Standard Operating Procedures
BLOOD TRANSFUSION SERVICES
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PREFACE

This collection of Standard Operating Procedures for BTS is a remarkable achievement. It has been elaborated in continuation of previous efforts under the National AIDS Control Programme, undertaken with the objective to improve blood safety and to curb the transmission of HIV. These SOPs will close an important safety gap in the blood transfusion system by helping to standardize laboratory practices and by providing concise guidance to the laboratory staff.

These SOPs are a step forward, as they deliver a comprehensive list of procedures required in the vein to vein transfusion chain, from donor management to compatibility testing and issuance of blood. These SOPs are also noteworthy for the methodology under which they were developed. The 12 sections addressed were distributed to specific working groups carefully identified and brought together to draw on the best professional competence in Pakistan.

The booklet describes procedures for donor management, collection of blood, TTI screening, immunohaematology, component preparation, storage, distribution to hospital blood banks, reception of blood components, issuance of blood components, and discarding of blood components. A review of the book gives the reader a thorough overview of the essential procedures required in a blood banking service, along with well-written details of each standard operating procedure. Information is arranged methodically so that any reader dealing with one specific department of blood banking may be able to access only the material of relevance to him.

Although these SOPs reflect both the recommendations of international literature and local best practices, there is no denial that individual institutions must develop their own standard operating procedures and the manufacturers’ instructions may supersede the guidance given in this document. This year, further work has been done to validate the document through pretesting by a local team of experts in suitable facilities. As chairperson of the validation panel, I am satisfied with the entire process. Findings based on feedback of the operators and observations made by the pre-testing team have been incorporated into this final version as recommended by the panel. This edition includes an additional chapter with SOPs for the Clinical Transfusion Chain, which will be a great help for the prescribers and staff responsible for bed-side transfusion to develop their transfusion protocols.

I am confident that there will be many grateful readers who gain a broader perspective of standard blood banking procedures and their application in their respective institutions. The authors, editors and the pre-testing team merit our recognition for this important contribution to quality management for V2V transfusion chain, which has to be taken up by blood establishments and complemented with appropriate training, supervision and documentation. This SOP collection then will fulfill the purpose for which it was developed – to improve the quality and safety of blood in Pakistan.

Prof. Hasan Abbas Zaheer
National Coordinator, Safe Blood Transfusion Programme
Ministry of National Health Services, Regulation & Coordination
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, revising or validating their own SOPs.

These SOPs have been generated with a novel methodology. The process started with a field analysis, in which the programme 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’¹. 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 Centres, Hospital Blood Banks, and the ‘end users’, i.e. 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 at 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 and conducted as a joint 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 represented their respective specializations, 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 In-charge, 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.

¹ Pls. refer to ‘Appendix 1: SOP Flyer with Critical Control Points’ at the end of the document.
In the current phase of the programme, SOPs developed were pre-tested and validated\(^2\) in order to assess their applicability in the future Regional Blood Centres and attached HBBs. The Technical Cooperation Team has contributed to the process through methodology and follow-up. The revised edition of SOPs for BTS 2015 includes both the recommended changes and a third section, comprising SOPs for the Clinical Transfusion Chain, developed by an additional working group. The SOPs will thus serve as a technical input for performing both Blood Bank and Clinical Transfusion processes. The booklet may also be used as a training resource for BTS technical staff to be trained under guidance and supervision of their Blood Bank In-charges. Some extra work would be required to convert the SOPs into a real technical handbook\(^3\) on blood banking processes. First examples were shared with the working group and met with a positive response. The respective Blood Transfusion Authority, RBC and Hospital Blood Bank managers together with the Hospital Transfusion Committees will ensure implementation in their respective areas.

It is pertinent to acknowledge the support and encouragement received from the National Coordinator SBTP, Prof. Hasan Abbas Zaheer, and our SBT Programme Managers, including Dr. Irum Gilani (AJK), Prof. Dr. Nadeem Samad Sheikh (Balochistan), Prof. Dr. Muhammad Tahir Khan (KP), Dr. Zafar Iqbal (Punjab), and Dr. Zahid Hasan Ansari (Sindh), 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 in 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. We are obliged to all of them.

Islamabad, December 2015

Paul Kohorst
Uzma Anjum
Usman Waheed
Editors

\(^2\) Pls. refer to Appendix 2: Pictorial view of WG Meetings SOP Development, Pre-testing and Validation

\(^3\) Pls. refer to some examples of ‘SOP as Training Modules1 & 2’ in Appendices 3
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Donor Management Department
Standard Operating Procedures

DONOR MANAGEMENT DEPARTMENT

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1. SCOPE AND APPLICATION
This SOP describes the documentation of the screening process according to the selection criteria of 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, he/she should confirm that the criteria are being fulfilled.

A Registered Nurse is responsible to make sure all forms are properly filled. She is also responsible to take the vitals, weight of the donor and enter the results in the relevant form. A 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 behaviour, 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 Centre, 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 and measure weight of the donor at the time of registration. (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. Take donor's signature on Donor History Questionnaire Form and keep it as a record.

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Date: Date: Date:
STANDARD OPERATING PROCEDURE

DONOR MANAGEMENT DEPARTMENT

HAEMOGLOBIN SCREENING BY CuSO₄ METHOD

<table>
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<tr>
<th>BTS/SOP/TP/02a</th>
<th>REGIONAL BLOOD CENTER</th>
<th>Version: 2.0</th>
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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 anaemic donor. The primary purpose of haemoglobin screening is donor protection, preventing an anaemic 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 (CuSO₄) 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 CuSO₄ 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 ¾ 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 from 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 are fairly common and may cause inappropriate deferral.

8. PREPARATION OF CUSO₄ STOCK SOLUTION

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

8.1 Dissolve 170 gm. crystalline CuSO₄ 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 520 ml of stock solution into 480 ml distilled water (Working Solution).
8.4 Check Specific Gravity of CuSO₄ 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 ± 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
2. 13.0 g/dl
3. 12.5 g/dl
4. 12.0 g/dl
5. 11.5 g/dl
6. 11.0 g/dl

<table>
<thead>
<tr>
<th>STOCK WORKING SOLUTION</th>
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<tr>
<td>Powder</td>
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<td>Mfr./Batch No.</td>
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9. DOCUMENTATION
Please enter the donor’s haemoglobin result in the Physical Examination Form or in the BT MIS.

