallel with forward ABO typing for group confirmation Mismatch transfusion of ABO/D blood group can cause fatal transfusion reactions and sensitization against transfused D positive antigens in Rh D negative individuals especially in child bearing age females where it may cause haemolytic disease on the new born.

### 2. RESPONSIBILITY

In the Immunohaematology Laboratory following staffs are responsible for this procedure:

Technician is responsible to perform the ABO grouping and RhD typing of donors

Technologist is responsible to verify the results

Medical Officer is responsible to supervise the procedure and to rule out any blood group discrepancy by further workup

It is the responsibility of all staff performing the ABO grouping and D typing to ensure that quality controlled reagents and proper cell concentrations are used.

### 3. PRINCIPLE

ABO system is the only system in which there is a reciprocal relationship between the antigen on the red cells and the naturally occurring antibodies in the serum. Routine grouping of donors must therefore include both red blood cells and serum tests, each serving as check on the other.

#### Forward Blood Grouping/Cell Grouping/Front Type Grouping

Known antibodies (commercially prepared anti A and anti B) are reacted with unknown antigens on the red blood cells of a patient or donor is called Forward Blood Grouping/Cell Grouping/Front Type Grouping.

Direct agglutination of unknown antigens on red cells (of patient/donor) with a particular reagent (known anti A or anti B) indicates the presence of corresponding antigen, and the blood group is termed as "A"/"B" or "AB". No agglutination indicates the absence of A, B or AB antigens and the blood group is termed as "O".

### **Reverse Blood Grouping/Serum Grouping/Back Type Grouping**

Unknown antibodies (anti A/anti B or both) present in the donor / patient serum or plasma are reacted with known red cell antigens (A, B and O red cells) is called reverse/serum/back typing grouping. All normal individuals have naturally occurring antibodies opposite to their antigens present on the red cells. For example; agglutination of the donor or patient serum/plasma with A cells indicates that the blood group is B, agglutination with B cells indicates A group, agglutination with both A and B cells indicates O group and no agglutination with A or B cells indicates that the blood group is AB. Donor or patient serum/plasma should not show any agglutination with O red cell of reverse blood grouping. If agglutination is seen with O cells than blood group should be considered as a discrepant and further workup is necessary (for confirmation of Bombay or allo/auto antibodies).

After ABO Blood Group System Rh D is the most immunogenic. The expression of Rh "D" positive or Rh "D" negative is based on the agglutination with anti D antisera.

For all donors who are typed Rh-D-negative, a weak D test must always being performed (see BTS/SOP/TP/24a).

A microtest plate is considered ad a matrix of 96 wells ("short" test tubes) in a fixed format of 12 columns (1 to12H) and 8 rows (A to H). The principle that apply to haemagglutination in test tubes also apply to tests in microtest plates

## **4. MATERIAL**

### **4.1 EQUIPMENT**

- Refrigerator to store samples and reagents at 2- 6°C
- Dispensers (optional): Semi-automated devices for dispensing equal volumes to a row of wells
- Microtest plate rotator
- Microtest plate readers (optional): Automated photometric devices that read the results by the light absorbance in U-shaped bottom wells to differentiate between positive and negative tests. The microprocessor component of the reader interprets the reactions and prints the blood testing results
- Special plate carriers are required to fit common table-top centrifuges
- Magnifying glass for microtest plates
- Incubator

## 4.2 SPECIMEN

- Automated methods may require the use of samples drawn from donor into a specific anticoagulant (K<sub>3</sub>-EDTA)
- Test red cells suspended in saline (2-3%)

## 4.3 REAGENTS

- Anti A, Anti-B anti-sera
- 2-3%% suspension of group A<sub>1</sub>, B reagent red cells
- 6% Albumin Control Reagent ( Rh Control)
- Use only Anti-D reagents approved for use in microtest plate tests<sup>2</sup>
- Isotonic (0.9%) saline

## 4.4 GLASS WARE

- Rigid or flexible U-shaped bottom microtest plates

## 4.5 MISCELLANEOUS

- Rubber teats for Pasteur pipettes
- Permanent Markers
- Timer
- Disposal box
- 2 plastic beakers
- Aluminum racks to hold sample tubes

## 5. PROCEDURE

### 5.1 RED BLOOD CELLS TESTING / FORWARD GROUP TESTING

- 1 Place 1 drop of anti-A, 1 drop of anti-B and 1 drop of donor's plasma/serumin separate clean wells of a U-bottom microtest plate.
- 2 Add 1 drop of 2-3% saline suspension of red cells to each well containing blood typing reagent.
- 3 Mix the contents of the wells by gently rotating the plate on the microtest plate rotator.
- 4 Centrifuge the plate at the appropriate conditions established for the centrifuge.\*
- 5 Resuspend the red cell buttons by gently manually tapping the plate or with the aid of a mechanical shakerRead, interpret, and record results. Compare red cell test results with those obtained in testing serum or plasma.

### 5.2 SERUM TESTING/REVERSE GROUP TESTING

- 1 Add 1 drop of serum or plasma under test to each well.
- 2 Add 1 drop of 2-3% suspension of A<sub>1</sub>, B and O reagent red cells to separate clean wells of a U-bottom microtest plate.

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<sup>2</sup> Consult the manufacturer's instructions for specific reagents, equipment, and proper controls

- 3 Mix the contents of the wells by gently tapping the slides of the plate or on a microtest plate rotator.
- 4 Centrifuge the plate at the appropriate conditions established for the centrifuge.\*
- 5 Re-suspend the red cell buttons by manually tapping the plate or with the aid of a mechanical shaker, read, interpret, and record results. Compare test results on serum or plasma with those obtained in testing red cells.

### 5.3 Rh D GROUP TESTING

- 1 Place 1 drop of anti-D reagent into a clean well of the microtest plate. If the reagent requires use of an Rh control, add 1 drop of the control to a second well.
- 2 Add 1 drop of a 2-3% suspension of red cells to each well.
- 3 Mix the contents of the wells by gently tapping the slides of the plate or on a microtest plate rotator.
- 4 Centrifuge the plate at the appropriate conditions established for the centrifuge.\*
- 5 Re-suspend the red cell buttons by manually tapping the plate or with the aid of a mechanical shaker. Examine for agglutination, read, interpret, and record the results.
- 6 To enhance weak reactions, incubate negative tests at 37° C in the incubator for 15 to 30 minutes and repeat steps 4 to 6.

## 6. RESULTS

Presence (+) or absence (-) of agglutination/haemolysis in ABO grouping

Presence (+) or absence (-) of agglutination in Rh D Typing

Confirm the ABO cell grouping results with those obtained in serum/reverse grouping and vice versa

All Rh-D-negative results must be retested with a weak D test (*cf. BTS/SOP/TP/24a*)

**Presence of “Weak D” in blood bank:** As a donor he/she should be considered as Rh-D-Positive

## 7. INTERPRETATION OF RESULT

- 7.1 Agglutination in any well of red blood cells tests and agglutination or haemolysis in serum test constitutes a positive test result. The expected agglutination reaction for positive tests are 3+ to 4+. (*cf. Annex 3*)
- 7.2 A smooth suspension of red cells after re-suspension of the cells button is a negative test result.
- 7.3 The interpretation of ABO group is as follows:

Reaction of Red Cells with Antisera /Forward Group Typing				Reaction of Serum with reagent Red Cells/Reverse Group Typing			Interpretation of group	
Anti-A	Anti-B	Rh D	auto	A1 Cells	B Cells	O Cells	ABO	Rh D
+++	-	+++	-	-	+++	-	A	Pos
-	+++	+++	-	+++	-	-	B	Pos
+++	+++	+++	-	-	-	-	AB	Pos
-	-	+++	-	+++	+++	-	O	Pos
+++	-	-	-	-	+++	-	A	Neg*
-	+++	-	-	+++	-	-	B	Neg*

+++	+++	-	-	-	-	-	AB	Neg*
-	-	-	-	+++	+++	-	O	Neg*
Positive(+++)= Agglutination/lysis/Mixed Field								
Negative(-)= No Agglutination/lysis/Mixed Field								
* Proceed with weak D (Du) Typing using indirect anti-globulin technique in case of donor blood sample.(Refer to SOP of weak D Test; BTS/SOP/TP/24a)								

7.4 If any of the following discrepancies occur, the Sample should be handed over to the Medical Officer In charge (cf. SOP- 22).

*There is a positive reaction in the reverse grouping with O cells*

*D- Control is positive*

*Auto- control is positive*

*There is a discrepancy between the forward and reverse ABO blood grouping*

*There is a discrepancy between the results of the two wells for Rh D grouping*

7.5 Any discrepancy between results on cell and serum or plasma tests should be resolved before an interpretation is recorded for the patient's or donor's ABO group.

## 8. DOCUMENTATION

Enter the results of donor grouping in the donor record register and BT IS.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

IMMUNOHAEMATOLOGY		
IDENTIFICATION OF WEAK ABO TYPES		
BTS/SOP/TP/22	REGIONAL BLOOD CENTRE	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure is performed to determine the subtypes of blood group A. In the laboratory A antigen is tested against anti-A<sub>1</sub> Lectin to prove presence of a high number of A antigens on the cell surface (e.g. A<sub>1</sub> cells)..

### 2. RESPONSIBILITY

Laboratory Technician is responsible to perform the grouping.

Laboratory Technologist is responsible to verify the results.

Medical Officer is responsible to supervise the procedure and to rule out any blood group discrepancy by further workup.

It is the responsibility of all staff performing the ABO grouping to ensure that quality controlled reagents and proper cell concentrations are used.

### 3. PRINCIPLE

The anti-A<sub>1</sub> lectin reagent will cause agglutination red cells, which carry the A<sub>1</sub> antigen, after centrifugation. If there is no agglutination, it indicates the presence of A<sub>2</sub> antigen.

### 4. MATERIAL

- Table top centrifuge
- Test tubes (glass)
- Normal Saline
- Positive and negative control red cells

### 5. PROCEDURE

- 1 Prepare a 3-5% suspension of washed test red cells in saline.
- 2 Place in a labeled test tube: 1 drop of Anti-A<sub>1</sub> reagent and 1 drop test red cell suspension.
- 3 Mix thoroughly and then centrifuge for 15 seconds at 3400 rpm.
- 4 Gently re-suspend red cell button and read macroscopically for agglutination.

## 6. RESULTS

Presence of agglutination indicates a positive reaction (A1 antigen is present)

Absence of agglutination indicates negative reaction (A1 antigen is absent)

To confirm the reactivity of anti-sera, use positive and negative red cells with every batch.

## 7. DOCUMENTATION

Enter the results of donor grouping in the donor record register and BT IS.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>IMMUNOHAEMATOLOGY LABORATORY</b>		
<b>IDENTIFICATION OF WEAK Rh-D TYPES (weak D)</b>		
<b>BTS/SOP/TP/24</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies for the identification of weak Rh-D types. Weak D is sometimes immunogenic, hence a donor carrying low levels of D antigen on their red blood cell are able to sensitize their recipient.

## 2. RESPONSIBILITY

In the Immunohaematology Laboratory following staffs are responsible for this procedure:

Technician is responsible to perform the weak RhD typing of donors

Technologist is responsible to verify the results

Medical Officer is responsible to supervise the procedure and to rule out any discrepancy by further workup

## 3. PRINCIPLE

Some weak D antigens are only recognized by an indirect anti-globulin (IAT) procedure. Detection of weak D is required when typing donor units for all donors who are Rh-D-negative, but this is not required for pre-transfusion testing of patient's samples

## 4. MATERIAL

### 4.1 EQUIPMENT

- Refrigerator to store samples and reagents at +2 to +6°C
- Table top centrifuge
- Incubator

### 4.2 SPECIMEN

- Anti-coagulated (K<sub>3</sub>EDTA tube) blood samples of donors.
- Test red cells suspended in native serum/plasma or saline.

### 4.3 REAGENT

- Suitable anti-D reagent for weak D testing ((Consult the manufacturer's package for test procedures and appropriate controls).

- Antihuman globulin reagent (Coomb's Reagent).
- IgG-coated control cells (*Check Cells: cf. Annex 5*).

#### 4.4 GLASS WARE

- Test tubes (10x75mm)
- Pasteur pipettes

#### 4.5 MISCELLANEOUS

- Rubber teats
- Permanent Markers
- Timer
- Disposal box
- 2 plastic beakers
- Aluminum racks to hold sample tubes

### 5. PROCEDURE

#### 5.1 METHOD FOR TESTING WEAK D

- 1 Label 2 test tubes with donor and test identification.
- 2 Arrange the test tubes in a row.
- 3 Place 1 drop of anti-D in a clean and labeled test tube.
- 4 Place 2 drops of the appropriate control reagent in a second, labeled test tube.
- 5 To each tube, add 1 drop of 2-5% saline-suspended red cells of the donor.
- 6 Mix and incubate the test and control tubes according to the reagent manufacturer's directions. This is typically 15 to 30 minutes at 37°C.
- 7 Centrifuge at 3400 rpm for 15-seconds-(or as specified by manufacturer)
- 8 Gently re-suspend the red cell button & examine for agglutination (*cf. Annex 2*).
- 9 If agglutination is absent, wash the red cells 3 times with normal saline.
- 10 Discard all saline than add 2 drops of anti-globulin reagent (Coomb's reagent)
- 11 Mix gently and centrifuge.
- 12 Gently re-suspend and examine for agglutination, grade, and record.
- 13 Add IgG-coated control cells (check cells) to confirm the validity of negative anti-globulin tests.

### 6. RESULT

Presence (+) or absence (-) of agglutination in weak D grouping

Confirm the validity of negative antiglobulin test with those results obtained by adding IgG-coated control cells (must always be positive)

### 7. INTERPRETATION

- 7.1 Agglutination in the anti-D tube, combined with a smooth suspension in the control tube, indicates that the red cells are D-positive.
- 7.2 No agglutination of the red cells in both the anti-D and the control tubes is a negative test result.
- 7.3 It is permissible to use a direct antiglobulin test (DAT) on the test cells as a control (because Positive DAT causes false Positive weak D test), but an indirect

antiglobulin procedure with an Rh control reagent is preferable because this ensures that all reagent components that might cause a false- positive result are excluded.

7.4 Agglutination at any phase in the control tube invalidates the test and no interpretation can be made.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

IMMUNOHAEMATOLOGY		
RED CELL ANTIBODY SCREENING AND IDENTIFICATION		
BTS/SOP/TP/25-26a	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies to all testing that requires red cell antibody screening for donor blood samples.

## 2. RESPONSIBILITY

It is the responsibility of the technician/technologist in the Immunohaematology laboratory to perform the antibody screen using proper, commercially available, screening cells. One technician performs all tests and another technologist checks it. If any unexpected blood group antibody is detected, inform the Medical Officer for further interpretation.

## 3. PRINCIPLE

The antibody screen test is used in the detection of unexpected immune blood group antibodies. In this test, the screening cells are combined with serum/plasma under investigation. The addition of a potentiating medium enzyme/22% Bovine Albumin helps to promote the interaction of red cells and antibodies allowing antibody/antigen reactions to occur. Positive reactions (haemolysis or agglutination) in any tests indicate the presence of allo-antibody or auto antibody in the serum.

## 4. EQUIPMENT AND MATERIAL

### 4.1 Equipment

- Refrigerator to store samples & reagents at +2 to +6°C
- Table top centrifuge.
- Automated cell washer (Optional: for automatic cell washing)
- Incubator

### 4.2 Specimen

- Donor's red cell and serum sample

### 4.3 Reagents

- Screening cells Papain 1% or Bromelain enzyme
- 22% Bovine albumin
- Antihuman globulin reagent (AHG)

- IgG sensitized control cells (Coombs Control cells)
- 0.9% saline
- Distilled water

#### **4.4 Glassware**

- Glass Tubes
- Adjusters pipettes 10-100ul

#### **4.5 Miscellaneous**

- Disposal box
- 2 glass beakers. (disposable)
- Aluminum racks to hold serum and Coombs' tubes

### **5. PROCEDURE**

#### ANTIBODY SCREENING

Antibody screening is a technique to detect the presence or absence of antibody in donor serum or plasma. It is done by:

- (1) Immediate spin method which detect the presence of cold reacting antibodies &
- (2) IAT method in which 22% Bovine Albumin/Papain/Bromelin enzyme may be added to enhance the reaction and is done to detect the presence or absence of warm reacting antibodies.

Preferably for screening of antibodies a 3 cell panel is used, but a two cell panel can be used too.

#### ANTIBODY IDENTIFICATION

Antibody identification is a technique to detect the type of antibody present in the patient/donor serum or plasma. It is done by

- (1) Immediate spin method which detects the type of cold reacting antibody like anti M, anti N, anti P, anti lewis, anti lutheran, etc. OR
- (2) IAT method detects the type of warm reacting antibodies like anti D, anti C, anti c, anti E, anti e, anti K, anti k, anti Fya, anti Fyb, anti Jka, anti Jkb, etc.

For identification of antibodies normally cell panels of 10 to 12 different red cell suspensions are used. They can be procured as enzyme-treated too (papaine or bromeline)

#### **5.1 METHOD FOR ANTIBODY SCREENING**

- 1 Allow all reagents to reach at room temp.
- 2 Label three clean glass tubes as I, II & III along with patient ID Number.

- 3 Centrifuge donor/patient blood sample at 3400 rpm for 5 minutes to obtain clear serum.
- 4 Dispense one drop of 3% screening cell suspension I, II and III in the respective labeled tubes I, II and III.
- 5 Add 2 drops of patient serum to all the tubes.
- 6 Mix gently.
- 7 Centrifuge all tubes at 3400 rpm for 15 seconds.
- 8 Gently take out the tubes so as not to disturb the cell button.
- 9 Observe macroscopically for haemolysis & then for agglutination by gentle shaking.
- 10 Grade all positive reactions and record them on the antigram.
- 11 wash all the tubes 3 times.
- 12 After the third wash decant supernatant completely.
- 13 Add 1-2 drops of polyspecific Coombs reagent to the "cell button" and mix gently.
- 14 Centrifuge all tubes at 3400 rpm for 15 seconds.
- 15 Take out all the tubes gently, read macroscopically.
- 16 Grade and record all results on the antigram.
- 17 Add one drop of check cells as quality control to all the negative results tubes. A negative reaction with the check cells invalidates the whole of the procedure and test should be repeated.

## 5.2 METHOD FOR ANTIBODY IDENTIFICATION

The method used for identification of immune blood group antibodies is exactly the same as used for screening of immune antibodies (see 5.1). For identification more cells are used (a normally available panel consists of 10 to 12 different cell suspensions). Choices can be made for enzyme treated cells or normal red blood cells according to antibody reaction strength

NOTE: enzymes will destroy some blood group antigens; you have kept that always in mind!

## 6. INTERPRETATION OF RESULT

POSITIVE RESULT: Haemolysis / Agglutination of red cells / Mixed Field.

NEGATIVE RESULT: No Haemolysis / No Agglutination of red cells (cf. Annex 2&3).

### NOTE:

- Screening cells and Identification cells in a kit should be of the same lot number or expiry date
- Haemolysed screening or identification should not be used
- All steps should be done immediately
- Never use plastic tubes as it adsorbed IgG antibody which can lead to false negative results
- Haemolysed patient blood sample should not be used. If there is haemolysis going on in the patient then check the size of cell button after centrifugation at 3400 rpm and match the colour of supernatant with the original blood sample. If the colour of the supernatant becomes darker than the original sample it means haemolysis had occurred during incubation at 37°C
- Tubes should be shaken gently

- Use clean glassware
- After addition of IgG-sensitized cells (Coombs Control Cells) to a negative test, the presence of agglutination indicates that the AHG reagent was added and was working properly. If negative result was obtained it shows that the AHG reagent was either not added to the AHG was not working properly (inactivated by improper washing)
- Use all reagents according to the manufacturer advice

## 7. DOCUMENTATION

Results of donor unit antibody screen are entered in the donor grouping register and Blood Transfusion Information System.

Results of patients antibody screen are entered in the patient grouping register, blood group requisition form, serial case number register and HMIS.

All records are initialed by the technician who has performed the test and by the Doctor/Technologist who has checked the results

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>IMMUNOHAEMATOLOGY DEPARTMENT</b>		
<b>TITLE: CROSS MATCH (SALINE/BOVINE-ALBUMIN/IAT)</b>		
<b>BTS/SOP/WP/26b</b>	<b>REGIONAL BLOOD CENTER</b>	<b>Version: 1.0</b>
Valid from:	Effective Date	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure is applied for compatibility testing of all patients requiring transfusion. There are two types of cross match, i.e. Major and Minor. Routinely major cross match is done in which donor red cells are cross matched with patient serum/plasma to detect incomplete antibodies in the patient serum/plasma (including IAT phase). Minor cross match is done when transfusion reaction is observed and is done by taking patient red cells which are cross match with the donor plasma to detect antibodies in the donor plasma. Incompatible blood units should never being used for transfusion.

## 2. RESPONSIBILITY

It is the responsibility of the technician in the immunohaematology laboratory to perform compatibility testing to demonstrate ABO Incompatibility and document the results. If any unexpected antibody is detected, the Medical officer should be informed for further investigation.

## 3. PRINCIPLE

Red cells possess a variety of antigens, in homozygous and heterozygous expressions, for identifying corresponding antibodies in the patients sample, donor red cells are tested against the patient's serum or plasma. The reaction between a specific antigen and its specific antibody is noticed by the presence of agglutination or haemolysis. Positive reaction in any test indicates incompatibility.

## 4. MATERIAL

### 4.1 EQUIPMENT

- Refrigerator to store samples & reagents at +2<sup>0</sup> to +6<sup>0</sup>C
- Tabletop centrifuge
- Automated cell washer

### 4.2 SPECIMEN

- Patient's serum or plasma. Patient sample should not be older than 3 days
- Donor red cells acquired from the blood packs intended to be transfused

### **4.3 REAGENTS**

- Polyspecific Antihuman globulin reagent (anti-IgG+anti-C3d)
- IgG sensitised control cells
- 0.9% saline

### **4.4 GLASSWARE**

- Pasteur pipettes
- Glass tubes

### **4.5 MISCELLANEOUS**

- Disposal box
- 2 glass beakers
- Aluminum racks to hold serum and coombs' tubes

## **5. PROCEDURE**

### **5.1 SALINE ROOM TEMPERATURE IMMEDIATE SPIN**

Saline room temperature is done to detect Major ABO incompatibility and complete (IgM) antibodies/cold antibodies like M, N, S, P, Lewis, Lutheran, etc. This cross match method can be done in emergency issue of blood (in emergency situations).

- 1 Label a clean glass tube.
- 2 Prepare 3% red cell suspension of donor red cells (60 microliters of washed red cells and 2 ml 0.9% normal saline) OR Prepare 5% red cell suspension of donor red cells (100 microliters of washed red cells in 2 ml 0.9% normal saline).
- 3 Centrifuge patient blood at 3400 rpm for 5 minutes to get clear serum.
- 4 Dispense 2 drops of patient serum into the labeled glass tube.
- 5 Add one drop of 3% or 5% donor red cell suspension to the tube containing patient serum.
- 6 Centrifuge immediately at 3400 rpm for 15 seconds.
- 7 Take out the tube gently.
- 8 Observe for haemolysis and then for agglutination by gentle shaking the tube.
- 9 Grade and record results.
- 10 Always continue with AHG phase, even in emergency situations, but in this case blood packs can be released after this phase).

### **5.2 AHG/COOMBS PHASE**

AHG/Coombs test is done to detect the presence of unexpected incomplete (IgG) antibodies in patients blood which can cause destruction of the transfused donor red cells. Coombs phase should always be included in the cross match. Continue with the tubes used in 5.1 saline room temperature immediate spin.

- 1 Optional: 2 drops of 22% Bovine Albumin can be added to this tube as enhancement media.
- 2 Incubate the tube at 37 °C for 45 minutes for saline/IAT or for 30 minutes if 22% Bovine Albumin is added.

- 3 Take out the tube and centrifuge at 3400 rpm for 15 seconds, than observe haemolysis and agglutination by gentle shaking the tube.
  - 4 If no agglutination/haemolysis is seen (grade and record results) then wash the tube 3 times to remove unbound antibodies.
  - 5 After the 3<sup>rd</sup> wash discard all saline/supernatant by gentle tapping on tissue paper.
  - 6 Add 2 drops of polyspecific coombs reagent.
  - 7 Mix & centrifuge immediately at 3400 rpm for 15 seconds.
  - 8 Take out the tube gently, observe macroscopically for haemolysis and agglutination by gentle shaking.
  - 9 Grade and record results.
- 10 To all negative results add one drop of “Coombs Control Cells” to validate the results. (Dispense one drop of check cells to the negative result tube and centrifuge immediately at 3400 rpm. Take out the tube and gently disperse the cell button, this time agglutination should be present at least 1+ or 2+. (The free AHG/Coombs reagent in the test tube causes agglutination of the check cells/sensitized cells). This validates that the Coombs reagent was working properly and cells were properly washed after incubation).

## 6. INTERPRETATION OF RESULT

POSITIVE RESULT: Haemolysis/Agglutination of red cells / Mixed Field is incompatible cross match.

NEGATIVE RESULT: No Haemolysis / No Agglutination of red cells is compatible crossmatch.

### NOTE:

- All steps should be done immediately
- Never use plastic tubes for cross match as it adsorbed IgG antibody which can lead to false negative results
- Haemolysed patient blood sample should not be used. If there is haemolysis going on in the patient then monitor the size of cell button after incubation at 37 °C by centrifugation at 3400 rpm and the supernatant colour should be matched with the original blood sample. If the colour of the supernatant becomes darker then the original sample it means haemolysis had occurred during incubation at 37°C
- Shaking should be done gently
- Haemolysed bag should not be selected for cross match
- Use clean glasswares
- Use all reagents according to the manufactures advice

### Limitations

The saline/enzyme cross match will not:

*Detect incomplete antibody*

*Ensure normal donor's red blood cell survival*

*Detection of antibodies connected to low level presence of antigens (as with heterozygous expressed blood groups like Fy<sup>a</sup>/Fy<sup>b</sup>)*

## 7. DOCUMENTATION

Enter results in cross-match register and compatibility report form

All records are initialed by technician who performed the test and the technologist who has verified the result.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

## Annex 1

### CELL WASHING PROCEDURE

1. Dispense 4-5 drops of whole blood or packed red cells in a 4 cc tube.
2. Fill the tube  $\frac{3}{4}$  full with 0.9% saline to resuspend the cells.
3. Centrifuge the tubes for 2 to 3 minutes at 3400 rpm.
4. Discard maximum supernatant fluid/saline by a plastic dropper.
5. Repeat this washing procedure three times, every time save red cells sediment.
6. Deposit at the bottom of the tube is washed cells.

### 5% RED CELL SUSPESION PROCEDURE

1. Take 100 micro-liters of "Washed Red Cells" in a clean labeled test tube.
2. Add 2 ml of 0.9% normal saline (1:20 ratio) to make 5% red cell suspension.
3. Mix thoroughly and this suspension can be used for 12 hours.

### 3% RED CELL SUSPESION PROCEDURE

1. Take 60 micro-liters of "Washed Red Cells" in a clean labeled test tube.
2. Add 2 ml of 0.9% normal saline (1:20 ratio) to make 3% red cell suspension.
3. Mix thoroughly and this suspension can be used for 12 hours.
4. Store at 2-8°C when not in use.

## Annex 2

<b>READING AND GRADING TUBE AGGLUTINATION</b>
1. Gently shake or tilt the tube to resuspend the red cell button in the tube. The tilt technique uses the meniscus to gently dislodge the red cell button from the wall of the tube.
2. Observe the way that cells are dispersed from the red cell button.
3. Record reactivity by comparing the agglutinates to the descriptions in the following table.
4. The reactivity should be assessed when the red cells have been completely resuspended from the button.

## Annex 3

<b>INTERPRETATION OF AGGLUTINATION REACTION</b>		
<b>Agglutination Grading/Score</b>		
<b>Macroscopically Observed Findings</b>	<b>Designation(USA)</b>	<b>Score(UK)</b>
One solid agglutinate, background clear and no free cells	4+	12
Several large agglutinates, background clear and no free cells	3+	10
Medium-size agglutinates, background turbid and many free cells	2+	8
Small agglutinates, background turbid and too many free cells	1+	5
Few tiny agglutinates, turbid background and all free cells	1+w	4
Barely visible agglutination, turbid background and almost all free cells	W+ or +/-	2
No agglutination	0	0
Mixtures of agglutinated and unagglutinated red cells	Mixed field	
<b>Haemolysis Grading</b>		<b>Plasma/Serum</b>
Complete Haemolysis	Haemolysis	Coca cola colour/cherry red colour
Partial Haemolysis	Partial Haemolysis	Light pink colour

## Annex 4

<b>PREPARATION OF 6% BOVINE ALBUMIN</b>	
<b>SCOPE:</b>	6% Bovine Albumin is isotonic to serum and contains no antibodies. It is used in R <sub>h</sub> Control and weak D Control
<b>Requirement:</b>	<ol style="list-style-type: none"> <li>1. 22% Bovine Albumin (stock)</li> <li>2. 0.9% normal saline</li> <li>3. Adjustable Jester (100-1000µL)</li> <li>4. Tips</li> <li>5. Sterilized dropper vials</li> <li>6. Labels and pen</li> </ol>
<b>Calculation: For making 6% Bovine albumin</b>	
Formula: $C_1V_1=C_2V_2$	C1 = Initial Concentration = 22% V1 = Initial Volume =? C2 = Final Concentration = 6% V2 = Final Volume 5 ml OR 5000 µL
$C_1V_1 = C_2V_2$ $V_1 = \frac{C_2V_2}{C_1}$ $V_1 = \frac{6 \times 5}{22}$ $V_1 = \frac{30}{22}$ <p>V1 = 1.364 ml OR 1364 µL</p> <p>Volume of diluent can be calculated as follows:</p> <p>Volume of diluent = V2 – V1</p> <p>Diluent volume (V2) = 5000 µL – 1364 µL            Diluent volume (V2) = 3636 µL OR 3.6 ml</p>	<p><b>Preparation:</b></p> <ol style="list-style-type: none"> <li>1. Take sterilized dropper vial.</li> <li>2. Using jester, pour 3636 µL OR 3.6 ml (round figure) 0.9% normal saline in it.</li> <li>3. Add 1364 µL OR 1.4 ml (round figure) 22% Bovine Albumin.</li> <li>4. Mix Well.</li> <li>5. Label it with 6% Albumin, Manufacturing and Expiry Dates and initials of the technician who made this.</li> </ol> <p><u>Note:</u> Store at 2-8°C when not in use.</p>

## Annex 5

<b>PREPARATION OF CHECK CELLS</b>	
<b>PRINCIPLE</b>	<p>IgG coated check cells are used to validate all tests using antihuman globulin reagent (AHG). Check cells ensures;</p> <ul style="list-style-type: none"> <li>• AHG reagent was added</li> <li>• AHG reagent was active</li> <li>• Washing was complete (all unbound proteins removed).</li> </ul>
<b>REAGENTS AND EQUIPMENT</b>	<ul style="list-style-type: none"> <li>• 12 x 75 mm test tubes</li> <li>• Anti-D human IgG type</li> <li>• Normal Saline</li> <li>• Centrifuge</li> <li>• Water bath</li> <li>• Alsever's Solution</li> <li>• Adjustable Jester 10-100ul</li> <li>• Tips</li> <li>• Labels and pen</li> <li>• Aliquots from known O Rh Positive donor units (segments) from 3-5 donors.</li> </ul>
<b>PROCEDURE</b>	<ul style="list-style-type: none"> <li>• Label a clean glass tube.</li> <li>• Dispense 100ul of whole blood from five different O Positive blood bags to make a pool of 5.</li> <li>• Add 4 drops of Anti-D human IgG type to the pool cells.</li> <li>• Mix the contents and incubate at 37°C for 30 minutes, during incubation gently shake the tube after every 5 minutes.</li> <li>• After incubation, wash the tube 4-6 times with normal saline.</li> <li>• Make 5% in Alsever's solution (500 µL packed sensitized red cells to 9500 µL Alsever's solution in a sterilized dropper bottle) OR Normal Saline can be used instead of Alsever's Solution.</li> <li>• Label the vial with check cells; manufacturing and expiry dates and initials of the technician who prepared them.</li> </ul>
<b>STORAGE TEMPERATURE</b>	2-6 <sup>0</sup> C when not in used.

<b>EXPIRY DATE</b>	<p>In Alsever's Solution 3-4 weeks.</p> <p>In normal saline 12-24 hours</p>
<b>QUALITY CONTROL</b>	<ol style="list-style-type: none"> <li>1. Label two tubes one as positive control and the other as negative control.</li> <li>2. In positive control tube dispense one drop of AHG and one drop Check Cells while in negative control tube dispense one drop of normal saline and one drop check cells.</li> <li>3. Centrifuge both the tubes in a calibrated centrifuge at 3400 rpm for 15 seconds.</li> <li>4. Positive control should give 1+ to 2+ results and negative control should be negative.</li> <li>5. Results should be documented on the daily QC sheet.</li> </ol>

## Annex 6 ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

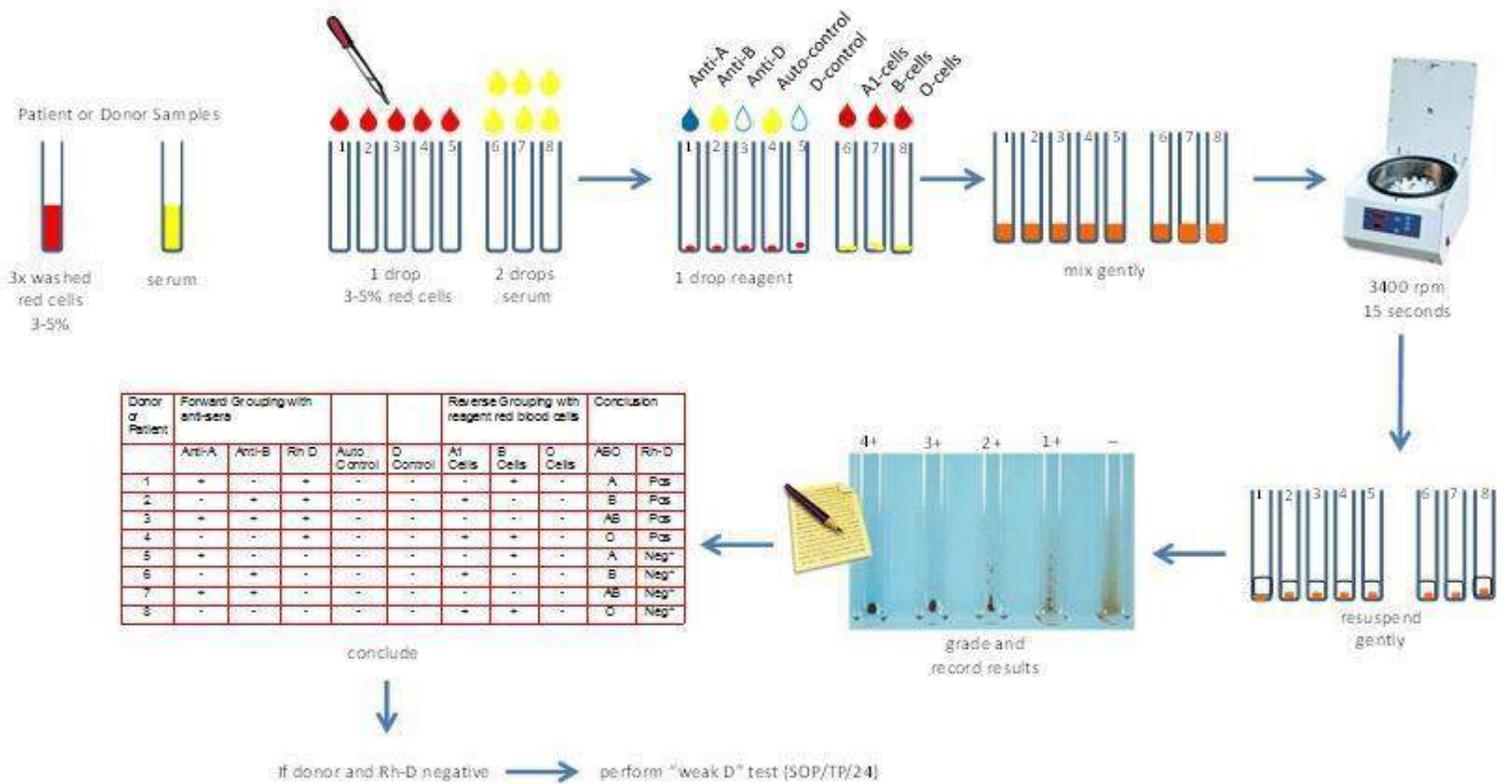
FORWARD GROUPING				REVERSE GROUPING				POSSIBLE CAUSES	RESOLUTION STEPS
Patient	Anti-A	Anti-B	Anti-AB	A1 Cells	B Cells	O Cells	Auto Control		
1.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Newborn with group "O" or elderly patient Patient may have hypogammaglobulinemia, or agammaglobulinemia May be taking immune-suppressive drugs	Check age of patient incubate at RT for 30 min or at 4°C for 15 min for weak antigens Immuno-globulin levels Drugs History
2.	4+	Neg	4+	1+	4+	Neg	Neg	Subgroup of A; probable A <sub>2</sub> group with Anti-A <sub>1</sub> Allo antibody	Use anti A1 Lectin Antibody screening and identification
3.	4+	4+	4+	2+	2+	2+	2+	1) Rouleaux (multiple myeloma patient; or patients given plasma expanders) 2) Cold autoantibody (probable group AB with an auto anti-I) 3) Cold autoantibody with underlying cold or RT reacting alloantibody (probable group AB with an auto anti-I and a high-frequency cold antibody (e.g. anti-P <sub>b</sub> , anti-M, anti-Le <sup>b</sup> ))	1) Wash red cells; use saline replacement technique 2) Perform cold auto absorption technique or use rabbit erythrocyte stroma (REST) absorb. 3) Perform cold autoabsorption technique or REST, and run panel on absorbed serum; select reverse cells lacking antigen for identified alloantibody; repeat reverse group on absorbed serum to determine true ABO group. 4) Use Pre warm technique

## ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

FORWARD GROUPING			REVERSE GROUPING					Auto Control	POSSIBLE CAUSES	RESOLUTION STEPS
Patient	Anti-A	Anti-B	Anti-A, B	A <sub>1</sub> Cells	B Cells	O Cells				
4.	3+	4+	4+	1+	Neg	Neg	Neg	Subgroup of AB; probable A <sub>2</sub> B with anti-A <sub>1</sub> <small>Alloantibody</small>	Use anti-A <sub>1</sub> lectin Antibody Sc & Id	
5.	Neg	Neg	Neg	4+	4+	4+	Neg	O <sub>h</sub> Bombay	Test with anti-H lectin;	
6.	Neg	Neg	2+	2+	4+	Neg	Neg	Subgroup of A; probable A <sub>x</sub> with anti-A <sub>1</sub>	Perform saliva studies Or absorption / elution	
7.	4+	2+	4+	Neg	4+	Neg	Neg	Group A with an "acquired B" antigen	Check patient history for lower gastrointestinal problem or septicemia; use modified BS-I lectin if available, or acidify anti-B typing reagent to pH 6.0 by adding 1 or 2 drops of 1N HCl to 1 ml of anti-B antisera, and measure with a pH meter (this acidified anti-B antisera would agglutinate only true B antigens and not acquire B antigens)	
8.	4+	4+	4+	2+	Neg	2+	Neg	Alloantibody (like anti Lea, anti P1, anti M & anti N)	Perform antibody screen and panel Selection of antigen negative reverse grouping cells for A & O	

## Annex 7

### ABO Grouping & Rh Typing by Tube Method



## Annex 8

ABO and Rh-D BLOOD GROUPING

Date:

	Donor ID										Anti-A	Anit-B	Anti-D	Auto	Anti-D	A1	B	O	conclusion
	cont	cont	cell	cell	cell	cont	cont	cell	cell	cell									
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			
13																			
14																			
15																			
16																			
17																			
18																			
19																			
20																			

Analysed by:

Checked by:

# **Blood Processing Laboratory**

# Standard Operating Procedures

## BLOOD PROCESSING LABORATORY

### COMPONENT PRODUCTION

- |   |     |
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| 1. Preparation of Red Blood Cells Concentrate (SOP/WP/27)         | 109 |
| 2. Preparation of Fresh Frozen Plasma/Cryoprecipitate (SOP/WP/28) | 111 |
| 3. Preparation of Platelets (SOP/WP/29)                           | 114 |
| 4. Labeling of Blood Bags and Blood Components (SOP/WP/30)        | 116 |

### ANNEXES

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| Annex 1. Formula for Calculating RCF | 131 |
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## STANDARD OPERATING PROCEDURE

BLOOD PROCESSING LABORATORY		
PREPARATION OF RED BLOOD CELL CONCENTRATES (RCC)		
BTS/SOP/WP/27	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

For appropriate use of blood it is necessary to use the components as per the need rather than using whole blood only. Red Blood Cell Concentrates, Fresh Frozen Plasma and Platelets are separated by using triple bags.

### 2. RESPONSIBILITY

It is the responsibility of the technician in the blood processing laboratory to separate components from whole blood collected in triple bags system.

### 3. PRINCIPLE

Red Blood Cells are obtained by removal of supernatant plasma from centrifuged Whole Blood. The volume of plasma removed determines the haematocrit of the component. When RBCs are preserved in CPDA-1, maximal viability during storage requires an appropriate ratio of cells to preservative. A haematocrit of 80% or lower in CPDA-1 RBC units ensures the presence of adequate glucose for red cell metabolism for up to 35 days of storage.

### 4. EQUIPMENT AND MATERIAL

Tube sealer/clamp

Refrigerated Bucket centrifuge with plastic inserts

Manual Plasma Extractor/Expresser

Electronic weighing scale or double pan weighing balance

Triple bags of average 450 ml ( $\pm$  10%) capacity with CPDA-1 as anticoagulant

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

### 5. PROCEDURE

Preparation of Red Blood Cells Concentrates using triple bags system without additive solution

5.1 Process the whole blood for component preparation within 6 hours of venipuncture.

5.2 Weigh the primary blood bag and record the weight in the register.

- 5.3 Label and check the satellite bag with the same donor unit number as that on the primary bag.
- 5.4 Keep the bags erect for 30-45 minutes.
- 5.5 Balance the two sets of triple bags system in the plastic inserts on a two pan weighing scale.
- 5.6 Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge.
- 5.7 Position the bags in buckets parallel to the direction of the spin. Centrifuge/soft spin the bags at 2000 rpm<sup>3</sup> for 10 minutes at 20o C.
- 5.8 Keep the primary bags containing centrifuged blood on a plasma expresser. Release the spring, allowing the plate of the expresser to contract the bag which expresses the plasma into the satellite bag.
- 5.9 Break the seal of the tubing connecting to the satellite bag; the plasma will now flow into the satellite bag.
- 5.10 leave 50-60 ml plasma along with the red cells.
- 5.11 seal the tubing between the primary bag and the satellite bag in tthree places, remove the bag by breaking the middle seal.
- 5.12 Mix the contents thoroughly and.
- 5.13 Weigh the RCC bag and record the weight in the register.
- 5.14 Label the primary bag as Red Blood Cells concentrates and keep it in quarantine storage until the results of TTI testing are available.

## 6. DOCUMENTATION

Enter following details in the Component Register

- a) *Date and time of separation*
- b) *Unit number*
- c) *Type of bag used, with batch number and manufacturer's name*
- d) *Weights of whole blood and RCC*
- e) *Date of expiry*
- f) *Type of centrifuge and speed used*
- g) *Blood group and serology code*

Enter in stock register of red cells after the testing is completed and the units are labeled.  
Incident reporting:

If there are any problems encountered during the component processing enter the incident report form and inform the supervisor/medical officer in charge.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

---

<sup>3</sup> Standardize the speed of the centrifuge as it depends on the type of bag, the amount of blood collected and centrifuge in use (*cf. Annex 1*).



# STANDARD OPERATING PROCEDURE

BLOOD PROCESSING LABORATORY		
PREPARATION OF FRESH FROZEN PLASMA /CRYOPRECIPITATE		
BTS/SOP/WP/28	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

For appropriate use of blood it is necessary to use the components as per the need rather than using whole blood only. Red Blood cells, FFP and Platelets are separated by using triple bags. When the plasma frozen at below  $-30^{\circ}\text{C}$  and it is thawed at  $+4^{\circ}\text{C}$  then after a process Cryoglobulin remains as a precipitate which is called Cryoprecipitate. It contains mainly Factor-VIII and fibrinogen.

## 2. RESPONSIBILITY

It is the responsibility of the technician in the blood processing laboratory to separate components from whole blood collected in triple bags system.

## 3. PRINCIPLE

*Plasma, Fresh Frozen* is a component for transfusion or for fractionation prepared either from *Whole Blood* or from plasma collected by aphaeresis, frozen within 6 hours after collection and to a temperature that will adequately maintain the labile coagulation factors in a functional state. Coagulation Factor-VIII is concentrated from freshly collected plasma by cryoprecipitation. Cryoprecipitation is accomplished by slow thawing of FFP at  $+1^{\circ}\text{C}$  to  $+6^{\circ}\text{C}$ .

## 4. EQUIPMENT AND MATERIAL

- Freshly collected whole blood in a blood bag with integrally attached transfer packs.
- Tube sealer/clamp.
- Refrigerated bucket centrifuge with plastic inserts.
- Manual Plasma Expresser.
- Electronic weighing scale/Double pan weighing balance.
- FFP Thawing Bath ( $+2^{\circ}\text{C}$  to  $+6^{\circ}\text{C}$ ).
- Triple bags of average 450ml ( $\pm 10\%$ ) capacity with CPDA-1 as anticoagulant.
- Plasma deep freezer upright below  $-30^{\circ}\text{C}$ .
- Manuals of all equipment for reference regarding use and maintenance of each equipment item.

## 5. PROCEDURE

### 5.1 Preparation of FFP using triple bags:

- 1 Process the whole blood collected within 6 hours of venipuncture for the preparation of Red Blood cell concentrates (*cf. SOP-WP-27*).
- 2 Weigh the primary blood bag and record the weight in the register.
- 3 Label and check the satellite bag with the same donor unit number as that on the primary bag
- 4 Note the weight of the secondary or satellite bag containing platelet rich plasma (PRP) and record in the register
- 5 Balance the two sets of satellite bags in the plastic inserts
- 6 Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge
- 7 Position the bags in buckets parallel to the direction of the spin. Hard spin the bags at 4° C in refrigerated centrifuge at 3500 rpm<sup>4</sup> for 10 minutes
- 8 Place the centrifuged bag on to the plasma expresser stand
- 9 Express the plasma into another attached empty bag leaving 50-60 ml plasma along with the red cells
- 10 Seal the tubing three times and cut the tubing on the middle seal of the plasma bag short (1") to avoid breakage during frozen storage
- 11 Affix a Fresh Frozen Plasma (FFP) component label and record the volume of plasma on the label
- 12 Keep the plasma bag in the quarantine storage in the plasma deep freezer at a temperature below – 30°C
- 13 Transfer to plasma deep freezer in issue area when the TTI tests results are available.

### 5.2 Preparation of Cryoprecipitate:

- 1 The basic material is platelet poor fresh frozen plasma prepared according to 5.1, but with a satellite bag still connected (third bag). The plasma should be free of red cells. Use the plasma frozen at a temperature below -30°C preferably within a day or two of freezing
- 2 Keep the segment of the bags for potential cryo-preparation longer
- 3 Fill the cryobath with daily with tap water
- 4 Maintain the temperature of water bath in continuous circular motion at +9° C
- 5 Keep the frozen plasma bags in this cryobath. When the plasma is thawed, place the bags in centrifuge inserts and balance the inserts on weighing scale
- 6 Keep the position of the bags in inserts parallel
- 7 Hard spin the buckets at 3500rpm<sup>2</sup> for 15 minutes at +4° C

---

<sup>4</sup> Standardize the speed of the centrifuge as it depends on the type of bag, the amount of blood collected and centrifuge in use (*cf. Annex 1*).

- 8 Place the plasma bag on expresser and separate supernatant cryo poor plasma into the attached transfer bag leaving approximately 15-25 ml as cryoprecipitate suspension in the original bag (use scale)
- 9 Seal the tubing and separate the cryoprecipitate and the cryo poor plasma bags.
- 10 Weigh the cryo and plasma bags and record
- 11 Refreeze the cryoprecipitate immediately, i.e. within 1 hour of thawing
- 12 The plasma separated is F-VIII deficient plasma. Both the bags are kept in quarantine till the tests are completed
- 13 Label, enter the inventory and place them in deep freezer at below  $-30^{\circ}\text{C}$  in issue area after test results are available

## 14 DOCUMENTATION

Enter following details in the Component Register

- a. *Date and time of separation*
- b. *Unit number*
- c. *Type of bag used, with batch number and manufacturer's name*
- d. *Weights of blood components*
- e. *Date of expiry of different components*
- f. *Type of centrifuge and speed used*
- g. *Blood group and serology code*

Enter in stock register of FFP/Cryoprecipitate after the testing is completed and the units are labeled.

Incident reporting:

If there are any problems encountered during the component processing enter the incident report form and inform the supervisor/ medical officer in charge.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

BLOOD PROCESSING LABORATORY		
PREPARATION OF PLATELETS		
BTS/SOP/WP/29	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 2 years

### 1. SCOPE AND APPLICATION

For appropriate use of blood it is necessary to use the components as per the need rather than using whole blood only. From triple bags packed cells and platelets are separated.

### 2. RESPONSIBILITY

It is the responsibility of the technician working in blood processing laboratory to separate components from whole blood collected in triple bags system.

### 3. PRINCIPLE

Platelets are prepared by the platelet–rich plasma (PRP) method in which PRP is separated from Whole blood by “soft spin” centrifugation, the platelets are concentrated by “hard spin” centrifugation and the supernatant plasma is subsequently removed.

### 4. EQUIPMENT AND MATERIAL

- Freshly collected Whole Blood with integrally attached transfer packs.
- Tube sealer/clamp.
- Refrigerated centrifuge with plastic inserts.
- Manual Plasma Extractor.
- Electronic weighing scale.
- Double pan weighing balance.
- Platelet incubator/agitator at room temperature.
- Manuals of all equipment for reference regarding use and maintenance of each equipment.

### 5. PROCEDURE

#### 5.1 Preparation of platelet concentrates using triple bags system without additive solution:

- 1 Process the whole blood for component preparation within 6 hours of venipuncture.
- 2 Do not chill the blood at any time before or during platelet separation.

- 3 Weigh the primary blood bag and record the weight in the register.
- 4 Label and check the satellite bag with the same donor unit number as that on the primary bag
- 5 Note the weight of the secondary or satellite bag containing platelet rich plasma (PRP) and record in the register.
- 6 Centrifuge the blood using soft spin at 2000 rpm<sup>5</sup> for 10 minutes at 20°C.
- 7 Express the PRP into the satellite bag intended for platelet storage leaving 50-60 ml plasma along with the red cells.
- 8 Mix the contents thoroughly and seal the tubing between the primary bag and the satellite bag in three places; break the middle seal
- 9 Keep the primary bags containing packed cells in quarantine storage in the blood bank refrigerator at 2 to 6°C
- 10 Centrifuge the platelet rich plasma (PRP) and satellite bag at 20°C using hard Spin at 5000 rpm<sup>3</sup> for 10 minutes after balancing the inserts.
- 11 Express the platelet-poor plasma into the 2<sup>nd</sup> satellite bag leaving 50-60 ml plasma along with the platelets (use scale).
- 12 Seal the tubing at three spots; break the middle seal.
- 13 Leave the platelet concentrates at room temperature on the laminar flow for 1 hour, keeping the label side down.
- 14 Mix the contents of the bag manually to achieve uniform resuspension.
- 15 Place the units to quarantine storage in the incubator at 20°C to 24°C on the lower shelf with continuous gentle agitation.
- 16 After the required test results are available place the platelet concentrates in the upper shelf of the agitator for use (or separate agitator).

## 6. DOCUMENTATION

Enter following details in the Component Register

- a. *Date and time of separation.*
- b. *Unit number.*
- c. *Type of bag used, with batch number and manufacturer's name.*
- d. *Weights of blood components.*
- e. *Date of expiry of different components.*
- f. *Type of centrifuge and speed used.*
- g. *Blood group and serology code.*

Enter in stock register of red cells, FFP and platelets after the testing is completed and the units are labeled.

### Incident reporting:

If there are any problems encountered during the component processing enter the incident report form and inform the supervisor/ medical officer in charge.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

<sup>5</sup> Standardize the speed of the centrifuge as it depends on the type of bag, the amount of blood collected and centrifuge in use (*cf. Annex 1*).



# STANDARD OPERATING PROCEDURE

BLOOD PROCESSING LABORATORY		
LABELING OF BLOOG BAGS AND BLOOD COMPONENTS		
BTS/SOP/WP/30	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

The blood after it is collected remains in quarantine and is released for transfusion only after all tests (blood grouping and TTI screening) are completed. Before these blood bags are taken onto inventory for use they are labeled with relevant information of their identity like unique identity number, ABO and RhD blood group, the name of the blood component and expiry date.

The label is required for identification and retrieval of blood units for use, disposal and follow up in case of adverse reactions.

## 2. RESPONSIBILITY

It is the responsibility of the technician in the Blood Processing Laboratory to label the blood and blood components units.

## 3. MATERIAL

Preprinted adhesive labels for all components printed as per regulatory requirement. The labels are printed and barcoded for all components as per blood groups. Negative labels also have the same labels except the printing with contour text for ABO and black background with white text for Rh-D type.

## 4. PROCEDURE

- 4.1 After collection and processing whole blood and components, units remain in quarantine storage areas.
- 4.2 Once all the reports of blood group and TTI testing are ready, place the bags on a table in chronological order.
- 4.3 Segregate those which are found reactive for any TTI or found unsuitable for use and keep them in the area for disposal. Leave those found suitable for use on the bench for labeling.
- 4.4 Verify the following information, label on the bag as appropriate:

- a. *the facility identification number*
  - b. *the unique identity number*
  - c. *the name of the blood component*
  - d. *the ABO and RhD group*
  - e. *Blood group phenotypes other than ABO and RhD (optional)*
  - f. *the date of collection/donation*
  - g. *the date of expiry*
  - h. *the name of the anticoagulant solution*
  - i. *additional component information: irradiated, etc. (if appropriate);*
  - j. *the volume or weight of the blood component;*
  - k. *the temperature of storage.*
- 4.5 After the bags are labeled, ask a second technician to double check the number and group on the bags tallying them with the records
- 4.6 Enter all labeled bags group wise in the stock book which is also maintained group wise. In the stock book keep a footnote for any autologous blood that is reserved for the patient's own use
- 4.7 Label FFP and Cryo-deficient plasma, and Platelet concentrates in the same manner. Cryoprecipitate labels do not indicate blood groups
- 4.8 The expiry date depends on the type of bag and component
- 4.9 RCC, with CPDA-1: 35 days
- 4.10 Platelet concentrate: 3 days in PVC bags, 5 days in special bags
- 4.11 FFP and Cryo: 1 year
- 4.12 The day of blood collection is considered the day zero for calculating the expiry dates

## 5. DOCUMENTATION

Enter all labeled bag numbers in the inventory of units for use.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

# **Storage of Blood Components**

# Standard Operating Procedures

## STORAGE AND DISTRIBUTION OF BLOOD COMPONENTS

### STORAGE OF BLOOD COMPONENTS

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## STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
STORAGE OF RED CELL CONCENTRATES		
BTS/SOP/WP/31	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the storage period.