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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-haemoglobin. It is one of the easiest and rapid methods for pre-donation haemoglobin estimation.

4. MATERIAL
- Haemoglobinometer
- Weighing Scale
- 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 analyser.

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 temporarily, 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 or in BT MIS (regardless of whether or not the donor passed the haemoglobin test)
1. **SCOPE AND APPLICATION**

The purpose of this procedure is to provide education to donors about the donation process and high risk behaviour 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 2*)
- 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 behaviour.
5.11 Provision of the educational material in the waiting area.
5.12 Use of wall posters/LCD (electронically) displaying the information.

6. DOCUMENTATION

Request the donors to sign the consent form (cf. Annex 3a) if they feel they are safe donors and willing to donate.

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<td>Date:</td>
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</tbody>
</table>
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 a separate donor examination room to ensure privacy. 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*) & Donor Consent Form (*cf. Annex 3a*)

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.

- 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 or temporarily for the period mentioned below:

### DONOR DEFERRAL CRITERIA (TEMPORARY/PERMANENT)

<table>
<thead>
<tr>
<th>CONDITIONS</th>
<th>DEFERRAL TYPE/PERIOD</th>
</tr>
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<tbody>
<tr>
<td>1. Abortion</td>
<td>6 months</td>
</tr>
<tr>
<td>2. Antibiotics</td>
<td>14 days after completion of treatment</td>
</tr>
<tr>
<td>3. Asthma</td>
<td>14 days after full recovery from acute exacerbation &amp; 14 days after completion of course of oral/injectable steroids.</td>
</tr>
<tr>
<td>4. Blood Transfusion Recipient</td>
<td>1 year following transfusion. Defer permanently if on regular treatment with plasma derived products.</td>
</tr>
<tr>
<td>5. CVS</td>
<td>Accept surgically corrected simple congenital cardiac malformation with no residual defect. Permanent – all other (e.g. Angina, CAD, arrhythmia valve replacement, etc.)</td>
</tr>
<tr>
<td>6. CNS</td>
<td>Epilepsy – Accept if off-medication and seizure free for 3 years. Permanent deferral – all others (including schizophrenia, convulsions &amp; fainting spells)</td>
</tr>
<tr>
<td>7. Chicken Pox</td>
<td>14 days following full recovery</td>
</tr>
<tr>
<td>8. Dental Treatment</td>
<td>24 hours after simple procedures. 7 days after extraction</td>
</tr>
<tr>
<td>9. Diabetes</td>
<td>Accept if controlled by diet/oral medication. Permanently defer if on insulin or if multi-organ involvement</td>
</tr>
<tr>
<td>10. Diagnostic Procedures</td>
<td>12 months following invasive diagnostic procedure</td>
</tr>
<tr>
<td>11. Injectable Drug Use</td>
<td>Permanent</td>
</tr>
<tr>
<td>12. Endocrine Disorders</td>
<td>Permanent</td>
</tr>
<tr>
<td>13. Fever (nonspecific)</td>
<td>14 days after full recovery</td>
</tr>
<tr>
<td>14. Dengue</td>
<td>6 months</td>
</tr>
<tr>
<td>15. Hepatitis</td>
<td>Permanent deferral</td>
</tr>
<tr>
<td>16. Carriers of HIV, HBV, HCV</td>
<td>Permanent</td>
</tr>
<tr>
<td>17. Herpes</td>
<td>28 days following full recovery</td>
</tr>
<tr>
<td>18. Hypertension</td>
<td>Accept stable, uncomplicated, controlled by medication. Permanent deferral if cardiac/renal involvement. If medicine has recently been changed, defer for 28 days after B.P stabilizes</td>
</tr>
<tr>
<td>19. Flu</td>
<td>14 days after full recovery</td>
</tr>
<tr>
<td>20. Measles/Mumps</td>
<td>14 days after full recovery</td>
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<td>21. Pregnancy</td>
<td>6 months after delivery or termination</td>
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<td>---</td>
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</tr>
<tr>
<td>22.</td>
<td>Lactation</td>
</tr>
</tbody>
</table>
| 23. | Malaria | 3 months (endemic)  
3 years (non-endemic) |
| 24. | Rheumatic Fever | 2 years following attack with no evidence of chronic heart disease. The latter complication is a cause for permanent deferral. |
| 25. | Surgery | Until fully recovered and fit to be donors, typically about 12 months.  
Permanent – Neurosurgery |
| 26. | Skin Diseases | Accept – mild disease, venepuncture site unaffected, lesions not infected. Defer - contagious |
| 27. | Salmonella, Streptococcal/ Staphylococcal Infection | 28 days following recovery |
| 28. | Malignant Diseases/Cancer Including Polycythaemia Vera, Leukaemia etc. | Permanent deferral |
| 29. | Tropical Diseases | 6 months following return from tropical areas. |
| 30. | Typhoid Fever | 12 months after recovery |
| 31. | Kidney Disease | Acute glomerulonephritis 5 years deferral period following complete recovery |
| 32. | Abnormal Bleeding Tendencies | Permanent deferral |
| 33. | Unexplained weight loss | Permanent deferral |
| 34. | Thrombosis (Arterial, Recurrent Venous) | Permanent deferral |
| 35. | Tuberculosis | 2 years following confirmation of cure |
| 36. | Endoscopy with Biopsy by using Flexible Instruments, Inoculation Injury, Acupuncture, Tattooing/Body Piercing, Mucosal Splash with Blood or Tissue | 12 months |
| 37. | Thalassemia Trait | Accept provided well and haemoglobin above required lower limit |
| 38. | Chronic Anaemia of Unknown Cause or Associated with Systemic Disease, e.g. Renal Failure, Rheumatoid, etc. | Defer permanently |
| 39. | Secondary Erythrocytosis | Accept, provided a diagnosis of PRV is excluded. |