### 2. RESPONSIBILITY

It is the responsibility of the technical staff in the blood processing laboratory to keep the units in the quarantine storage. The technologist who labels the units after the testing and processing is responsible to transfer the labeled units to their respective storage areas.

### 3. MATERIAL REQUIRED

- Blood Bank Refrigerator (360 bag Capacity) with glass front. (+2°C to +6°C)

### 4. PROCEDURE

- 4.1 All untested units should be kept in the quarantine area.
- 4.2 After testing is over (blood grouping and TTI screening), release the fully tested bags to their appropriate storage areas. Transfer those suitable for clinical use from quarantine area to the stock area after labeling according to SOP: BTS/SOP/WP/30: "LABELING OF BLOOD BAGS AND BLOOD COMPONENTS"
- 4.3 Label those found unsuitable for use with a biohazard label and keep for disposal.
- 4.4 Keep whole blood and Red Cell concentrates at a controlled temperature between +2°C to +6°C.
- 4.5 Arrange the blood units inside the fridge properly to allow easy circulation of cold wave.
- 4.6 Each shelf of the refrigerator is reserved for a particular group having its label stuck on the outer side. Arrange the blood bags in chronological order according to the expiry dates in the shelves (FIFO=first in, first out). This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required.
- 4.7 In this way, the blood collected is issued first and thus chances of stored blood reaching expiry date are minimized.
- 4.8 Store blood collected in CPDA-1 without additive solution and the red cell concentrates (RCC) separated in a closed system up to 35 days from time of collection.

- 4.9 Take due care to maintain sterility of all components by keeping all storage areas clean.
- 4.10 Monitor to ensure the storage conditions to be appropriate and keep monitoring the temperature of all refrigerators with continuous automatic graphic recorder or by electronic data recording systems.
- 4.11 Make sure the alarm system is working.
- 4.12 Carry out physical stock taking every night and rewrite the inventory.

**5. DOCUMENTATION**

Record all whole blood and RCC released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
TITLE: STORAGE OF FRESH FROZEN PLASMA/CRYOPRECIPITATE		
BTS/SOP/WP/32	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE & APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the whole storage period.

## 2. RESPONSIBILITY

It is the responsibility of the technical staff in the blood processing laboratory to keep the units in the quarantine storage. The technologist who labels the units after the testing and processing is responsible to transfer the labeled units in their respective storage areas.

## 3. EQUIPMENT REQUIRED

- Plasma Deep Freezer Upright at a temperature below  $-30^{\circ}\text{C}$

## 4. PROCEDURE

- 4.1 Keep all untested units in the quarantine storage in the plasma deep freezer placed in the blood processing laboratory.
- 4.2 After testing is over (blood grouping and TTI screening), release the fully tested bags to their appropriate storage areas. Transfer those suitable for clinical use from quarantine area to the stock area after labeling according to SOP: BTS/SOP/WP/30: "LABELING OF BLOOG BAGS AND BLOOD COMPONENTS"
- 4.3 Label those found unsuitable for use with a biohazard label and keep for disposal.
- 4.4 Store Fresh Frozen Plasma/cryoprecipitate in deep freezer below  $-33^{\circ}\text{C}$  for one year.
- 4.5 Arrange the blood bags in chronological order, group wise and according to the expiry dates on the shelves (FIFO=first in, first out). This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required.
- 4.6 Keep Fresh Frozen Plasma, cryoprecipitate and Factor-VIII deficient plasma bags in over wrap bags and then arrange in plastic trays in the Deep Freezer immediately after separation.
- 4.7 Use Fresh Frozen Plasma/Cryoprecipitate as soon as possible after thawing in order to preserve labile factors.
- 4.8 Do not refreeze FFP/Cryoprecipitate once thawed.
- 4.9 Take due care to maintain sterility of all components by keeping all storage areas clean.

- 4.13 Monitor to ensure the storage conditions to be appropriate and keep monitoring the temperature of all storage areas with continuous automatic graphic recorder or by electronic data recording systems.
- 4.10 Make sure that the alarm system is working.
- 4.11 After labeling the plasma bags, enter the unit numbers group wise in the stock register.
- 4.12 Carry out physical stock taking every night and rewrite the inventory.

**5. DOCUMENTATION**

Record all blood/components released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
STORAGE OF PLATELETS		
BTS/SOP/WP/33	REGIONAL BLOOD CENTER	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE & APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the whole storage period.

## 2. RESPONSIBILITY

It is the responsibility of the technical staff in the blood processing laboratory to keep the units in the quarantine storage. The technologist who labels the units after the testing and processing is responsible to transfer the labeled units in their respective storage areas.

## 3. MATERIAL REQUIRED

Platelet incubator  
Platelet agitator

## 4. PROCEDURE

- 4.1 All untested units should be kept in the quarantine area, i.e. in the lower shelf of Platelet Incubator.
- 4.2 After the required test results are available, transfer the platelet concentrates from quarantine area to the stock area, i.e. in the upper shelf of the agitator for use.
- 4.3 Label those found unsuitable for use with a biohazard label and keep for disposal.
- 4.4 Store Platelets in the platelet incubator at +20<sup>0</sup>C to +24<sup>0</sup>C under constant and gentle agitation.
- 4.5 Arrange the blood bags in chronological order, group wise and according to the expiry dates in the shelves. This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required.
- 4.6 Store platelet concentrates in a closed system up to 5 days.
- 4.7 When an open system has been used for preparation of platelets, the storage time must not exceed 6 hours.
- 4.8 Take due care to maintain sterility of all components by keeping all storage areas clean.
- 4.9 Monitor to ensure the storage conditions to be appropriate and correct for each product. Monitor the temperature of agitator on manual charts recorder or by electronic data recording systems.
- 4.10 Carry out physical stock taking every night and rewrite the inventory.

## 5. DOCUMENTATION

Record all blood/components released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

# **Distribution to HBB**



# STANDARD OPERATING PROCEDURE

DISTRIBUTION TO HBB		
RECEPTION AND DOCUMENTATION OF REQUESTS FROM HBB		
BTS/SOP/WP/34	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure ensures that the request forms are received from the hospital blood bank on weekly basis.

## 2. RESPONSIBILITY

Issuing Clerk from the Inventory and Distribution sections of Regional Blood Centre is responsible to receive the request form and document in the entry register or BTIS.

## 3. MATERIAL

- Request Form
- Stock Register
- Entry Register

## 4. PROCEDURE

- 4.1 Record the hospital name, number, blood groups and product names required, in an entry register and/or BTIS.
- 4.2 Check the stock register for the availability of units.
- 4.3 Issue the blood and blood products according to the established policy.

## 5. DOCUMENTATION

Document the following:

- *Hospital name/Health facility unique ID*
- *Number, blood group and products required*
- *Initials of the issuing clerk*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

DISTRIBUTION TO HBB		
DISPATCHING AND TRANSPORTING COMPONENTS		
BTS/SOP/WP/35/36/37	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applies to all blood and blood components that needs to be transported to Hospital Blood Banks. Validated shipping containers suited for different products are critical to this process.

### 2. RESPONSIBILITY

Technician from Regional Blood Centre is responsible to carry out dispatching, and transportation, including temperature monitoring of the transport boxes.

### 3. PROCEDURE

#### 3.1 Red Cell Concentrates

- 1 Select an appropriate transport container. Visually inspect the integrity of the outer of the blood container, the strap and the inner side of the container. Also ensure the inner container is clean and dry
- 2 Document transport container inspection on release voucher (form in duplicate).
- 3 Remove any existing transport labels
- 4 Fill out a transport label and place into an envelope attached to the transport container.
- 5 Remove the appropriate blood/blood component from the refrigerator.
- 6 List the donor unit number, ABO/Rh type, product name and expiry date of each unit to be transported on the release voucher (in duplicate)
- 7 Place the units in the container and finalize with recording the time, name and signature of packer on both forms
- 8 Place a gel pack that has been stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for at least 24 hours on top of the red cells in the transport container.
- 9 Close the container and transport to the Hospital Blood Bank.
- 10 Transportation between facilities should not exceed the validated transport time for the transport containers used.
- 11 The hospital blood bank will sign for receiving the requested blood products on the second form
- 12 The duplicate form must be brought back to the BTS and stored in the appropriate way.

### **3.2 Fresh Frozen Plasma**

- 1 Select an appropriate transport container. Visually inspect the integrity of the exterior of the blood container, the strap and the inner side of the container. Also ensure the inner container is clean and dry
- 2 Document transport container inspection on release voucher (form in duplicate).
- 3 Remove any existing transport labels
- 4 Fill out a transport label and place into an envelope attached to the transport container.
- 5 Remove the appropriate fresh frozen plasmas from the freezer
- 6 List the donor unit number, ABO/Rh type, product name and expiry date of each unit to be transported on the release voucher (in duplicate)
- 7 Place the units in the container and finalize with recording the time, name and signature of packer on both forms
- 8 Place a gel pack that has been stored at  $-25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  or lower for at least 24 hours
- 9 Close the container and transport immediately to the Hospital Blood Bank.
- 13 Transportation between facilities should not exceed the validated transport time for the transport containers used.
- 10 The hospital blood bank will sign for receiving the requested blood products on the second form
- 11 The duplicate form must be brought back to the BTS and stored in the appropriate way.

### **3.3 Platelet Concentrates**

- 1 Select an appropriate transport container. Visually inspect the integrity of the outer of the blood container, the strap and the inner side of the container. Also ensure the inner container is clean and dry
- 2 Document transport container inspection on release voucher (form in duplicate).
- 3 Remove any existing transport labels
- 4 Fill out a transport label and place into an envelope attached to the transport container.
- 5 Remove the appropriate platelet concentrates from the agitator.
- 6 List the donor unit number, ABO/Rh type, product name and expiry date of each unit to be transported on the release voucher (in duplicate)
- 7 Place the units in the container and finalize with recording the time, name and signature of packer on both form
- 8 Place a second gel pack that has been stored at  $20\text{-}24^{\circ}\text{C}$  for at least 24 hours on top of the bag of platelet concentrates
- 9 Close the container and transport immediately to the Hospital Blood Bank.
- 14 Transportation between facilities should not exceed the validated transport time for the transport containers used.

#### **NOTE:**

Use temperature monitoring devices in one or more shipping containers in each shipment of blood and blood products as documented evidence that environmental specifications have been met.

#### 4. DOCUMENTATION

The transportation container must be labeled with a minimum of the following information:

- a) *Contents (blood components)*
- b) *Originating location*
- c) *Destination location*
- d) *Any cautions or descriptions for the containers*

Records that maintain the chain of traceability must be kept so that it is possible to trace all blood components from their source to final disposition. This includes:

- a) *the name of the facility receiving the blood components*
- b) *a unique tracking number for the distribution*
- c) *the type of blood components to be distributed*
- d) *the donation number of each blood component*
- e) *the total number of items distributed*
- f) *the date and time of transportation and receiving at the hospital*
- g) *special instructions that pertain to the transportation or unit(s) within the transport the signature of the person responsible for packing the transportation container*

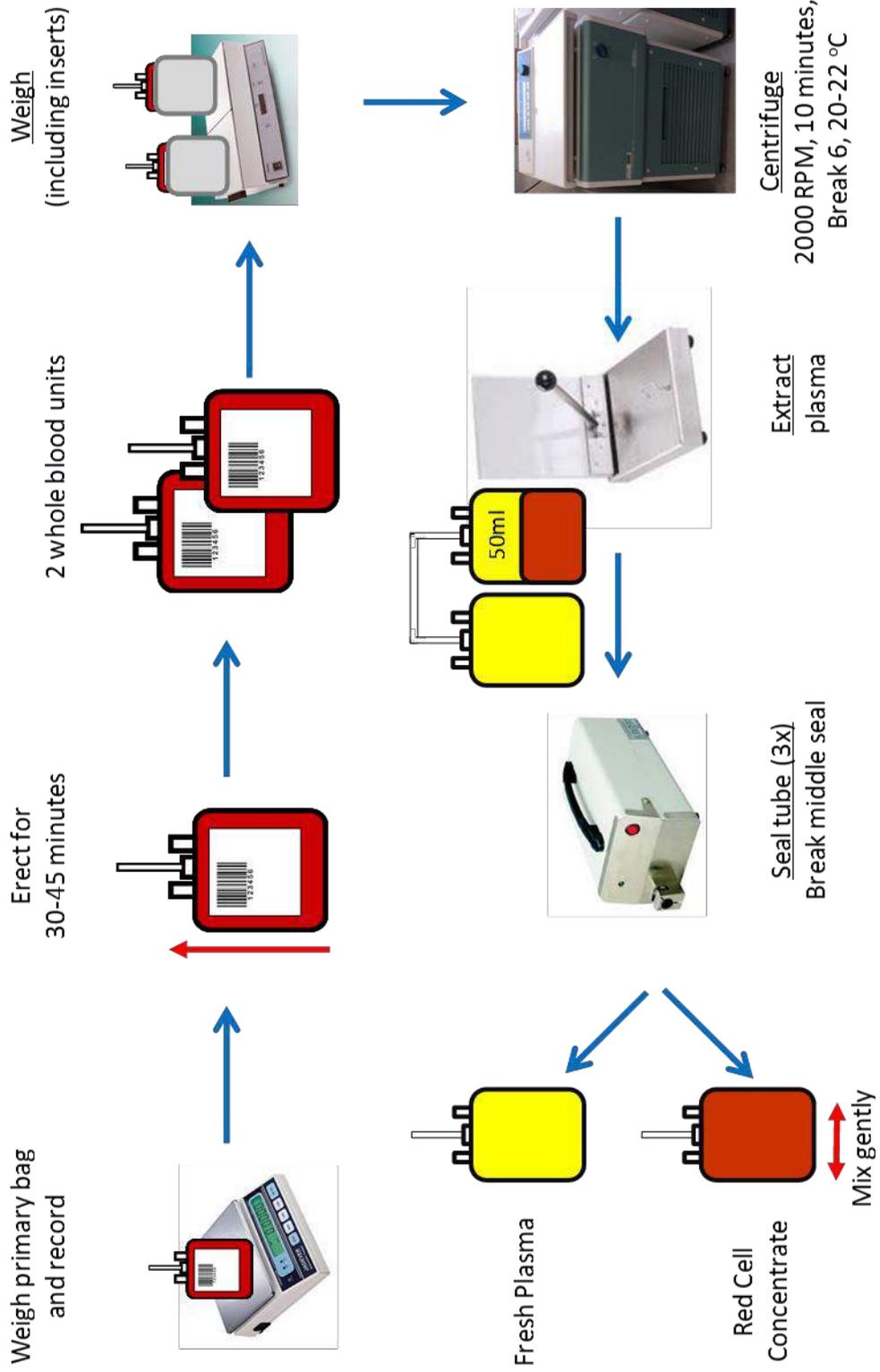
Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

## Annex 1

Formula for calculating relative centrifugal force (RCF)	
$\text{RCF} = 28.38 \times R \times (\text{RPM}/1000)^2$	$\text{RPM} = \sqrt{[\text{RCF}/(28.38 R)]} \times 1000$
Where: <b>RCF</b> = relative centrifugal force ( $\times g$ ) <b>R</b> = radius in inches <b>RPM</b> = revolutions per minute	

# PREPARATION OF RED BLOOD CELLS CONCENTRATES (RCC)

Sop: BTS/SOP/WP/27



# Standard Operating Procedures

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# **Reception of Blood Components**



## STANDARD OPERATING PROCEDURE

<b>RECEPTION OF BLOOD COMPONENTS</b>		
<b>TITLE: RECEPTION AND INSPECTION OF BLOOD BAGS</b>		
<b>BTS/SOP/WP/38-39</b>	<b>HOSPITAL BLOOD BANK</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure ensures that the products received are documented and visually inspected for signs of damage, contamination, spoilage and haemolysis.

### 2. RESPONSIBILITY

Technician from hospital Blood Bank is responsible to carry out the documentation and inspection of the units received.

### 3. MATERIAL

- Blood products to be inspected
- Entry register

### 4. PROCEDURE

- 4.1 Record the date, unit number and product type of units received in a record register and BTIS.
- 4.2 Check each product whether it is in date (within expiry date).
- 4.3 Inspect the blood units visually for the following:
  - a) *Appearance: No leakage, no discoloration and no clot.*
  - b) *Inspect the FFP bag for signs of breakage*
  - c) *At least two segments of integral donor tubing is attached*
  - d) *The blood bag closure is undisturbed*
- 4.4 Any blood unit not meeting the above criteria must not be accepted and returned to the BTC with reasons

### 5. DOCUMENTATION

Document the following:

- a) *Tracking number of the transportation*
- b) *Unit number and type of blood components received*
- c) *Date and time of reception*
- d) *Any sign of spoilage*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

# **Storage of Blood Components**



## STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
STORAGE OF RED CELL CONCENTRATES		
BTS/SOP/WP/40	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the storage period.

### 2. RESPONSIBILITY

It is the responsibility of the technical staff in the hospital blood bank to keep the units in the respective storage areas.

### 3. MATERIAL REQUIRED

- Blood Bank Refrigerator (120 or 240 Bags Capacity) with glass front

### 4. PROCEDURE

- 4.1 All uncrossed and matched units should be kept in the storage area.
- 4.2 After incompatibility testing is over, release the fully compatible bags. Transfer those suitable for clinical use from storage area to the issuance area after labeling with the results of cross match.
- 4.3 Restore units unsuitable for transfusion in the storage area: the unit can be used for another patient.
- 4.4 If any spoilage is detected or expired units found label the unit with a biohazard label and keep for disposal. Record the spoilage.
- 4.5 Keep whole blood and Red Cell concentrates at a controlled temperature between +2°C to +6°C.
- 4.6 Arrange the blood unit inside the fridge properly to allow easy circulation of cold wave.
- 4.7 Each shelf of the refrigerator is reserved for a particular group having its label stuck on the outer side. Arrange the blood bags in chronological order according to the expiry dates in the shelves. This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required.
- 4.8 In this way, the blood collected first is issued first and thus chances of stored blood reaching expiry date are minimized (FIFO=first in first out).
- 4.9 Store blood collected in CPD-A1 without additive solution and the red cell concentrates separated up to 35 days from date of collection.

- 4.10 Take due care to maintain sterility of all components by keeping all storage areas clean.
- 4.11 Monitor to ensure the storage conditions to be appropriate and keep monitoring the temperature of all refrigerators with continuous automatic graphic recorder or by electronic data recording systems.
- 4.12 Make sure the alarm system is working.
- 4.13 Carry out physical stock taking every night and rewrite the inventory.

**5. DOCUMENTATION**

Record all whole blood and RCC released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
STORAGE OF FRESH FROZEN PLASMA/CRYOPRECIPITATE		
BTS/SOP/WP/41	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date	Review Period: 1 Year

### 1. SCOPE & APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the whole storage period.

### 2. RESPONSIBILITY

It is the responsibility of the technical staff in the hospital blood bank to keep the units in the storage area.

### 3. EQUIPMENT REQUIRED

- Plasma Deep Freezer Upright at a temperature below -30°C

### 4. PROCEDURE

- 4.1 Keep all units in the storage/stock area in the plasma deep freezer placed in the hospital blood bank.
- 4.2 If any spoilage is detected or expired units found label those found unsuitable for use with a biohazard label and keep for disposal. Record the spoilage.
- 4.3 Store Fresh Frozen Plasma/cryoprecipitate in deep freezer below -30°C or colder for one year.
- 4.4 Arrange the blood bags in chronological order, group wise and according to the expiry dates on the shelves. This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required. (FIFO = first in first out).
- 4.5 Keep Fresh Frozen Plasma, cryoprecipitate and Factor-VIII deficient plasma bags in over wrap bags and then arrange in plastic trays in the Deep Freezer immediately after reception in the hospital blood bank.
- 4.6 Use Fresh Frozen Plasma/Cryoprecipitate as soon as possible after thawing in order to preserve labile factors.
- 4.7 Do not refreeze FFP/Cryoprecipitate once thawed.
- 4.8 Take due care to maintain sterility of all components by keeping all storage areas clean.
- 4.9 Monitor to ensure the storage conditions to be appropriate and keep monitoring the temperature of all storage areas with continuous automatic graphic recorder, or by electronic data recording systems.
- 4.10 Make sure that the alarm system is working.
- 4.11 Carry out physical stock taking every night and rewrite the inventory.

## 5. DOCUMENTATION

Record all units released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
STORAGE OF PLATELETS		
BTS/SOP/WP/42	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE & APPLICATION

Blood components prepared are stored in conditions designed to preserve optimal viability and function during the whole storage period.

### 2. RESPONSIBILITY

It is the responsibility of the technical staff in the hospital blood bank to keep the units in the storage area.

### 3. MATERIAL REQUIRES

- Platelet incubator with agitator

### 4. PROCEDURE

- 4.1 If spoilage is detected or expired units found label those found unsuitable for use with a biohazard label and keep for disposal.
- 4.2 Store Platelets in the platelet incubator at +20°C to +24°C under constant and gentle agitation and the following conditions:
  - a. All labels on the packs face downwards.
  - b. No unit is allowed to lay on top (partly or totally) in another unit.
- 4.3 Arrange the blood bags in chronological order, group wise and according to the expiry dates in the shelves. This makes it very easy for the technologists on duty to remove the bags for issuing, whenever required (FIFO = first in first out).
- 4.4 Store platelet concentrates in a closed system up to 5 days.
- 4.5 When an open system has been used during preparation of platelets, the storage time must not exceed 6 hours.
- 4.6 Take due care to maintain sterility of all components by keeping all storage areas clean.
- 4.7 Monitor to ensure the storage conditions to be appropriate and correct for each product. Monitor the temperature of agitator on manual charts, or by electronic data recording systems.
- 4.8 Carry out physical stock taking every night and rewrite the inventory.

## 5. DOCUMENTATION

Record all units released for use as well as the unsuitable units to be discarded in the disposal register.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS		
THAWING OF FFP		
<b>BTS/SOP/WP/43</b>	<b>HOSPITAL BLOOD BANK</b>	<b>Version: 1.0</b>
<b>Valid from:</b>	Effective Date:	Review Period: 1 Year

## 1. SCOPE & APPLICATION

The Fresh Frozen Plasma (FFP) is thawed at warm temperature before issuance to the wards. If the thawed FFP is immediately transfused, it remains rich in clotting factors.

## 2. RESPONSIBILITY

Laboratory technicians in the hospital blood bank are responsible to thaw the plasma before issuance.

## 3. MATERIAL REQUIRES

- Plasma thawer

## 4. PROCEDURE

- 4.1 Adjust the temperature of the chamber at 36.5°C.
- 4.2 Press the Basket Access Button to raise the basket assembly if it is still lowered into the chamber bath.
- 4.3 Insert a frozen plasma bag into a Plasma Overwrap and place it into the basket assembly. Make sure that the metal finger tab on the top of the basket assembly is inserted through the slot in the top of the Plasma Overwrap.
- 4.4 Press the Time Set Button to advance through the pre-programmed times until the desired cycle time is selected.
- 4.5 Press the Start/Stop Button to begin the thawing cycle. The basket assembly will automatically lower into the chamber bath and begin agitating.
- 4.6 After the thawing cycle is completed the agitation will stop, the basket assembly will automatically lift out of the chamber, a tone will sound five times, and the cycle Time Indicator will reset itself in preparation for the next thawing cycle.
- 4.7 For QC, verify that the digital temperature controller is calibrated correctly allow the chamber temperature to stabilize and then take a temperature reading from a calibrated thermometer inside the chamber.

## 5. DOCUMENTATION

Record all FFPs thawed and issued for use.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

# **Issuance of Blood Components**



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
RECEIVING CLINICAL REQUEST FORMS AND BLOOD SAMPLES		
BTS/SOP/WP/44	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

The blood and blood components are used as per need of the patients. These are issued against the blood ordering request of an ordering physician after ensuring the compatibility and testing results. The purpose of pre-transfusion ordering and testing is to select blood components that will not harm the recipient and that will have acceptable survival when transfused.

### 2. RESPONSIBILITY

It is the responsibility of technician working on shift duty in hospital blood bank to receive the blood ordering request and patient's samples. It is confirmed by senior staff that the information on the labels and on the transfusion request is identical.

### 3. PRINCIPLE

Requests for blood and blood components are submitted in an electronic, or written format in order to prevent inappropriate use of blood and blood components. Requests must contain sufficient information for accurate recipient identification (ID). Collection of a properly labeled samples from the intended recipient is critical to safe blood transfusion. Most hemolytic reactions result from errors in sample or patient's identification, the person drawing the blood sample must identify the intended recipient in a positive manner. Requests for blood or blood components that 1) lack the required information, 2) are inaccurate, or 3) are illegible are not accepted. Verbal requests are acceptable in urgent situations but should be documented later.

### 4. MATERIAL

- Blood Request Form
- Inventory Register
- Cross-match register
- Patient's blood samples

## 5. PROCEDURE

### Receive Blood Ordering Request Form according to following check list:

- A. Check who ordered blood or blood components?  
Only a physician can initiate an order to administer blood or blood components.
- B. Check how blood or blood components are ordered?  
The physician's order must be written on a *Blood Request Form* (that is sent to the Blood Bank), and shall specify:
1. Patient's full name
  2. Patient's unique hospital number
  3. Patient's date of birth/age
  4. Patient's gender/sex
  5. Patient's diagnosis or clinical summary
  6. Patient's past transfusion/pregnancy history (if applicable)
  7. Type of desired blood component (e.g., RBCs, FFP/Cryoprecipitate, Platelets, etc.)
  8. Indication for transfusion
  9. Number of units to be transfused
  10. Date and Time when product is required
  11. Name of requesting physician
  12. Date and time of the request
  13. Signature of the prescribing physician
- C. Check samples  
Patient's blood samples are submitted to the Blood Bank along with the request form for blood or blood components
1. Patient's blood sample consists of one 5 mL red top (clot) tube and 5 ml purple top (K3EDTA) tube. In some cases less sample is allowed (e.g. children) in agreement with the physician
  2. Patient's blood samples must be obtained in such a way that identity of the patient is confirmed.
  3. Phlebotomist/staff nurse who draws patient's blood samples must do the following:
    - a. *Label sample tube with patient's first/last name, birth date and preferably the hospital admission number*  
*This must be done by blood drawer (phlebotomist) before leaving patient's bedside*  
**NOTE:** *Name/age on the tubes must perfectly match those on the Blood Request Form.*
    - b. *Mention date and time of sample collection*
    - c. *Signature of person taking sample*
  4. Confirm sample identity in the Blood Bank, If there is any doubt about identity of patient and specimen integrity e.g.:  
Label is not complete  
Sample mislabeled  
Information on label and blood request form do not match  
**NOTE:** *Incorrectly labeled samples must never be corrected in Blood Bank but a new sample must be drawn.*
  5. Appearance of sample

- a. Whenever possible, a haemolyzed sample should be replaced with a new specimen
- b. The same applies to markedly lipemic plasma
- 6. Age of sample:  
When samples are intended for use in cross-matching, they must be not older than 3 days (Exception: if patient has not been transfused or pregnant in the past 3 months)
- 7. Retaining and storing sample
  - a. Recipient's blood specimen and sample of donor's red blood cells must be sealed and stored in refrigerator (4-6 °C) for less than 7 days following transfusion.
  - b. Keeping patient's and donor's samples allows repeat or additional testing if patient experiences adverse effects of transfusion
- D. Check the availability of blood and blood components  
Check the inventory register for required blood group and/or components.  
Check physical availability in storage cabinets.
- E. Send the sample for pre-transfusion testing for ABO Group, Rh-D-type, antibody screening (if needed antibody identification) and compatibility testing.

## 6. DOCUMENTATION

Do initial with date and time on the form at receiver's column.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
ABO GROUPING AND Rh D TYPING BY TUBE TEST		
BTS/SOP/TP/45a	HOSPITAL BLOOD BANKS	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies to all those activities that are performed to determine the correct ABO group and Rh D type of a donor and ensuring the reliability of the results. This procedure describes the method of detection of the presence or absence of A, B & D antigens on red cells by using Anti-A, Anti-B and anti D antisera (antibodies) against the corresponding antigens. The Anti A & Anti B are monoclonal IgM antibodies specific against A & B red cell antigen. Anti D is also monoclonal which may be purely IgM or a blend of IgG and IgM (blend preferably). Reverse blood grouping should always be run in parallel with forward ABO typing for group confirmation Mismatch transfusion of ABO/D blood group can cause fatal transfusion reactions and sensitization against transfused D positive antigens in Rh D negative individuals especially in child bearing age females where it may cause haemolytic disease on the new born.

## 2. RESPONSIBILITY

In the Immunohaematology Laboratory following staffs are responsible for this procedure:

Trained Technician is responsible to perform the ABO grouping and RhD typing of donors.

Technologist is responsible to verify the results.

Medical Officer is responsible to supervise the procedure and to rule out any blood group discrepancy by further workup.

It is the responsibility of all staff performing the ABO grouping and D typing to ensure that quality controlled reagents, proper cell concentrations and calibrated centrifuges are used

## 3. PRINCIPLE

ABO system is the only system in which there is a reciprocal relationship between the antigen on the red cells and the naturally occurring antibodies in the serum. Routine grouping of donors must therefore include both red blood cells and serum tests, each serving as check on the other.

### Forward Blood Grouping / Cell Grouping / Front Type Grouping

Known antibodies (commercially prepared anti A and anti B) are reacted with unknown antigens on the red blood cells of a patient or donor is called Forward Blood Grouping / Cell Grouping / Front Type Grouping.

Direct agglutination of unknown antigens on red cells (of patient / donor) with a particular reagent (known anti A or anti B) indicates the presence of corresponding antigen, and the blood group is termed as “A” / “B” or “AB”. No agglutination indicates the absence of A, B or AB antigens and the blood group is termed as “O”.

#### Reverse Blood Grouping / Serum Grouping / Back Type Grouping

Unknown antibodies (anti A / anti B or both) present in the donor / patient serum or plasma are reacted with known red cell antigens (A, B and O red cells) is called reverse / serum / back typing grouping. All normal individuals have naturally occurring antibodies opposite to their antigens present on the red cells. For example; agglutination of the donor or patient serum / plasma with A cells indicates that the blood group is B, agglutination with B cells indicates A group, agglutination with both A and B cells indicates O group and no agglutination with A or B cells indicates that the blood group is AB. Donor or patient serum / plasma should not show any agglutination with O red cell of reverse blood grouping. If agglutination is seen with O cells than blood group should be considered as a discrepant and further workup is necessary (for confirmation of Bombay or allo / auto antibodies).

After ABO Blood Group System Rh D is the most immunogenic. The expression of Rh “D” positive or Rh “D” negative is based on the agglutination with anti D antisera.

## **4. MATERIAL**

### **4.1 EQUIPMENT**

- Refrigerator to store samples and reagents at 2- 6°C.
- Calibrated table top centrifuge.
- Lighted agglutination viewer

### **4.2 SPECIMEN**

- Two Blood Samples; properly labeled, 2-3 cc, one EDTA (purple), one clotted (red)
- Freshly drawn blood sample is preferred but it should not be older than 14 days.
- Blood sample should not be haemolysed

### **4.3 REAGENTS**

Commercially available monoclonal antisera: Anti-A, Anti-B, and Anti D<sup>6</sup>

- Rh control: 6% Bovine Albumin OR commercially available Rh control (*cf. Annex 4*)
- Fresh 0.9% normal saline in washing bottle.
- Prepare 3%-5% red cell suspension of donor red cells (60 microliters or one drop of washed packed red cells in 2 ml 0.9% normal saline) OR Prepare 5% red cell suspension of donor cells (100 microliters OR 2 drops of washed packed red cells in 2 ml 0.9% normal saline) for reverse grouping A<sub>1</sub>, B & O cells. All A/B/O reverse blood grouping cells should be Rh “D” negative to avoid agglutination with anti D formed in

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<sup>6</sup> Follow the manufacturer's instructions for the use of the reagent. Anti-D reagents that do not detect Dvi is to be selected for recipient's sample.)

sensitized individuals like pregnant women. Otherwise the anti D will react/agglutinate with all A/B/O positivewith reverse grouping cells.

#### 4.4 MISCELLANEOUS

- Adjustable pipet, 50 -100 microliters, OR plastic dropper
- Tips
- Test tubes (12 x 75mm).
- Test tube rack
- Permanent Markers
- Timer
- 2 plastic beakers.

### 5. PROCEDURE

#### 5.1 RED BLOOD CELLS TESTING / FORWARD GROUP TESTING

IMPORTANT: check the actual volumes/drops and procedure steps in the inserts provided with the antibodies; these can differ from lot to lot number!

- Label 4 clean test tubes with A, B, auto and D along donor ID (auto=auto control)
- Arrange the test tubes in a row
- Prepare cell suspension for cells being tested (*cf. Annex 1*)
- Dispense one drop of anti-A, anti-B, and anti-D in the appropriately labeled tubes A, B and D respectively.
- Dispense two drops of donor plasma or serum to the tube marked “auto”
- Add to each test tube one drop of a 3-5% red cell suspension to tubes labeled as A, B, auto and D.
- Mix the contents of the tubes gently and centrifuge immediately after balancing at 3400rpm for 15 seconds.
- Gently take out the tubes and re-suspend the red cell button.
- Examine individually each tube macroscopically for agglutination. (*cf. Annex 2*)
- Grade and record test results. (*cf. Annex 3*)

#### 5.2 SERUM TESTING / REVERSE GROUP TESTING

- 1 Label 3 clean test tubes with A1, B, and O along with unique donor ID
- 2 Arrange the test tubes in a row
- 3 Centrifuge donor blood specimen to get clear serum / plasma for reverse grouping.
- 4 Add 2 drops of donor serum in all tubes in the corresponding tube
- 5 Add one drop of known 3% or 5% reverse grouping red cell suspension of A, B & O cells to tubes labeled as A1, B and O.
- 6 Mix the contents and centrifuge all tubes immediately after balancing, at 3400 rpm for 15 seconds
- 7 Gently take out the tubes and re-suspend the red cell button.
- 8 Examine individually each tube macroscopically for haemolysis and agglutination (*cf. Annex 2 & 3*)
- 9 Grade and record test results.

### 5.3 CONTROLS FOR Rh D GROUPING

Rh Control should be tested in parallel with “Rh-D-Positive” cases for true identification of Rh D blood grouping and not due to auto antibodies.

- 1 Take a clean labeled test tube
- 2 Dispense one drop of 6% Bovine Albumin or commercially available Rh control
- 3 Add one drop of 3-5% donor specimen.
- 4 Mix and centrifuge at 3400 rpm for 15 seconds
- 5 Take out the tube gently, read macroscopically Document result.

#### Result Interpretation:

Results must be negative because 6% Bovine Albumin does not contain any antibodies. If the result is Positive than the case is referred to the shift incharge/head of the Hospital Blood Bank to solve Rh D discrepancy.

### 6. RESULTS OF ABO/RhD BLOOD GROUPING

POSITIVE: Agglutination / Mixed Field/ Haemolysis

NEGATIVE: No agglutination/No Mixed Field/No Haemolysis

Confirm the ABO cell grouping results with those obtained in serum/reverse grouping and vice versa.

### 7. INTERPRETATION

7.1 Agglutination/Mixed Field in any tube of Red Blood Cells tests and agglutination, mixed field or haemolysis in serum test constitutes a positive test result. The expected agglutination reaction for positive tests are 3+ to 4+.(**cf. Annex 2 & 3**)

7.2 A smooth suspension of Red Blood Cells after resuspension of Red Blood Cells button is a negative test result. The interpretation of ABO group is as follows:

Reaction of Red Cells with Antisera/ Forward Group Typing				Reaction of Serum with reagent Red Cells/Reverse Group Typing			Interpretation of group	
Anti-A	Anti-B	Rh D	auto	A1 Cells	B Cells	O Cells	ABO	Rh D
+++	-	+++	-	-	+++	-	A	Pos
-	+++	+++	-	+++	-	-	B	Pos
+++	+++	+++	-	-	-	-	AB	Pos
-	-	+++	-	+++	+++	-	O	Pos
+++	-	-	-	-	+++	-	A	Neg*
-	+++	-	-	+++	-	-	B	Neg*
+++	+++	-	-	-	-	-	AB	Neg*
-	-	-	-	+++	+++	-	O	Neg*
Positive(+++)= Agglutination/lysis/Mixed Field								
Negative(-)= No Agglutination/lysis/Mixed Field								
* Proceed with weak D (Du) Typing using indirect anti-globulin technique in case of donor blood sample.(Refer to SOP of weak D Test; BTS/SOP/TP/24a)								

- 7.3 If any of the following discrepancies occur, the sample should be handed over to the Medical Officer in charge: **(cf. SOP 22)**  
*There is a positive reaction in the reverse grouping with O cells.*  
*D- control is positive.*  
*Auto- control is positive.*  
*There is a discrepancy between the forward and reversed ABO blood grouping.*  
*There is a discrepancy between the results of the two tubes for Rh D grouping.*
- 7.4 Any discrepancy between results on cell and serum or plasma tests should be resolved before an interpretation is recorded for the donor's ABO group.