**PROPHYLACTIC IMMUNIZATION**

<table>
<thead>
<tr>
<th></th>
<th>DEFERRAL PERIOD</th>
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</thead>
<tbody>
<tr>
<td>Cholera, Typhoid, Diphtheria, Tetanus, Pertusis, Plague, G. Globin</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Rabies Vaccination</td>
<td>1 yr after vaccination</td>
</tr>
<tr>
<td>Medication</td>
<td>Indications</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Chicken Pox Vaccination</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Hepatitis B Immunoglobin</td>
<td>12 months</td>
</tr>
<tr>
<td>Hepatitis in Family or Close Contact</td>
<td>12 months</td>
</tr>
<tr>
<td>Treated Case of Syphilis/Gonorrhoea</td>
<td>12 months</td>
</tr>
<tr>
<td>Immunization for German Measles (Rubella), MMR (Measles, Mumps and Rubella), Chickenpox and Shingles</td>
<td>Wait for 4 weeks</td>
</tr>
<tr>
<td>Immunization for Mumps, Polio (by mouth) and Yellow Fever</td>
<td>Wait for 2 weeks</td>
</tr>
<tr>
<td>Immunization for Hepatitis B as long as you are not given the Immunization for Exposure to Hepatitis B.</td>
<td>Wait for 21 days</td>
</tr>
<tr>
<td>If vaccinated for Influenza, Tetanus or Meningitis, Provided Symptom Free and Fever Free</td>
<td>Acceptable</td>
</tr>
<tr>
<td>If Given HPV Vaccine (Example Gardasil)</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Isotretinoin</td>
<td>Usually given for severe acne</td>
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<tr>
<td>Antibiotics</td>
<td>For acute infections</td>
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<tr>
<td>Etretinate</td>
<td>Severe psoriasis</td>
</tr>
<tr>
<td>Acitretin</td>
<td>Severe psoriasis</td>
</tr>
<tr>
<td>Finasteride</td>
<td>Prostate gland enlargement</td>
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<tr>
<td>Dutasteride</td>
<td>Prostate enlargement</td>
</tr>
<tr>
<td>Statins</td>
<td>Lipid lowering</td>
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<tr>
<td>Aspirin has not been taken for Blood Donations</td>
<td>Platelet components should not be prepared using donations from donors who have taken aspirin within 5 days and NSAIDS within 48 hours.</td>
</tr>
<tr>
<td>NSAIDS</td>
<td>- Do -</td>
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</tbody>
</table>
5.5 Interview.
• Conduct a confidential interview to enquire about donors engagement in any risk behaviour
• 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.

<table>
<thead>
<tr>
<th>Written or revised by</th>
<th>Reviewed or approved by</th>
<th>Authorized by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date:</td>
<td>Date:</td>
<td>Date:</td>
</tr>
<tr>
<td>Name &amp; signature</td>
<td>Name &amp; signature</td>
<td>Name &amp; signature</td>
</tr>
</tbody>
</table>
1. SCOPE AND APPLICATION

This procedure applies to all activities that are required to perform a physical examination of the donor for confirming fulfilment 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) to be taken and also provides specific guidance on the clinical and physical evidence for screening a donor during the health assessment according to the eligibility criteria.

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 (usually taken at the time of registration).

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.

ELIGIBILITY CRITERIA

- Should be 18-65 years of age
- Must weigh at least 50 kg
- Should have a Hb of 13.5 gm/dl (males), 12.5 gm/dl (females)
- B.P 100-140/70-90 mm of Hg
- Pulse – Regular (60 – 100 beats/min)
- Body temperature <37.5°C or <98.6°F

4. MATERIAL

- Donor Physical Examination Form (cf. Annex 4)
- Donor Card
- Weighing scale
- Sphygmomanometer
- Stethoscope
- Clinical thermometer
5. PROCEDURE

Physical Examination:
- Take special note on ‘General Appearance’ in case of plethora, poor physique, debilitation, under nutrition, anaemia, 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.
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. ___________ ___________ ___________ ___________ ___________ ___________ ___________ ___________ ___________

Home Address: ____________________________________________________________

_________________________________________ Home Tel. No: ________________________

Office Address: ____________________________________________________________

_________________________________________ Office Tel. No: ________________________

Cell 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: _________________________________________________
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 centre 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 anaemic

**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 gonorrhoea 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
  • Diarrhoea 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!**

<table>
<thead>
<tr>
<th>Travel to or birth in other countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What happens after your donation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

Thank you for donating blood today! (Regional Blood Centre’s Name)

Donor’s Name: _______________________ Donor’s Signature: _____________________
### Annex 3

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

<table>
<thead>
<tr>
<th>Are you</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Feeling healthy and well today?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Currently taking an antibiotic or any other medication for an infection?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Have you taken aspirin or anything that has aspirin in it?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Currently taking or have you ever taken any medications mentioned on the Medication Deferral List? <em>(Cf. annex)</em></td>
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</tr>
<tr>
<td>5. Have you read and understood the educational material on donation process and information on AIDS (HIV infection) and Hepatitis?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**In the past 6 weeks**

| 6. Female donors: Have you been pregnant or are you pregnant now? | | |

**In the past 8 weeks H/O**

| 7. Donated blood, platelets or plasma? | | |
| 8. Vaccinations or other shots? | | |
| 9. Dental treatment or tooth extraction? | | |
| 10. Allergies or rashes | | |

**In the past 16 weeks**

| 11. Have you donated a double unit of red cells using an aphaeresis machine? | | |
| 12. Unexplained weight loss | | |