## 8. DOCUMENTATION

Enter the results of patient's grouping in the patient record register, blood group requisition form, serial case number register and BTIS.

### SOURCES OF ERRORS

	False Negative	False Positive
1.	Centrifugation time too short	Over centrifugation
2.	Reagent or Serum not added	Incorrect Interpretation
3.	Inappropriate ratio of serum / reagent to cells	Used dirty glass ware
4.	Wrong technique, not following manufacturer advice	Used contaminated reagents, cells, normal saline
5.	Haemolysis not identified as positive reaction	Cells contaminated with Wharton's jelly
6.	In correct interpretation	Incorrect interpretation
	Weak D Test not performed	False Positive weak D test, due to positive DAT

#### NOTE:

- 1 All reagents should be used according to the manufacture's advice within expiry date.
- 2 Do not run large batches, each batch should not be more than five samples.
- 3 Perform both Forward and Reverse Blood Grouping.
- 4 All reagents / antisera should be stored at 2 – 8 °C when not in use.
- 5 Quality control of all reagents cells / antisera should be performed on daily basis.
- 6 Use high titered antisera; titration of Anti A should be 1:256, Anti B 1:256 and Anti D 1:128. The expected agglutination reaction for positive tests with undiluted antisera are 3+ to 4+
- 7 Confirm possible Bombay blood group with Anti-H.
- 8 After centrifugation, all tubes should be read immediately as delay may cause dissociation of antigen antibody complex leading to false negative or weak positive results.

9 Discrepant results should be informed to the shift in charge or head of the blood bank.

10 All steps should be done immediately one after the other.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
EMERGENCY ABO GROUPING AND Rh D TYPING BY TILE METHOD		
BTS/SOP/TP/45b	HOSPITAL BLOOD BANKS	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 year

### 1. SCOPE AND APPLICATION

Tile method is used only in acute emergency conditions and only for patient's blood grouping; otherwise tube method is the method of choice. After using the tile method and releasing blood products ABO and Rh-D grouping must always be repeating with the tube method.

### 2. RESPONSIBILITY

In the Hospital Blood Bank following staffs are responsible for this procedure:

- Trained Technician is responsible to perform the ABO grouping and RhD typing of patients.
- Technologist is responsible to verify the results.

### 3. PRINCIPLE

#### Forward /cell /front grouping:

Direct agglutination of unknown red cells (patient/donor) with a particular reagent (anti-A or anti-B) indicates the presence of corresponding antigen or blood group. No agglutination indicates the absence of particular antigen or blood group against the known antibodies (anti-A or anti-B).

#### Reverse/serum/back grouping:

Unknown antibodies (anti-A/ anti-B or both) present in patient serum are reacted with known red cell antigens (A antigen or B antigen) is called reverse grouping. All individuals have naturally occurring ABO antibodies opposite to their antigens present on the red cells. For example, agglutination of the serum with A cells indicates that the blood group is 'B', agglutination with B cells indicates 'A' group, agglutination with both cells indicates 'O' group and no agglutination with A and B cells indicates that the blood group is 'AB'.

Rh-D-Grouping: After ABO system antigens, Rh.D is the most immunogenic (70%) that is transfusion of 'D' positive to a 'D' negative patient can produce potent antibodies in the patient and can cause severe transfusion reaction or haemolytic disease of newborn

## 4. MATERIAL

### 4.1 BLOOD SPECIMEN

- Blood Sample; properly labeled 2-3 ml in K3EDTA (purple) tube and/or 3-5 ml in Red top plain tube. Freshly drawn blood sample of a patient is preferred. Blood sample should not be haemolysed

### 4.2 REAGENT

- Antisera; Anti-A & Anti-B
- Reverse grouping cells (A<sub>1</sub>, B and O cells)
- Anti-D
- Normal Saline

### 4.3 MISCELLANEOUS

- Clean non greasy Tile
- Marker
- Adjustable pipet or Droppers
- Tips
- Tooth picks

## 5. PROCEDURE

- 4.1 Rinse the tile with normal saline.
- 4.2 Allow air drying or dry with a clean paper tissue
- 4.3 Label the Row with patient's sample ID.
- 4.4 Label three circles for Forward Grouping as A, B and D and three circles for Reverse Grouping as A<sub>1</sub>, B and O.
- 4.5 Centrifuge patient blood to get clear serum/ plasma.
- 4.6 Dispense one drop (50µL) of anti-A, anti-B and anti-D to the respective circles.
- 4.7 Dispense 2 drops of Patient's serum to each of the reverse grouping circles.
- 4.8 Add one drop (50µL) of patient's red cells to A, B and D circles each.
- 4.9 Add one drop A<sub>1</sub>, B and O cells to A<sub>1</sub>, B and O circles respectively.
- 4.10 Mix the contents of each circle using separate tooth pick for each in a circle of 2 cms in all directions.
- 4.11 Look for agglutination by gentle rotating the tile upwards and downwards.

## 6. INTERPRETATION OF RESULT

Agglutination is considered as positive result.

No agglutination indicates negative result.

### Disadvantages of Tile Method

- a. Haemolysis and mix field cannot be detected.
- b. Agglutination grading cannot be done.
- c. Weak ABO antigens (sub groups of ABO / new born) may not be detected because centrifugation is not done which enhances the antigen & antibody reaction.

- d. Delayed agglutination may be observed in cold weather where the tile is cold, this is because Rh-D antibodies are IgG type and react best at 37<sup>0</sup>C. .
- e. Contaminated tile may lead to false results.
- f. Washed cells are not used which may lead to false results.

Quality Control

- 1. All reagents should be used according to manufacturer’s advice.
- 2. Both forward and reverse grouping should be done in parallel.
- 3. Reagents and blood specimen should be stored at 2-8°C when not in use.
- 4. Q.C. of all reagents should be checked daily.
- 5. Anti-A1 lectin can be used where necessary.

**7. DOCUMENTATION**

Enter the results of patient’s grouping in the patient record register, blood group requisition form, serial case number register and BTIS. Make a remarks that tile-method was used.

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
CROSS MATCHING		
BTS/SOP/TP/46a	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 year

### 1. SCOPE AND APPLICATION

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

### 2. RESPONSIBILITY

It is the responsibility of technician to perform cross match and document the results on blood component issue form (*cf. Annex 11*) and component issuance register (*cf. Annex 9*). If any unexpected antibody is detected, do not issue the blood and perform additional screening of for the antibodies.

### 3. PRINCIPLE

This test is the most important and most frequently performed procedure in the routine hospital blood bank laboratory prior to any transfusion. The purpose of compatibility test is to ensure serological compatibility between the recipient's serum and the donor cells. The method used must demonstrate ABO incompatibility and clinically significant immune antibodies to red cell antigens.

### 4. MATERIAL

- Refrigerator to store samples and reagents at 2-6°C
- Tabletop centrifuge
- 22% bovine albumin or LISS suspension
- Coombs Control Cells
- Glass tubes 12x75mm
- 0.9% saline
- Distilled water
- Patient's serum
- Donor's red cells (obtained from a labeled segment of tubing originally attached to blood unit)

### 5. PROCEDURE

This test is used to detect antibodies in recipient serum against donor cells. It is done in three phases:

- 1 Saline Phase or Immediate Spin Phase (IS)
- 2 Albumin Phase or LISS 37°C Phase
- 3 Coomb's Phase or Indirect Anti-Human Globulin Phase

### **5.1 Saline Phase/Immediate Spin Phase (IS)**

- 1 Acquire donor segments from blood units to be cross matched. Label the cross matching tubes properly with the unit number of the blood bag.
- 2 Label clean glass tubes with the donor segments to be used.
- 3 Empty the donor segments in the corresponding glass tubes.
- 4 Wash the donor red cells 3 times with normal saline (or with an automated cell washer)
- 5 Prepare a 3-5% saline suspension of donor cells.
- 6 Take two test tubes and mark them both with patients ID. Mark one tube with "auto" this tube will be used for the auto control.
- 7 place two drops of recipient serum in each tube.
- 8 Add one drop of donor's 3-5% cell suspension in the first tube and mix.
- 9 Add one drop of patients 3-5% cell suspension in the second tube marked "auto".
- 10 Centrifuge at 3,000 rpm (1000xg) for 1 minute.
- 11 Examine the tubes for agglutination (haemolysis) against a good light source macroscopically.
- 12 If there is no agglutination then proceed to albumin phase or LISS 37<sup>0</sup>C.

### **5.2 Albumin Phase or LISS 37<sup>0</sup>C Phase**

- 1 Add two drops of 22% bovine albumin in both tubes and incubate for 30 minutes. (or add two drops of LISS in both tubes and incubate for at 37<sup>0</sup>C).
- 2 Centrifuge both tubes at 3,000 rpm (1000xg) for 15 to 45 seconds.
- 3 Examine for agglutination (haemolysis) in a good light source.
- 4 If there is no agglutination /haemolysis then proceed to Coombs or IAT phase.

### **5.3 Coomb Phase or Indirect anti-Human Globulin Phase**

- 1 Wash the cells of both tubes 3 times with normal saline. Decant the supernatant saline completely after the last wash. (or wash with an automated cell washer).
- 2 Add two drops of anti-human globulin (coomb's) serum in both tubes and mix.
- 3 Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g).
- 4 Note the agglutination (haemolysis) macroscopically against a good light source
- 5 If there is no agglutination add one drop of Coombs Control Cells.
- 6 Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g)
- 7 Note the agglutination macroscopically against a good light source. The agglutination must be at least 1-2+. If the result is negative, repeat the cross match procedure.
- 8 If there was no agglutination or haemolysis before the coombs control phase, the crossmatch is compatible and blood can be issued for transfusion.

## **6. INTERPRETATION**

Agglutination/haemolysis in any phase of the test indicates incompatibility.

Note: Save donor cells and patient serum for 7 days after crossmatch.

If Coombs Control Cells did not result in an agglutination causes are:

- Inactive anti-human globulin serum: use a new bottle antiserum
- Inactivated anti-human globulin serum due to improper washing: check washing procedure

## 7. DOCUMENTATION

Enter results in cross match register/component issue register (*cf. Annex 9*) and compatibility report form/blood component issue form (*cf. Annex 11 a/b*).

All records are initiated by technician who performed the test and the technologist who has verified the results.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
CROSS MATCHING BY GEL CARD TECHNIQUE		
BTS/SOP/TP/46b	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

The cross-match/compatibility testing is done to select safe blood for a patient, when transfused, does not cause any harm to the transfused (donor cells) and patient red cells. At least 75% of the transfused (donor) red cells should have acceptable survival rate.

## 2. RESPONSIBILITY

It is the responsibility of technician to perform cross match and document the results on blood component issue form (*cf. Annex-11*) and component issuance register (*cf. Annex 9*). If any unexpected antibody is detected, do not issue the blood and perform additional screening for antibodies.

## 3. PRINCIPLE

There are two types of cross match, i.e. Major and Minor. In major cross-match, donor red cells are cross matched with patient's serum/plasma to detect potent antibodies in the patient's serum/plasma and in minor cross match patient's red cells are cross matched with the donor's plasma to detect potent antibodies in the donor's plasma. Minor cross match is done in cases of transfusion reaction.

## 4. MATERIAL

### 4.1 BLOOD SAMPLE

- 3-5 ml K3EDTA (purple top) or clotted (red top) patient's blood sample collected in a clean labeled tube. The sample should not be haemolysed and not be more than 3 days old. All blood specimens should be stored at 2 - 6<sup>0</sup>C if required.
- donor blood sample in segment from blood bag (whole blood/packed red cells). Donor blood sample should be checked for any haemolysis before cross match. Haemolysed blood should not be selected for cross match.

### 4.2 REAGENTS/CARDS

- Polyspecific Coombs Microtyping Gel Cards
- Diluent or Modified LISS Solution

### 4.3 EQUIPMENTS

- Gel Card Incubator
- Gel Card Centrifuge
- Dispenser
- Disposable yellow tips
- Glass test tubes 12 x 75 mm

### 5. PROCEDURE

- 5.1 Allow all reagents to reach at room temperature.
- 5.2 Prepare 0.8% red cell suspension of 3 times washed donor red cells (1ml Diluent / LISS and 10-12 microliters packed red cells or 20-25 microliters whole blood).
- 5.3 Centrifuge patient blood to get clear serum / plasma.
- 5.4 Identify microtyping polyspecific Coombs gel card with the patient name / number and each microtube with the donor unit number.
- 5.5 Remove the aluminum foil on top of the gel card
- 5.6 Dispense 50 microliters of donor red cell suspension into appropriate microtube.
- 5.7 Add 25 microliters of patients serum or plasma to the microtube.
- 5.8 Incubate the microtyping card for 15 minutes at 37°C in the Incubator.
- 5.9 After incubation centrifuge the microtyping card for 10 minutes in the card centrifuge.
- 5.10 Take out the gel card and observe macroscopically for agglutination / mixed field and haemolysis throughout the gel column.
- 5.11 Record result.

### 6. INTERPRETATION

Positive reaction or incompatible result: Haemolysis/mix field/agglutination grade “4+ to weak” in the microtube indicates an incompatibility between donor and recipient.

Negative reaction: Clear settling of the red cells at the bottom of the microtube and no agglutination /mix field /haemolysis indicates that the donor and recipient are compatible.

#### Agglutination Grading



- 4+ = Trapping of red cells at the top of the gel.
- 3+ = Trapping of red cells in the upper half of the gel column.
- 2+ = Trapping of red cells throughout the gel column.
- 1+ = Trapping of red cells in the lower half of the gel column.
- Weak or +/- = Trapping of red cells in the lower half of lower half of the gel column.
- 0 or Negative = clear settling of red cells at the bottom of the gel column.

Mixed Field: Some agglutinate trap at the top of the gel and some clear settling of the red cells.

Haemolysis: Pinkish or reddish colour observed in the reaction chamber indicates haemolysis.

## 7. DOCUMENTATION

Enter results in cross match register/component issue register (*cf. Annex 9*) and compatibility report form/blood component issue form (*cf. Annex 11a*).

All records are initiated by technician who performed the test and the technologist who has verified the results.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
RED BLOOD CELL ANTIBODY SCREENING		
BTS/SOP/TP/47a	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applies to all testing that requires antibody screening, for patient's pre-transfusion in-compatibility testing.

### 2. RESPONSIBILITY

It is the responsibility of the technician/technologist to perform the antibody screen using proper cell concentrations. One technician performs all tests and another technologist checks it. If any unexpected blood group antibody is detected, inform the Medical Officer for further interpretation.

### 3. PRINCIPLE

The antibody screen test is used in the detection of unexpected blood group antibodies. In this test, the antibody-screening reagent red blood cells are exposed to serum under investigation. The addition of a potentiating medium enzyme/albumin helps to promote the interaction of red cells and antibodies allowing antibody/antigen reactions to occur. Positive reactions (haemolysis or agglutination) in any tests indicate the presence of allo-antibody or auto antibody in the serum.

### 4. EQUIPMENT AND MATERIAL

#### 4.1 Equipment

- Refrigerator to store samples & reagents at 2-6<sup>0</sup>c
- Deep Freezer to store enzyme papain cystein in frozen state
- Tabletop centrifuge
- Automated cell washer (for patient pre-transfusion and prenatal testing)
- Microscope
- Incubator

#### 4.2 Specimen

- patient's plasma or serum sample

#### 4.3 Reagents

- Antibody-screening reagent red blood cells (preferably a 3 cell reagent kit)
- Papain cystein
- 22% Bovine albumin or LISS suspension
- Antihuman globulin reagent (AHG)
- IgG sensitized control cells (Coombs Control Cells)
- 0.9% saline
- Distilled water

#### 4.4 Glassware

- Serum Test Tubes
- Coombs' Tubes (for patient pre-transfusion & prenatal testing)
- Micro Tubes
- Pasteur pipettes

#### 4.5 Miscellaneous

- Rubber teats
- Disposal box
- 2 plastic beakers
- Wooden blocks to hold micro tubes
- Aluminum racks to hold serum and coombs' tubes

### 5. PROCEDURE

- 5.1 Label 4 tubes with patient and test identification: 1,2,3 and auto.
- 5.2 Add two drops of test serum to each tube.
- 5.3 Add 1 drop of patient 3-5% cell suspension to the tube marked with "auto".
- 5.4 Add 3% suspension of the antibody-screening reagent red cells to tubes marked with 1, 2 or 3.
- 5.5 According to:
  - ✓ *Bovine – albumin procedure: Add 2 drop of 22% bovine albumin to all tubes.*
  - ✓ *LISS procedure: Add 2 drops of LISS solution to all tubes.*
  - ✓ *Enzyme procedure: Add 1 drop of papain cystein to all tubes.*
- 5.6 Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g).
- 5.7 Note the agglutination (haemolysis) macroscopically against a good light source.
- 5.8 Incubate the tubes 30 minutes in a water bath of 37 oC.
- 5.9 Mix the cells and serum gently, Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g).
- 5.10 Note the agglutination (haemolysis) macroscopically against a good light source.
- 5.11 Wash the cells of both tubes 3 times with normal saline. Decant the supernatant saline completely after the last wash. (or wash with an automated cell washer).
- 5.12 Add two drops of anti-human globulin (coomb's) serum in both tubes and mix.
- 5.13 Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g).
- 5.14 Note the agglutination (haemolysis) macroscopically against a good light source.
- 5.15 If there is no agglutination add one drop of Coombs Control Cells.
- 5.16 Centrifuge for 15 seconds to 1 minute at 3,000 rpm (1000g).
- 5.17 Note the agglutination macroscopically against a good light source. The agglutination must be at least 1-2+. If the result is negative, repeat the cross match procedure If enzyme method is being followed.

NOTE: Either enzyme, albumin or LISS method may be followed for detection of incomplete antibodies.