**In the past 12 months have you had**

| 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? | | |

**High risk behaviour/unusual sexual practices**

<p>| 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 gonorrhea? | | |</p>
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>24. A positive test for the HIV/AIDS virus?</td>
<td></td>
</tr>
<tr>
<td>25. Used needles to take drugs, steroid, or anything not prescribed by your doctor?</td>
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<tr>
<td>26. Hepatitis/Jaundice?</td>
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<tr>
<td>27. Sexually transmitted disease e.g. Syphilis</td>
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<tr>
<td>28. Tuberculosis?</td>
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<tr>
<td>29. Typhoid fever?</td>
<td></td>
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<tr>
<td>30. Rheumatic fever?</td>
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<tr>
<td>31. Any heart disease/hypertension?</td>
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<tr>
<td>32. Asthma?</td>
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<tr>
<td>33. Persistent cough and chest pain?</td>
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<tr>
<td>34. Any type of cancer, including Leukaemia?</td>
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<tr>
<td>35. Bleeding condition or a blood disease?</td>
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<tr>
<td>36. Epilepsy?</td>
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<tr>
<td>37. Diabetes?</td>
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<tr>
<td>38. Malaria/Dengue?</td>
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<tr>
<td>39. Leismaniasis?</td>
<td></td>
</tr>
<tr>
<td>40. Any foreign visit in recent past?</td>
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<tr>
<td>41. Any history of imprisonment?</td>
<td></td>
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</tbody>
</table>
Annex 3a

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 Centre 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 4

DONOR PHYSICAL EXAMINATION FORM

Donor Appearance and Inspection
Take special note in case of;
Yes No

Plethora ____________________________
Poor Physique ________________________
Debilitation _________________________
Under-nutrition _____________________
Anemia ______________________________
Jaundice ____________________________
Cyanosis ____________________________
Dyspnoea ____________________________
Mental Alertness _____________________
Intoxication from alcohol or drugs ________________

Skin Examination:
Any signs of needle marks and sclerotic veins on arms indicative of intravenous drug use. ________________
Presence of boils, purulent wounds or severe skin infections anywhere on the body. ________________

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: ____________________ (>12.5 gm/dl Female, >13.5 gm/dl Male)
Weight: _________________________ (>50 Kg)

Attending Medical Officer’s Signature:
## Annex 5

### DONOR RECORD

<table>
<thead>
<tr>
<th>Blood Bag No.</th>
<th>Donor’s Name</th>
<th>Donor’s Unique ID</th>
<th>Donor’s Blood Group</th>
<th>Donor’s Age/Sex</th>
<th>Donor’s Hb/B.P</th>
<th>Date &amp; Time of donation</th>
<th>Donor’s Address &amp; Tel. No.</th>
<th>Donor’s Screening Results</th>
<th>Post Donation Adverse Reaction(s)</th>
<th>Remarks/Signature</th>
</tr>
</thead>
<tbody>
<tr>
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</table>
Collection of Donation
Standard Operating Procedures

COLLECTION OF DONATION

1. Inspection of Blood Bags and Labelling (SOP/WP/06) 30
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3. Phlebotomy and Collection of whole Blood Donation (SOP/WP/08) 34
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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 labelling that need to survive multiple processing, testing, and storage steps through challenging environmental conditions. All labelling 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**

Labelling of blood units is a three-step process. The first step, performed by the phlebotomist, involves the appropriate labelling 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 is also performed by the laboratory staff when the blood is being issued 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* on the primary collection bag, prior to collection of blood.

5.5 Attach adhesive barcode label or manual labelling 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 centre).

5.6 Write down the collection date on 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 when it starts.

5.10 Label sampling test tubes which are used for blood grouping and TTI screening.

6. DOCUMENTATION

Make sure the bag labelling 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

<table>
<thead>
<tr>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written or revised by</td>
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<td>Authorized by</td>
</tr>
<tr>
<td>Date:</td>
<td>Date:</td>
<td>Date:</td>
</tr>
</tbody>
</table>
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 venepuncture.

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

3. **PRINCIPLE**
Although it is impossible to guarantee 100% disinfection 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 disinfect the venepuncture 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 venepuncture 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 venepuncture; then release the cuff. Scrub area at least 4 cm (1.5 inches) in all directions from the intended site of
venepuncture (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 venepuncture and moving outward in a concentric spiral, apply spirit swab; let stand for 30 seconds.

5.8 Allow the disinfected venepuncture 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 venepuncture site.

5.11 Cover the area with dry, sterile gauze until the time of venepuncture.

5.12 Dispose of used swab(s) into a waste bin meant for bio-hazardous material.

Note:

5.13 For donors sensitive to iodine (tincture or povidone preparations), another method (e.g., Chlora Prep 2% chlorhexidine and 70% isopropyl alcohol) should be chosen by the blood bank physician.

5.14 For donors sensitive to both iodine and chlorhexidine, a method using only isopropyl alcohol could be considered.

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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 venepuncture. A correct performance of venepuncture is essential for the quality and safety of the blood donation. Successful venepuncture results not only in safe collection of a full unit of blood suitable for separation of components with good quality yields, but also contributes to the comfort and satisfaction of the donors thus encouraging re-attendance.

2. **RESPONSIBILITY**

The phlebotomist is responsible for blood collection from the donor after verifying the donor screening details (like Hb, Wt, Ht, B.P, Pulse, Temp, etc.) checking the unit number labels and preparing the phlebotomy site. The Duty Medical Officer 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
- Blood tubing stripping device
- Adhesive Tape
- Sharp container
- Blood pressure cuff or Tourniquet
- Comfortable donor couch or chair
- Tennis ball
- SOP for preparation of venepuncture site
- SOP for Inspection and labelling 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 venepuncture site. (SOP/WP/07)

5.4 Cover the area with dry, sterile gauze until the time of venepuncture (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 in an automatic mixer.

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 completed 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 venepuncture 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 gauze.

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 Draw the samples for testing directly from the bleed line after clamping, sealing or cutting the line going to the bag at the end of the collection, or from deviation pouch of the collecting system at the beginning of the collection.

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 If Platelets are going to be produced then whole blood should be maintained at 22°C.

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

6. **DOCUMENTATION**

Record the following on the Bag labelling 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 venepuncture
f) Time at stop of venepuncture
g) Gross weight of unit
h) Initials of person doing venepuncture in the appropriate box

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1. SCOPE AND APPLICATION
This describes a procedure for collection of individual blood component. Apheresis is a procedure where whole blood from the healthy donor or patient is drawn, mixed with ACD anticoagulant and passed into the plastic disposable kit attached to the cell separator. Whole blood is separated into plasma, platelets, red cells, leucocytes & peripheral stem cells. The desired component is removed & the remaining blood is returned to the body.

2. RESPONSIBILITY
Trained Senior Medical Technologist is responsible for performing the aphaeresis procedure on the donor/patient under the supervision of a consultant/Medical Doctor.

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):
It requires two venepuncture sites as the blood "continuously drawn" from one site and after separation of the desired component, the remaining blood is simultaneously returned to the body though another site. The process is completed in short time.

Intermittent Flow Centrifugation (IFC):
It requires single venepuncture site. Intermittent flow centrifugation works in cycles, taking blood, spinning/processing it, separate the required component and then giving back the remaining blood to the donor in a bolus. 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 selective collection of plasma (to be frozen immediately within 6-8 hours as fresh frozen plasma (FFP) or therapeutic plasma exchange (TPE)
Erythrocytaphaeresis is the separation of erythrocytes from the whole blood
Plateletaphaeresis is the collection of platelets by aphaeresis
Leukaphaeresis is the removal of granulocytes or leukocytes for transfusion or for therapeutic purpose.

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, whereas 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. INVESTIGATION & MATERIAL
For Plasmapheresis:
Investigation before procedure,
Please check patients’
1. Blood Group
2. CBC
3. Serum Calcium
4. Serum Electrolytes
5. Serum Albumin
6. PT, APTT

Material required
For the Therapeutic Plasma Exchange:
In Haemonetic
KIT: LN 980E, 225ML Bowl Or LN 981E, 125 ML Bowl
- TPE Protocol Card
- Double Lumen should be placed
- Anticoagulant to be determined by physician/doctor

In Fresenius:
Kit: PL 1
- Double lumen should be placed.
- Substation fluid per physician order

Necessary measures before procedure
- Check Vital Signs of patient
- Check Blood Pressure
- Check written order of physician for plasma-aphaeresis
- Check identification of patient

For Platelets-aphaeresis
Investigation:
- Blood Group
- HB % ≥12gm/dl.
- HCT ≥40%
- Platelet count ≥200 u/l
- HBsAg, Anti HCV: Negative.
- HIV: Negative.
- TPHA: Negative.
- Age: 19 to 60 years.
- Donor physical/medical assessment

Material required:
- Aphaeresis Kit
  In Fresenius C4L, C5L
  In Haemonetic (series XN 995E/E2)
OR (LN 994CF).

- Haemonetics LDP Protocol card.
- Venepuncture material and extra clamps
- An adequate quantity of an anticoagulant solution
- Alcohol swabs
- Adhesive bandage/plasters

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:
   - Weight, Height, Sex, HCT, platelet yield and plasma volume to be drawn for platelet procedure and similarly for plasma collection or TPE (Therapeutic Plasma Exchange) procedures

5.3 Adjust the draw and return speed between 50 to 70 ml/min (both depend on the donor/patient condition)

5.4 Prick the donor/patient vein under aseptic technique. Connect the patient or donor with inlet line and outlet line. In Fresenius the blood will flow through double lumen into the chamber of equipment at a low speed (1000 rpm). The centrifuge speed is increased when interface detects blood in the separation chamber. In Hemonetic centrifuge bowl speed is (4200 rpm) as the collection of the blood starts.

5.5 Check the vital signs and blood pressure of patient half hourly during plasma aphaeresis and donor after completion of the procedure.

5.6 The machine stops the separation process once the target volume (pre-selected) has been achieved.

5.7 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/patient via the return line (Reinfusion in Fresenius).

5.8 In Haemonetics as return completes, the whole blood return to the donor/patient

5.9 For platelets in Fresenius de-airate the concentrate bags by pressing excessive air into the PC sample collection bag

5.10 For platelets thoroughly mix the concentrate in the open concentrate bag

5.11 For platelet disconnect the concentrate bag from the set and keep the concentrate on the agitator for one hour before issuance to the patients.

5.12 Place the blood bag into the controlled temperature storage and transfer to the processing site under conditions (temperature) appropriate for the component that is prepared (cf. SOP/WP/31-32-33).

5.13 In TPE procedure, end product should be discarded as per bio-hazard discard procedure.

5.14 In TPE procedure, check the vital signs of the patient

5.15 For instructions on attending the donor (cf. Post-donation Care SOP-11).
6. DOCUMENTATION

Record the following on the bag labelling form/ Donor Record/BT IS

a) All particulars of the donor/patient
b) Lab reports of the donor/patient
c) Anticoagulant consumed
d) For platelets; pre procedure platelet count, product bag platelet count and post donation platelet count, plasma volume in the product bag and platelet yield obtained
e) For plasma donor plasma volume collected
f) For TPE patients total volume exchange, replacement fluids name and volume,
g) Complications noted and its management
h) Vitals pre, during and post procedure
i) Kit name and lot number
j) Blood bag type, lot
k) Time at start of procedure
l) Time of completion of the procedure
m) Procedure performed by, supervised by

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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
- 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 blood sample at 3000 RPM for 2-3 minutes and send to TTI screening laboratory and Immunohematology Department or place at refrigeration temperature.

6. **DOCUMENTATION**

Record the following on the sample tube

- **a)** *Blood bag type*
- **b)** *Blood bag Number*
- **c)** *Donor’s unique ID*
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 venepuncture 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 stay in this area for at least 10-15 minutes during which let him/her to enjoy any drink and food like tea, coffee, juice, or any other refreshments.

5.6 Inspect the venepuncture 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 Avoid driving 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/her important contribution and encourage repeat donation (after at least 12 weeks of interval).

6. DOCUMENTATION

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

Record if any adverse reaction is noticed.

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STANDARD OPERATING PROCEDURE

COLLECTION OF DONATION

MANAGEMENT OF ADVERSE EFFECTS

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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 donor 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% 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 on the bed in a relaxing position
- Raise legs/feet and lower down head end
- If it happens during the collection of donation, then immediately withdraw the needle and secure the venepuncture site.
- Loosen tight clothing (belt, tie etc.)
- Ensure adequate airway
- Check vitals e.g. pulse and blood pressure
- When the donor settle down, inform him/her about the risk of delayed fainting and advise to avoid driving or resume work in the ensuing 12 hours
- If the reaction does not subdue within 10 minutes or if the donor has vomiting, seizures, tetany or urinary or focal incontinence; immediately inform DMO and shift the donor to E.R.