## 6. RESULTS

POSITIVE RESULT: Haemolysis / Agglutination of red cells / Mixed Field

NEGATIVE RESULT: No Haemolysis / No Agglutination of red cells (*cf. Annex 2 & 3*)

### NOTE:

- Screening cells and Identification cells in a kit should be of the same lot number or expiry date.
- Haemolysed screening or identification should not be used.
- All steps should be done immediately.
- Never use plastic tubes as it adsorbed IgG antibody which can lead to false negative results.
- Haemolysed patient blood sample should not be used. If there is haemolysis going on in the patient then check the size of cell button after centrifugation at 3400 rpm and match the colour of supernatant with the original blood sample. If the colour of the supernatant becomes darker than the original sample it means haemolysis had occurred during incubation at 37<sup>0</sup>C.
- Tubes should be shaken gently.
- Use clean glassware.
- After addition of IgG-sensitized cells (Coombs Control Cells) to a negative test, the presence of agglutination indicates that the AHG reagent was added and was working properly. If negative result was obtained it shows that the AHG reagent was either not added to the AHG was not working properly (inactivated by improper washing).
- Use all reagents according to the manufacturer advice.

If any of the screening cells react positive in one of the different phases inform the Medical Officer for further interpretation.

## 7. DOCUMENTATION

Results of donor unit antibody screen are entered in the donor grouping register and Blood Transfusion Information System.

Results of patients antibody screen are entered in the patient grouping register, blood group requisition form, serial case number register and HMIS.

All records are initialed by the technician who has performed the test and by the Technologist who has checked the results

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
DAT/DIRECT COOMB'S TEST BY TUBE TECHNIQUE		
BTS/SOP/WP/47b	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

The Direct Antiglobulin Test (DAT) is used to detect in-vivo sensitization of red blood cells. It is useful in the diagnosis of Autoimmune Haemolytic Anemia (AIHA), Haemolytic Disease of the Newborn (HDN), investigation of red cell sensitization by drugs and Haemolytic Transfusion Reaction (HTR).

### 2. RESPONSIBILITY

It is the responsibility of the technician/technologist to perform this test. One technician performs all tests and another technologist checks it. If any unexpected blood group antibody is detected, inform the Medical Officer for further interpretation.

### 3. PRINCIPLE

Direct Antiglobulin Test (DAT) is used to detect in-vivo sensitization of red cells with immunoglobulin, complement or both. An unrefrigerated, anti-coagulated blood sample (EDTA) is needed. The EDTA anticoagulant chelate calcium and magnesium ions that are essential for in-vitro complement activation, but do not affect complement components already bound to red blood cells following an immune reaction in-vivo).

### 4. MATERIAL REQUIRED

- Patient's blood specimen 2-3 ml freshly drawn K<sub>3</sub>EDTA sample, it should not be haemolysed
- 12 x 75 mm clean glass tubes
- Pipettes
- Tips
- Marker
- Test Tube Holder
- Fresh 0.9% normal saline
- Calibrated table top centrifuge
- Tissue or gauze piece
- Polyspecific AHG, Monospecific Anti-IgG and Anti-C<sub>3</sub>d
- Coombs Control Cells (Check Cells)
- Agglutination Viewer

## 5. PROCEDURE

- 5.1 Identify the patient correctly
- 5.2 Label tubes with patient's MR#
- 5.3 Wash the patients red cells 3 times, decant the last wash eluate
- 5.4 Make 3-5% red cell suspension of the sample to be tested
- 5.5 Dispense one drop of 3-5% suspension in the labeled tube
- 5.6 Add 2 drops of Polyspecific AHG
- 5.7 Mix and centrifuge at 3400 rpm for 15 seconds
- 5.8 Read, grade and record results
- 5.9 Validate negative result with check cells (add one drop check cells and centrifuge at 3400 rpm for 15 seconds)
- 5.10 Examine the cells for agglutination; grade and record the reactions

If DAT is positive with polyspecific AHG, test the sample with monospecific anti-IgG and anti-C<sub>3</sub>d separately to detect cause of sensitization, i.e. due IgG or C3d, follow the same procedure as for polyspecific AHG.

## 6. INTERPRETATION OF RESULT

POSITIVE: DAT is Positive when agglutination is observed. Monospecific Reagents are needed to confirm which globulins are present

NEGATIVE: DAT is negative when no agglutination is observed in the test phase and the check cells are agglutinated. If check cells are not agglutinated, the negative DAT results are considered invalid and the test must be repeated

### NOTE:

Use all reagents according to the manufacture advice!

All steps should be performed immediately one after the other (uninterrupted)!

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

# STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
ANTIBODY IDENTIFICATION		
BTS/SOP/WP/48	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applies to compatibility testing of all multi-transfused patients and transfusion recipients who currently demonstrate or have a history of clinically significant antibodies or have a positive screening for immune antibodies.

## 2. RESPONSIBILITY

It is the responsibility of the technician in the Immunohaematology laboratory to perform the antibody identification testing using quality controlled reagents and proper cell concentrations. One technician performs the tests and another technologist verifies it. If all results show an unexpected blood group antibody, inform the Medical Officer in charge to carry out further investigations.

## 3. PRINCIPLE

The cross match technique used in antibody identification permits detection of clinically significant antibodies caused by complete or incomplete antibodies that sensitize cells.

## 4. EQUIPMENT AND MATERIAL

### 4.1 Equipment:

- Refrigerator to store samples & reagents at 2<sup>o</sup>- 6<sup>o</sup>C
- Table top centrifuge
- Automated cell washer
- Water bath 37<sup>o</sup>C

### 4.2 Specimen:

- Patient's serum or plasma

### 4.3 Reagents:

- Antibody Identification panel
- 22% Bovine albumin
- Antihuman globulin reagent (anti-IgG+anti-C3d)/(Polyspecific)
- IgG sensitized control cells (*cf. Annex 5*)
- 0.9% saline

- Distilled water

#### 4.4 Glassware:

- Serum tubes
- Coombs' tubes (for patient pre-transfusion & prenatal testing)
- Micro tubes
- Pasteur pipettes
- Glass slides

#### 4.5 Miscellaneous:

- Rubber teats
- Disposal box
- 2 plastic beakers
- Wooden blocks to hold micro tubes
- Aluminum racks to hold serum and coombs' tubes

### 5. PROCEDURE

Note: Identification method is prescribed procedure.

- 5.1 Label tubes with patient and identification cell.
- 5.2 Add 2 drops of patient's serum to each tube.
- 5.3 Add 1 drop 3-5% suspension of the identification panel cells to each tube.
- 5.4 Add 2 drop of 22% bovine albumin and mix well.
- 5.5 Mix well and Centrifuge at 3000 rpm (1000xg) for 1 minute.
- 5.6 Examine the tubes for haemolysis.
- 5.7 Gently re-suspend red cell buttons and examine for agglutination.
- 5.8 Grade and record test results immediately.
- 5.9 Incubate at 37<sup>o</sup>C for minimum 30 minutes.
- 5.10 Examine the tubes for haemolysis.
- 5.11 Mix well and Centrifuge at 3000 rpm (1000xg) for 1 minute.
- 5.12 Gently re-suspend red cell buttons and examine for agglutination.
- 5.13 Wash the cells 3 times with saline. Decant completely after last wash.(washing can be done manually or in automated cell washer).
- 5.14 Add 2 drops of antihuman globulin reagent to the dry cell button.
- 5.15 Mix well and Centrifuge at 3000 rpm (1000xg) for 1 minute.
- 5.16 Examine the tubes for haemolysis.
- 5.17 Gently re-suspend red cell buttons and examine for agglutination.
- 5.18 Grade and record test results immediately.
- 5.19 Let another technologist check the results.
- 5.20 To all negative antiglobulin tests add 1 drop of IgG-sensitized control cells.
- 5.21 Centrifuge, re-suspend and read for agglutination. Grade and record test results. After the addition of IgG-sensitized control cells to a negative test, the presence of agglutination indicates that the AHG serum added was capable of reacting and that the negative antiglobulin test is valid.

## 6. INTERPRETATION OF RESULT

Haemolysis or agglutination indicates the presence of an antibody. This result is interpreted as POSITIVE.

Absence of agglutination and haemolysis is a negative test result and no antibody is present. This result is interpreted as NEGATIVE.

If the IgG-sensitized control cells added to confirm the activity of the polyspecific reagent show only weak (1+/2+). If no agglutination is seen the test is invalid and must be repeated.

## 7. LIMITATIONS

The anti-globulin cross- match will not:

- a) *Detect error in Rh typing*
- b) *Prevent immunization of the recipient*
- c) *Ensure normal red blood cell survival*
- d) *Detect some weakly reactive antibodies*

## 8. 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 technician who performed the test and the one who verified the results sign all records.

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



# STANDARD OPERATING PROCEDURE

<b>ISSUANCE OF BLOOD COMPONENTS</b>		
<b>FILTRATION OF RED CELL CONCENTRATES</b>		
<b>BTS/SOP/WP/49</b>	<b>HOSPITAL BLOOD BANK</b>	<b>Version: 1.0</b>
Valid from:	Effective Date:	Review Period: 1 Year

## 1. SCOPE AND APPLICATION

This procedure applied to the filtration of red cells before transfusion to remove more than 99% of any residual leucocytes for the prevention of febrile transfusion reactions or HLA-immunization.

## 2. RESPONSIBILITY

It is the responsibility of the HBB technician to prepare filtered red cells from packed blood cells.

## 3. PRINCIPLE

The leucocyte removal filters contain multiple layers of synthetic polyester nonwoven fibers that selectively retain white cells while allowing red cells and/or platelets to flow through. Lymphocytes and monocytes are passively held in the filter, while granulocytes in addition are also trapped by adhesion.

## 4. MATERIAL

- In-line filters attached to the blood bag

## 5. PROCEDURE

- 5.1 Perform pre-storage leukocyte reduction by in-line blood filters soon after whole blood collection and component production.
- 5.2 In case the blood is collected without the in-line leukocyte reduction filter, a filter can be attached to the tubing by a sterile connection device.

## 6. DOCUMENTATION

Enter following details in the Component Register:

- a) *Date and time of preparation*
- b) *Unit number*
- c) *Blood group*
- d) *Type of component preparation (filtering)*

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:



## STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS		
WASHING of RED CELL CONCENTRATES		
BTS/SOP/WP/50	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

This procedure applied to the washing of red cells before transfusion to remove any residual plasma.

### 2. RESPONSIBILITY

It is the responsibility of the HBB technician to prepare washed red cells from 'Red Cell Concentrates'.

### 3. PRINCIPLE

Washed red cells are derived from secondary processing of a red cell component with sequential washing and re-suspension of the red cells in an additive solution. This removes leucocytes.

### 4. MATERIAL

- Tube sealer
- Plasma expresser
- Electronic weighing scale
- Refrigerated centrifuge
- 0.9% Saline

### 5. PROCEDURE

- 5.1 Undertake the washing procedure only after the proposed unit is found to be compatible with recipient.
- 5.2 **Balance the blood bag with an insert, and then place in the centrifuge bucket.**
- 5.3 Spin the bag at 3500 rpm for 10 minutes at 4<sup>0</sup> C or the buckets are centrifuged as per programme.
- 5.4 Remove the supernatant plasma completely in a transfer bag using an plasma expresser under laminar flow.
- 5.5 Connect the bag with a sterile 0.9% saline bag using a transfer set.
- 5.6 Record batch number and expiry dates of saline in use.
- 5.7 Introduce approximately 200 ml of saline into the RCC bag and mix thoroughly and balance and centrifuge again (3500 rpm for 10 minutes at 4<sup>0</sup> C).

- 5.8 Transfer the supernatant saline with some plasma into a transfer bag using the expresser under laminar flow.
- 5.9 Disconnect the transfer bag, seal and discard.
- 5.10 Repeat the washing with saline twice more (total three times) exactly in the same manner as described above. In the end keep 25-30ml saline with the red cells in the bag.
- 5.11 Seal the final three times washed red cell unit with three seals and break the middle seal.
- 5.12 Weigh the bag and record details in the register.
- 5.13 Store the washed packed red cell unit at 2-6°C and use within 24 hours of washing.
- 5.14 Use this blood only for the patient for which requested. If not used discard after 24 hours with standard disposal protocol, after subjecting small sample for bacteriological examination.

## 6. DOCUMENTATION

Enter following details in the Component Register:

- a) *Date and time of separation*
- b) *Unit number*
- c) *Blood group and compatibility record*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

# **Distribution to Wards**



## STANDARD OPERATING PROCEDURE

DISTRIBUTION TO WARD		
LABELING		
BTS/SOP/WP/51	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

Before these blood bags are taken to the ward for use they are labeled with relevant information about recipient's/patient's unique identity number, ABO and RhD blood group, compatibility testing results, etc. The label is required for identification and retrieval of blood units for ward use, disposal and follow up in case of adverse reactions.

### 2. RESPONSIBILITY

It is the responsibility of the laboratory technician working on shift duty in HBB to label the blood and blood components units after performing the blood grouping and compatibility testing with patient's blood.

It is the responsibility of second technician to check the labels and tags before issuance of the blood bag.

### 3. MATERIAL REQUIRED

Preprinted adhesive labels for all components are used as per regulatory requirement which indicates:

Recipient's/Patient's full name and birth date/age  
Patient's unique hospital number  
Patient's ABO group and Rh type  
Compatibility test results

### 4. PROCEDURE (LABELING OF BLOOD COMPONENTS FOR WARD USE)

- 4.1 After incompatibility testing, blood units are shifted to remain in the section of cross-matched components inventory ready for use.
- 4.2 Ensure that the compatible units have been labeled with patient's ABO, Rh blood group type and compatibility testing results.
- 4.3 Place the patient unique ID/reference no. on the bag for whom the bag has been cross matched.
- 4.4 Make entries in blood component issuance form and register (*cf. Annex 9 & 11*).
- 4.5 Mention date and time and result of cross-match.

- 4.6 After the bags are labeled, ask a second technician to double check the labels on the bags tallying them with the records on the blood component issuance form and register.
- 4.7 Before issuing blood:
- a) *Inspect each unit for any signs of deterioration*
  - b) *Mention date and time of issue*
  - c) *Identity of person issuing blood*
  - d) *Identity and record of person who picked up blood, or to whom blood was delivered*
  - e) *Instruct the individual to take the unit straight to Ward for transfusion*
- 4.8 Final identification of recipient and blood container rests with transfusionist, who must identify patient and donor unit and certify that identifying information on forms, tags, and labels are in agreement.
- 4.9 After transfusion, a record of transfusion becomes part of patient's medical record and duplicate copy of Blood Component Issue form is placed in *BB* (cf. Annex11).

## 5. DOCUMENTATION

Make following entries in the issue register:

- a) *Blood bag No. and blood group*
- b) *Patient's Name and hospital ID*
- c) *Patient's Blood Group*
- d) *Type and no. of components issued*
- e) *Date and time of issuance*
- f) *Patient's cross-match result*
- g) *Donor's screening result's*
- h) *Signature of technician who issues*
- i) *Signature of collector/receiver*

Name & signature	Name & signature	Name & signature
Written or revised by	Reviewed or approved by	Authorized by
Date:	Date:	Date:

## **Discarding of blood Components**



## STANDARD OPERATING PROCEDURE

DISCARDING OF BLOOD COMPONENTS		
HANDLING RETURNED/EXPIRED BLOOD COMPONENTS		
BTS/SOP/WP/52	HOSPITAL BLOOD BANK	Version: 1.0
Valid from:	Effective Date:	Review Period: 1 Year

### 1. SCOPE AND APPLICATION

The technologists have the responsibility and duty to see that the blood is not wasted and made available to another patient of the same group. This is achieved by first-in-first-out (FIFO) policy. Expired blood and blood components will never be used for transfusion.

### 2. RESPONSIBILITY

It is the responsibility of the HBB staff to see that the blood which has returned and not used is once again cross matched and made safe for transfusion to another patient. They also have to look for expired bags and to be sent for incineration.

### 3. MATERIAL REQUIRED

- Blood component Issuance Register (*cf. Annex 9*)
- Inventory Register

### 4. PROCEDURE

- 4.1 When blood is released from the Blood Bank to operation theatre or ward of the hospital or outside for transfusion, sometimes for some reason or the other, it may not be required by the patient and it is returned to the blood bank. If this unit of blood or blood component arrives within half an hour, it could be reused for another patient. Take care to see that this unit of blood is kept erect in the cold room to look out for haemolysis. If there is no haemolysis seen after spinning or standing, issue this unit safely to another patient.
- 4.2 In case of FFP, which comes to the blood bank unused, issue to another patient if there is a demand for that particular group immediately within 6 hours of the first issue. If no call arises, then use it later as FVIII deficient plasma.
- 4.3 Check the inventory and send any blood unit that has been expired to the incinerator.

### 5. DOCUMENTATION

Make entries of returned units against the issue in the issue register  
Re-enter the unit in the inventory before reissue  
Make a entry of the expired blood unit sent to incinerator

Name & signature Written or revised by	Name & signature Reviewed or approved by	Name & signature Authorized by
Date:	Date:	Date:

## Annex 1

## ANNEXES

### CELL WASHING PROCEDURE

7. Dispense 4-5 drops of whole blood or packed red cells in a 4 cctube.
8. Fill the tube  $\frac{3}{4}$  full with 0.9% saline to resuspend the cells.
9. Centrifuge the tubes for 2 to 3 minutes at 3400 rpm.
10. Discard maximum supernatant fluid/saline by a plastic dropper.
11. Repeat this washing procedure three times, every time save red cells sediment.
12. Deposit at the bottom of the tube is washed cells.

### 5% RED CELL SUSPESION PROCEDURE

4. Take 100 micro-liters of "Washed Red Cells" in a clean labeled test tube.
5. Add 2 ml of 0.9% normal saline (1:20 ratio) to make 5% red cell suspension.
6. Mix thoroughly and this suspension can be used for 12 hours.

### 3% RED CELL SUSPESION PROCEDURE

1. Take 60 micro-liters of "Washed Red Cells" in a clean labeled test tube.
2. Add 2 ml of 0.9% normal saline (1:20 ratio) to make 3% red cell suspension.
3. Mix thoroughly and this suspension can be used for 12 hours.
4. Store at 2-8°C when not in use.

## Annex 2

<b>READING AND GRADING TUBE AGGLUTINATION</b>
5. Gently shake or tilt the tube to resuspend the red cell button in the tube. The tilt technique uses the meniscus to gently dislodge the red cell button from the wall of the tube.
6. Observe the way that cells are dispersed from the red cell button.
7. Record reactivity by comparing the agglutinates to the descriptions in the following table.
8. The reactivity should be assessed when the red cells have been completely resuspended from the button.