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
- Immediately inform the duty medical officer and shift the donor to ER

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% Saline or 5% Dextrose Saline infusions if hypotension is prolonged
• In a very severe reaction the donor may be shifted to nearby hospital for further management

### 6. DOCUMENTATION

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

List of donor adverse effects *(cf. Annex 2)*

Donor vigilance form *(cf. Annex 3)*

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.
ANNEXES

Annex 1

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 Centre to see a doctor.
Annex 2

LIST OF DONOR ADVERSE EFFECTS

1. VASOVAGAL REACTION
   - Cold extremities/chills
   - Convulsions
   - Feeling of Warmth
   - Hypotension
   - Light headedness/Dizziness
   - Urination/Loss of bladder/bowel control
   - LOC<60 seconds
   - LOC > 60 seconds
   - Nausea/Vomiting
   - Pallor (pale skin or lips)
   - Rapid pulse
   - Slow pulse
   - Sweating
   - Tetany
   - Twitching
   - Weakness

2. SYSTEMIC ALLERGIC REACTION/ANAPHYLAXIS
   - Anxiousness, restlessness
   - Arrhythmia
   - Cyanosis
   - Generalized hives
   - Generalized rash
   - High blood pressure
   - Laryngeal edema with stridor (noisy breathing)
   - Low blood pressure
   - Pulmonary edema
   - Rapid Pulse
   - Slow pulse
   - Scratchy feeling in throat
   - Shortness of breath
   - Sneezing and nasal congestion
   - Swollen throat, tongue, eyes and face
   - Wheezing

3. HYPERVENTILATION

4. MEDICAL EMERGENCY
   - Cardiac
   - Respiratory
   - Stroke

5. LOCAL SITE REACTION
   - Itching at insertion or bandage site
   - Rash/hives at insertion or bandage site
   - Redness at insertion or bandage site
   - Multiple pricks
   - Bruising or Haematoma
Annex 3

BLOOD DONOR VIGILANCE FORM

Name: ____________________________ Age/Sex: ____________ Donor Unique ID: ____________

Donation Date: ____________________ Donation type: ☐ Replacement ☐ Voluntary

Donor Hb (gm/dL): ____________ Donor Weight (kg): ____________ Donor Height (cm): ____________

Frequency of Donation: ☐ First time ☐ No. of earlier donations __________________________

Reaction began at: ____________ Reaction ended at: ____________ Reaction recovery time: ____________

Type of Adverse Effects (Mark sign and symptoms observed)

1. Vasovagal
   - Cold extremities/Chills ☐
   - Convulsions ☐
   - Feeling of warmth ☐
   - Hypotension ☐
   - Lightheadedness/Dizziness ☐
   - Urination/Loss of bladder/bowel control ☐
   - LOC < 60 seconds ☐
   - LOC > 60 seconds ☐
   - Nausea/Vomiting ☐
   - Pallor (pal skin or lips) ☐
   - Rapid pulse ☐
   - Slow pulse ☐
   - Sweating ☐
   - Tetany ☐
   - Twitching ☐
   - Weakness ☐

2. Systemic Allergic Reaction/Anaphylaxis
   - Anxiousness, restlessness ☐
   - Arrhythmia ☐
   - Cyanosis ☐
   - Generalized hives ☐
   - Generalized rash ☐
   - High blood pressure ☐
   - Laryngeal edema with stridor (noisy breathing) ☐
   - Low blood pressure ☐
   - Pulmonary edema ☐
   - Rapid pulse ☐
   - Slow pulse ☐
   - Scratchy feeling in throat ☐
   - Shortness of breath ☐
   - Sneezing and nasal congestion ☐
   - Swollen throat, tongue, eyes, and face ☐
   - Wheezing ☐

3. Hyperventilation

4. Medical Emergency
   - Cardiac ☐
   - Respiratory ☐
   - Stroke ☐

5. Local Site Reaction
   - Itching at insertion or bandage site ☐
   - Rash/hives at insertion or bandage site ☐
   - Redness at insertion or bandage site ☐
   - Double or multiple pricks ☐
   - Bruising ☐
   - Haematoma ☐

Vital Signs (VS)

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Vital signs are required in Vasovagal, Anaphylaxis, Hyperventilation, and Medical Emergencies

Deferral ☐ Yes ☐ No ☐ if Yes, Temporary ☐ Permanent ☐

Donation taken by: ______________ Reaction reported by: ______________ Date: ______________
TTI Screening Laboratory
Standard Operating Procedures

TTI SCREENING

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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 a technician working on sample receiving desk of the TTI screening laboratory to ensure correct/properly labelled samples with proper ID are received from donation collection area.

3. MATERIAL
- Blood sample with donor ID
- Sample record entry register

4. PROCEDURE
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 labelling or any sign of deterioration, is not accepted.
4. Record the sample with ID in a separate entry register.
5. Centrifuge the sample at 3000 RPM for 2-3 minutes 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 or BT MIS and initial with date and time on the form at receiver's column.
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 of TTI screening lab: Responsible to verify the results and to check possible changes in kit insert of 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 (H₂SO₄)
5. **PROCEDURE**

**IMPORTANT:** Check the actual volumes and procedure steps provided with the test kit; this can differ from one lot to another.

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, apheresis 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°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 Concentrated 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 +120 µ concentrate conjugate).

5.12 Add 100µl of diluted Conjugate in each well, except the well for Blank, and seal.

5.13 Incubate at 37°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°C for 30 minutes (in dark at the bench)

5.18 Colour develops. Add 100 µl stopping solution (N/10 H₂SO₄) to each well, mix gently on the microwell shaker and wait for 3-4 minutes.

5.19 Interpret the results using semi-automatic 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 10,000 rpm for 10 minutes. 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 or Donor Screening Register.

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.

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</table>
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 volume for blood & reagent and time of interpretation of results according to the manufacturer advice provided with the test kit; this can differ from one lot to another.

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 or Donor Screening Register.

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STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY

ANTI HCV ANTIBODY TESTING BY ELISA METHOD

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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 colour 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 (H₂SO₄)

5. **PROCEDURE**

IMPORTANT: Check the actual volumes and procedure steps provided with the test kit; this can differ from one lot to another.
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 to each well including well for blank.
5.17 Incubate the plate at 22°C for 30 minutes (in dark at the bench).
5.18 When colour develops. Add 100µl Stopping Solution (N/10 H₂SO₄) to each well and wait for 3-4 minutes.
5.19 Interpret the results using semi-automatic 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 [=]’10 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 10,000 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

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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 colour 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 volume for blood & reagent and time of interpretation of results according to the manufacturer advice provided with the test kit; this can differ from one lot to another
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 minutes. Do retesting with the same and with 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 or Donor Screening Register.

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STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY

ANTI HIV ANTIBODY TESTING BY ELISA METHOD

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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 immunosorbert 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 and to check possible changes in kit insert of 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 (H₂SO₄)
- HIV Reagent Test Kit
5. PROCEDURE

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

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 H₂SO₄) 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 minutes. Do retesting with the same and with 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.
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 colour 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 volumes for the blood & reagent and time of interpretation of results according to the manufacturer advice provided with the test kit; this can differ from one lot to another.
5.1 Centrifuge the sample at 3400 rpm for 2-3 minutes (if not already done)
5.2 Bring all the specimens and kit contents at room temperature (18-25°C) before performing the test.
5.3 Remove the protective foil cover.
5.4 Label the test strip with client identification number.
5.5 Add 25 µl of the sample to the sample well.
5.6 Add 1 full drop (40 µl) of buffer and start the stop watch.
5.7 Examine macroscopically (after 15 minutes) for the appearance of colour line in the test region.
5.8 Handle the device as infectious material and dispose them accordingly.
5.9 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 pink colour line appears in the control region (C)
INVALID: Absence of line in the control region (C); retest these samples

Note: Retest all samples showing grey zone after ultra-centrifugation at 10,000 rpm for 10 minutes. Do retesting with the same and with 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 or Donor Screening Register.
STANDARD OPERATING PROCEDURE

TTI SCREENING LABORATORY

SYPHILIS TESTING BY RPR

BTS/SOP/TP/18a

REGIONAL BLOOD CENTER

Version: 2.0

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 and to check possible changes in kit insert of 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 one lot to another

5.1 Allow the samples and reagents to reach room temperature.
5.2 Place 50 µl of the sample and one drop of each positive and negative controls on the separate circles on the disposable card.
5.3 Swirl the carbon coated antigen bottle and place one drop (20 µl) to 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 manually 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’.

Note: A positive RPR test should be followed by another type of test (TPHA testing) for its verification.

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

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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 and to check possible changes in kit insert of 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 colour line will appear in the test line region, indicating a positive result. If the specimen does not contains TP antibodies, a colour line will not appear in the test line region, indicating a negative result. To serve as a procedural control, a colour 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 colour 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 colour 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.

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<th>Name &amp; signature</th>
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<tr>
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<td>Date:</td>
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</table>
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

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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.
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 as 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 30 minutes 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.

<table>
<thead>
<tr>
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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 malarial parasites. The test is highly accurate and is capable of detecting as few as 50-100 parasites per µL of blood; results are achieved within 20 minutes. The presence of Plasmodium species, in the blood sample, the pLDH (Lactate dehydrogenase from the parasite) captured by the conjugate in the test device and 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 10µl 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 (10µl).
5.4 Add this blood to the 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 in the control band and Pf.

NEGATIVE REACTION
No detectable pLDH in the sample.

<table>
<thead>
<tr>
<th>P Band</th>
<th>Pf Band</th>
<th>Control Band</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Negative for all types of malaria spp.</td>
</tr>
<tr>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Positive for Plasmodium falciparum +/- (P. vivax/P. ovale/P. malariae)</td>
</tr>
<tr>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Positive for Plasmodium sp. P. vivax/P. ovale/P. malariae)</td>
</tr>
</tbody>
</table>

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.
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 Blood Transfusion Information System.

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 send disposal as bio-hazards material in 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.
Immunohaematology
Standard Operating Procedures

IMMUNOHAEMATOLOGY

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Annex 8. Blood Grouping Form 112
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 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 of 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 at the same time
- 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 control 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) react 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 then 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
- Plastic or glass test tubes (preferably)

4.2 SPECIMEN

One blood sample; properly labeled, either EDTA or CPDA1 or red top plain tube up to the mark on tube
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

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

---

1 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 of washed red cells in 1940µl 0.9% Saline) OR Prepare 5% red cell suspension of donor cells (100 microliters of washed red cells in 1900µl 0.9% Saline) for reverse grouping A, B & O cells.

• Known ABO reverse grouping cells. All A/B/O reverse blood grouping cells should be Rh “D” negative (i.e. A negative, B negative & O 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 Rh D 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. Prepare 3-5% cell suspension for cells being tested. (cf. Annex-1)
2. Label 4 clean glass test tubes with A, B, auto and D along donor ID (auto= auto control).
3. Arrange the test tubes in a row.
4. Dispense one drop of anti-A, anti-B, and anti-D in the appropriately labelled 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 labelled 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.
10. Grade and record test results. (cf. Annex-3)

5.2 SERUM TESTING / REVERSE GROUP TESTING

1. Centrifuge donor blood specimen to get clear serum/plasma for reverse grouping.
2. Label 3 clean test tubes with A1, B, and O along with unique donor ID.
3. Arrange the test tubes in a row.
4. Add 2 drops of donor serum in all tubes in the corresponding tube.
5. Add one drop of 3-5% reverse grouping red cell suspension of known A, B & O cells to tubes labelled 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.

NOTE: Auto-control should be performed ONLY in ABO discrepancy cases (when AB Rh D positive is observed in forward grouping and all tubes show positive results in reverse grouping as well)

5.3 CONTROLS FOR Rh D GROUPING
Rh Control is the negative control for Anti-D, it 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 labelled 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 and document the result.