## Annex 3

<b>INTERPRETATION OF AGGLUTINATION REACTION</b>		
<b>Agglutination Grading/Score</b>		
<b>Macroscopically Observed Findings</b>	<b>Designation(USA)</b>	<b>Score(UK)</b>
One solid agglutinate, background clear and no free cells	4+	12
Several large agglutinates, background clear and no free cells	3+	10
Medium-size agglutinates, background turbid and many free cells	2+	8
Small agglutinates, background turbid and too many free cells	1+	5
Few tiny agglutinates, turbid background and all free cells	1+w	4
Barely visible agglutination, turbid background and almost all free cells	W+ or +/-	2
No agglutination	0	0
Mixtures of agglutinated and unagglutinated red cells	Mixed field	
<b>Haemolysis Grading</b>		<b>Plasma/Serum</b>
Complete Haemolysis	Haemolysis	Coca cola colour/cherry red colour
Partial Haemolysis	Partial Haemolysis	Light pink colour

## Annex 4

<b>PREPARATION OF 6% BOVINE ALBUMIN</b>	
SCOPE:	6% Bovine Albumin is isotonic to serum and contains no antibodies. It is used in R <sub>h</sub> Control and weak D Control
Requirement:	7. 22% Bovine Albumin (stock) 8. 0.9% normal saline 9. Adjustable Jester (100-1000µL) 10. Tips 11. Sterilized dropper vials 12. Labels and pen
Calculation: For making 6% Bovine albumin	
Formula:= $C_1V_1=C_2V_2$	C1 = Initial Concentration = 22% V1 = Initial Volume =? C2 = Final Concentration = 6% V2 = Final Volume 5 ml OR 5000 µL
$C_1V_1 = C_2V_2$ $V_1 = \frac{C_2V_2}{C_1}$ $V_1 = \frac{6 \times 5}{22}$ $V_1 = \frac{30}{22}$ <p>V1 = 1.364 ml OR 1364 µL</p> <p>Volume of diluent can be calculated as follows:</p> <p>Volume of diluent = V2 – V1</p> <p>Diluent volume (V2) = 5000 µL – 1364 µL            Diluent volume (V2) = 3636 µL OR 3.6 ml</p>	Preparation: <ol style="list-style-type: none"> <li>6. Take sterilized dropper vial.</li> <li>7. Using jester, pour 3636 µL OR 3.6 ml (round figure) 0.9% normal saline in it.</li> <li>8. Add 1364 µL OR 1.4 ml (round figure) 22% Bovine Albumin.</li> <li>9. Mix Well.</li> <li>10. Label it with 6% Albumin, Manufacturing and Expiry Dates and initials of the technician who made this.</li> </ol> <p><u>Note:</u> Store at 2-8°C when not in use.</p>

## Annex 5

<b>PREPARATION OF CHECK CELLS</b>	
<b>PRINCIPLE</b>	<p>IgG coated check cells are used to validate all tests using antihuman globulin reagent (AHG). Check cells ensures;</p> <ul style="list-style-type: none"> <li>• AHG reagent was added</li> <li>• AHG reagent was active</li> <li>• Washing was complete (all unbound proteins removed).</li> </ul>
<b>REAGENTS AND EQUIPMENT</b>	<ul style="list-style-type: none"> <li>• 12 x 75 mm test tubes</li> <li>• Anti-D human IgG type</li> <li>• Normal Saline</li> <li>• Centrifuge</li> <li>• Water bath</li> <li>• Alsever's Solution</li> <li>• Adjustable Jester 10-100ul</li> <li>• Tips</li> <li>• Labels and pen</li> <li>• Aliquots from known O Rh Positive donor units (segments) from 3-5 donors.</li> </ul>
<b>PROCEDURE</b>	<ul style="list-style-type: none"> <li>• Label a clean glass tube.</li> <li>• Dispense 100ul of whole blood from five different O Positive blood bags to make a pool of 5.</li> <li>• Add 4 drops of Anti-D human IgG type to the pool cells.</li> <li>• Mix the contents and incubate at 37°C for 30 minutes, during incubation gently shake the tube after every 5 minutes.</li> <li>• After incubation, wash the tube 4-6 times with normal saline.</li> <li>• Make 5% in Alsever's solution (500 µL packed sensitized red cells to 9500 µL Alsever's solution in a sterilized dropper bottle) OR Normal Saline can be used instead of Alsever's Solution.</li> <li>• Label the vial with check cells; manufacturing and expiry dates and initials of the technician who prepared</li> </ul>
<b>STORAGE TEMPERATURE</b>	2-6 <sup>0</sup> C when not in used.
<b>EXPIRY DATE</b>	In Alsever's Solution 3-4 weeks. In normal saline 12-24 hours

**QUALITY CONTROL**

- Label two tubes one as positive control and the other as negative control.
- In positive control tube dispense one drop of AHG and one drop Check Cells while in negative control tube dispense one drop of normal saline and one drop check cells.
- Centrifuge both the tubes in a calibrated centrifuge at 3400 rpm for 15 seconds.
- Positive control should give 1+ to 2+ results and negative control should be negative.
- Results should be documented on the daily QC sheet.

## Annex 6 ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

FORWARD GROUPING				REVERSE GROUPING				Auto Control	POSSIBLE CAUSES	RESOLUTION STEPS
Patient	Anti-A	Anti-B	Anti-AB	A1 Cells	B Cells	O Cells				
1.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Newborn with group "O" or elderly patient Patient may have hypogammaglobulinemia, or agammaglobulinemia May be taking immune-suppressive drugs	Check age of patient incubate at RT for 30 min or at 4°C for 15 min for weak antigens Immuno-globulin levels Drugs History	
2.	4+	Neg	4+	1+	4+	Neg	Neg	Subgroup of A; probable A <sub>2</sub> group with Anti-A <sub>1</sub> Allo antibody	Use anti A1 Lectin Antibody screening and identification	
3.	4+	4+	4+	2+	2+	2+	2+	1) Rouleaux (multiple myeloma patient; or patients given plasma expanders) 2) Cold autoantibody (probable group AB with an auto anti-I) 3) Cold autoantibody with underlying cold or RT reacting alloantibody (probable group AB with an auto anti-I and a high-frequency cold antibody (e.g. anti-P <sub>b</sub> , anti-M, anti-Le <sup>b</sup> ))	1) Wash red cells; use saline replacement technique. 2) Perform cold auto absorption technique or use rabbit erythrocyte stroma (REST) absorb. 3) Perform cold autoabsorption technique or REST, and run panel on absorbed serum; select reverse cells lacking antigen for identified alloantibody; repeat reverse group on absorbed serum to determine true ABO group. 4) Use Pre warm technique	

## ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

### FORWARD GROUPING

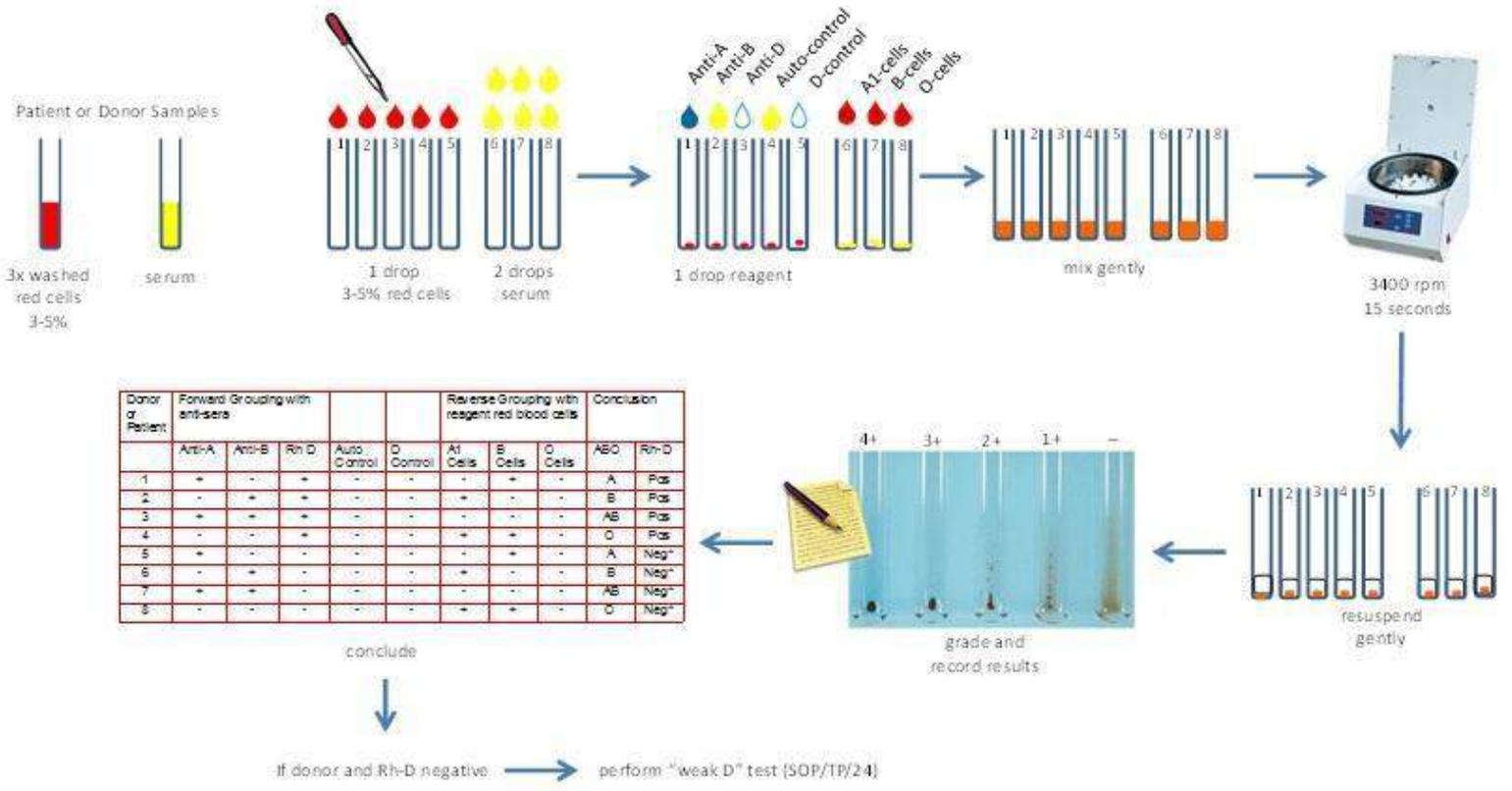
### REVERSE GROUPING

Patient	Anti-A	Anti-B	Anti-A, B	A <sub>1</sub> Cells	B Cells	O Cells	Auto Control	POSSIBLE CAUSES	RESOLUTION STEPS
4.	3+	4+	4+	1+	Neg	Neg	Neg	Subgroup of AB; probable A <sub>2</sub> B with anti-A <sub>1</sub> <small>Alloantibody</small>	Use anti-A <sub>1</sub> lectin Antibody Sc & Id
5.	Neg	Neg	Neg	4+	4+	4+	Neg	O <sub>h</sub> Bombay	Test with anti-H lectin;
6.	Neg	Neg	2+	2+	4+	Neg	Neg	Subgroup of A; probable A <sub>x</sub> with anti-A <sub>1</sub>	Perform saliva studies Or absorption / elution
7.	4+	2+	4+	Neg	4+	Neg	Neg	Group A with an "acquired B" antigen	Check patient history for lower gastrointestinal problem or septicemia; use modified BS-I lectin if available, or acidify anti-B typing reagent to pH 6.0 by adding 1 or 2 drops of 1N HCl to 1 ml of anti-B antisera, and measure with a pH meter (this acidified anti-B antisera would agglutinate only true B antigens and not acquire B antigens)
8.	4+	4+	4+	2+	Neg	2+	Neg	Alloantibody (like anti Lea, anti P1, anti M & anti N)	Perform antibody screen and panel Selection of antigen negative reverse grouping cells for A & O

# Annex 7

## Blood Grouping Flow Chart

### ABO Grouping & Rh Typing by Tube Method



**Annex 8**

**Blood Grouping Form**

ABO and Rh-D BLOOD GROUPING

Date:

	Donor ID										Anti-A	Anti-B	Anti-D	Auto cont	Anti-D cont	A1 cell	B cell	O cell	conclusion
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			
13																			
14																			
15																			
16																			
17																			
18																			
19																			
20																			

Analysed by:

Checked by:

**Annex 9**

**Component Issuance Register**

Blood Bag No.	Blood Bag Group	Patient's unique hospital ID	Patient's Name/Age/Sex	Patient's Blood Group	Type of component issued	Date and Time of Issuance	Cross match Result			Donor's Screening					Hand-over to	Remarks/Signature
							Saline /immediate spin phase	Albumin/LISS 37°C	Coombs/Anti Human Globulin phase	HBs Ag	HCV Ab	HIV Ab	Syphilis	Malaria		

**Annex-10**

**BLOOD REQUEST FORM**

**Patient's Identification Data**

Patient's Unique Hospital # \_\_\_\_\_ Location \_\_\_\_\_

Name \_\_\_\_\_ S/O, D/O \_\_\_\_\_

Age/DOB \_\_\_\_\_ Sex \_\_\_\_\_

**Details of Blood Requisition**

Name of Attending Physician \_\_\_\_\_

Name of Requesting Physician \_\_\_\_\_

**Required for**

<input type="checkbox"/> Emergency
<input type="checkbox"/> Planned Surgery/Transfusion

**No. of Units Required**

Date

Time

**Type of Desired Blood Component**

<input type="checkbox"/> Whole Blood	<input type="checkbox"/> Un-crossmatch "O" Negative Blood
<input type="checkbox"/> Red cell Concentrate	<input type="checkbox"/> Group Specific un-crossmatched Blood
<input type="checkbox"/> Platelet Concentrate	<input type="checkbox"/> Gamma Irradiated
<input type="checkbox"/> Fresh Frozen Plasma	
<input type="checkbox"/> Cryoprecipitate	

**Clinical Diagnosis (Indication for Transfusion)**

\_\_\_\_\_

**Baseline Investigations of Patient**

<input type="checkbox"/> Haemoglobin	
<input type="checkbox"/> Platelet Count	
<input type="checkbox"/> Prothrombin Time	
<input type="checkbox"/> Partial Thrombin Plastin Time	

**Past Transfusion History**

Previously Identified Blood Group \_\_\_\_\_ Irregular Antibodies \_\_\_\_\_

Last Transfusion Date \_\_\_\_\_

Previous Adverse Reaction : Type \_\_\_\_\_

Date \_\_\_\_\_

**Instructions:**

- ✓ 5ml of patient's clotted blood in red top tube (properly labeled) should accompany this requisition.
- ✓ Form to be completed and signed by requesting physician.

Requesting Physician Name and Signature:	Receiving Technician Name and Signature:
Date/Time:	Date/Time:

## Annex-11

### BLOOD COMPONENT (Whole Blood/RCC) ISSUE FORM

#### Patient's Identification Data

Patient's Unique Hospital # \_\_\_\_\_ Location \_\_\_\_\_

Patient's Name \_\_\_\_\_ S/O, D/O \_\_\_\_\_

Age/DOB \_\_\_\_\_ Sex \_\_\_\_\_

#### CROSS MATCH REPORT

Patient Blood Group	Donor Blood Group	Donor Bag No.
<input type="checkbox"/> Saline Phase/Immediate spin phase	<input type="checkbox"/> <b>Compatible</b>	Screening Results <b>Negative</b> for <input type="checkbox"/> HBs Ag <input type="checkbox"/> Anti HCV <input type="checkbox"/> Anti HIV <input type="checkbox"/> Malarial Parasite <input type="checkbox"/> Syphilis
<input type="checkbox"/> Albumin Phase/LISS 37°C	<input type="checkbox"/> <b>Compatible</b>	
<input type="checkbox"/> Coombs Phase/Anti Human Globulin phase	<input type="checkbox"/> <b>Compatible</b>	

Date / Time of Issue \_\_\_\_\_

Issuing Technologist \_\_\_\_\_ Verifying Technologist \_\_\_\_\_

Blood Bag(s) Received By \_\_\_\_\_

#### TRANSFUSION RECORD (To be filled by Duty MO/RN)

Vital Signs	Pre-Transfusion	Time	During Transfusion	Time	Post - Transfusion	Time
<input type="checkbox"/> Temp						
<input type="checkbox"/> Pulse						
<input type="checkbox"/> B.P mm/Hg						
<input type="checkbox"/> Duty MO						
<input type="checkbox"/> Duty RN						

Transfusion Started at \_\_\_\_\_ Transfusion completed at \_\_\_\_\_

Transfusion discontinued at \_\_\_\_\_ Reason \_\_\_\_\_

#### INSTRUCTIONS

- ✓ Before starting the transfusion of this unit, verify patient's identity, blood group and Rh, and donor unit number as per information given on transfusion form, blood unit, its tag, and labels.
- ✓ After completion of transfusion please return the empty bag(s) along with the duplicate copy of this form to blood bank.
- ✓ In case of transfusion reaction stop administration of blood and initiate transfusion reaction investigation procedure.

## Annex-12

### BLOOD COMPONENT (FFP/Platelet) ISSUE FORM

#### Patient's Identification Data

Patient's Unique Hospital # \_\_\_\_\_ Location \_\_\_\_\_

Patient's Name \_\_\_\_\_ S/O, D/O \_\_\_\_\_

Age/DOB \_\_\_\_\_ Sex \_\_\_\_\_

<b>Patient Blood Group</b>	<b>Donor Blood Group</b>
----------------------------	--------------------------

<b>Donor Bag No.</b>
Screening Results <b>Negative</b> for
<input type="checkbox"/> HBs Ag
<input type="checkbox"/> Anti HCV
<input type="checkbox"/> Anti HIV
<input type="checkbox"/> Malarial Parasite
<input type="checkbox"/> Syphilis

Date / Time of Issue \_\_\_\_\_

Issuing Technologist \_\_\_\_\_ Verifying  
Technologist \_\_\_\_\_

Blood Bag(s) Received

By \_\_\_\_\_

#### TRANSFUSION RECORD (To be filled by Duty MO/RN)

Vital Signs	Pre- Transfusion	Time	During Transfusion	Time	Post - Transfusion	Time
<input type="checkbox"/> Temp						
<input type="checkbox"/> Pulse						
<input type="checkbox"/> B.P mm/Hg						
<input type="checkbox"/> Duty MO						
<input type="checkbox"/> Duty RN						

Transfusion Started at \_\_\_\_\_ Transfusion completed at \_\_\_\_\_

Transfusion discontinued at \_\_\_\_\_ Reason \_\_\_\_\_

#### INSTRUCTIONS

- ✓ Before starting the transfusion of this unit, verify patient's identity, blood group and Rh, and donor unit number as per information given on transfusion form, blood unit, its tag, and labels.
- ✓ After completion of transfusion please return the empty bag(s) along with the duplicate copy of this form to blood bank.
- ✓ In case of transfusion reaction stop administration of blood and initiate transfusion reaction investigation procedure.

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