Result Interpretation: Results must be negative because 6% Bovine Albumin does not contain any antibodies. If the result is Positive then 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 or 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. Annex 2&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:

<table>
<thead>
<tr>
<th>Reaction of Red Cells with Antiseras</th>
<th>Reaction of Serum with reagent Red Cells/Reverse Group Typing</th>
<th>Interpretation of Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>A1 Cells</td>
<td>B Cells</td>
</tr>
<tr>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>-</td>
<td>+++</td>
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</tbody>
</table>
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

<table>
<thead>
<tr>
<th>False Negative</th>
<th>False Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Centrifugation time too short</td>
<td>Over centrifugation</td>
</tr>
<tr>
<td>2. Reagent or Serum not added</td>
<td>Incorrect Interpretation</td>
</tr>
<tr>
<td>3. Inappropriate ratio of serum / reagent to cells</td>
<td>Used dirty glass ware</td>
</tr>
<tr>
<td>4. Wrong technique, not following manufacturer advice</td>
<td>Used contaminated reagents, cells, 0.9% saline</td>
</tr>
<tr>
<td>5. Haemolysis not identified as positive reaction</td>
<td>Cells contaminated with Wharton’s jelly</td>
</tr>
<tr>
<td>6. Incorrect interpretation</td>
<td>Incorrect interpretation</td>
</tr>
<tr>
<td>7. Weak D Test not performed</td>
<td>False Positive weak D test, due to positive DAT</td>
</tr>
<tr>
<td>8. QC failure of antisera</td>
<td></td>
</tr>
</tbody>
</table>

**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. 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.

Presence of 'Weak D' in blood bank: As donor he/she should be considered as Rh-D-Positive (cf. BTS/SOP/TP/24a)
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 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 of 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) react 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 to 12H) 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$_3$-EDTA)
- Test red cells suspended in saline (2-3%)

4.3 REAGENTS

- Anti A, Anti-B anti-sera
- 2-3% suspension of group A, B reagent red cells
- 6% Albumin Control Reagent (Rh Control)
- Use only Anti-D reagents approved for use in microtest plate tests
- 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
- Aluminium racks to hold sample tubes
- Needle discarader/red or yellow bio-hazard waste box

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/serum in 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 shaker.
Read, 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 A1, B and O reagent red cells to separate clean wells of a U-bottom microtest plate.

---

* 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:

<table>
<thead>
<tr>
<th>Reaction of Red Cells with Antisera /Forward Group Typing</th>
<th>Reaction of Serum with reagent Red Cells/Reverse Group Typing</th>
<th>Interpretation of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Rh D</td>
</tr>
<tr>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
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</tbody>
</table>


|+++|+++| - | - | - | - | 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.

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<th>Name &amp; signature</th>
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</table>
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\textsubscript{1} Lectin to prove presence of A antigens on the cell surface (e.g. A\textsubscript{1} 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 control reagents and proper cell concentrations are used.

3. **PRINCIPLE**

The anti-A\textsubscript{1} lectin reagent will cause agglutination red cells, which carry the A\textsubscript{1} antigen, after centrifugation. If there is no agglutination, it indicates the presence of A\textsubscript{2} antigen.

4. **MATERIAL**

- Table top centrifuge
- Test tubes (glass)
- 0.9 \% 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 labelled test tube: 1 drop of Anti-A\textsubscript{1} 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.
5. To confirm the reactivity of anti-sera, use positive and negative red cells with every batch.
6. RESULTS

Presence of agglutination indicates a positive reaction (A1 antigen is present). Absence of agglutination indicates negative reaction (A1 antigen is absent) and blood group is labeled as A₂.

7. DOCUMENTATION

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

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</table>
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$_3$EDTA tube)/CPDA1 blood samples of donors.
- 3-5% red cell suspension of the test sample.

4.3 **REAGENT**

- Anti-D IgG type 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
- Aluminium 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 labelled test tube.
4. Place 2 drops of the appropriate control reagent in a second, labelled test tube.
5. To each tube, add 1 drop of 3-5% red cell suspension 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 0.9% saline.
10. After final washing, add 2 drops of anti-human globulin reagent (Coomb's reagent) & re-suspend the cell button.
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-human 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
Anti-globulin procedure with an Rh control reagent (commercially prepared 6% bovine albumin or 0.9% saline) 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.
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**

- Patient/recipients’ red cell and serum sample

4.3 **Reagents**

- Untreated screening cells
- 22% Bovine albumin/LISS (Low Ionic Strength Saline)
- Polyspecific 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)
• Aluminium 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, N, S, P, Lewis, Luthran, etc. OR

(2) IAT method detects the type of warm reacting antibodies like anti Rh, anti kell, anti-Kidd and anti-duffy, etc.

For identification of antibodies normally cell panels of 10 to 11 different red cell suspensions are used. They can be procured as enzyme-treated too (papain 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, I I & I I I 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 labelled 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 If negative then add 2-3 drops of bovine albumin or LISS to all the tubes.
12 Incubate at 37°C for 30 minutes. (10 minutes in case of LISS)
13 After incubation, centrifuge at 3400 rpm for 15 seconds, gently dispense the cell button, grade & record all results.
14 Wash all the tubes 3 times with normal saline.
15 After the third wash decant supernatant completely.
16 Add 1-2 drops of polyspecific Coombs reagent to the "cell button" and mix gently.
17 Centrifuge all tubes at 3400 rpm for 15 seconds.
18 Take out all the tubes gently, read macroscopically.
19 Grade and record all results on the antigram.
20 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 37ºC

- 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 initialled by the technician who has performed the test and by the Doctor/Technologist who has checked the results

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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 patient’s 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°C to 6°C
- Tabletop centrifuge
- Automated cell washer

4.2 **SPECIMEN**

- 2-3 ml 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 PHASE/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 for the issuance of blood in emergency situations.

1. Take a test tube (preferably glass tube) and label with patient’s/donor ID
2. Prepare 3-5% red cell suspension of donor red cells (*cf. Annex 1*)
3. Centrifuge patient blood at 3400 rpm for 5 minutes to get clear serum
4. Dispense 2 drops of patient serum into the labelled 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 ALBUMIN/37°C PHASE

1. Add 2 drops of Bovine Albumin or LISS and incubate the tube for 30-45 minutes for albumin and 10-15 minutes for LISS at 37°C
2. Take out tubes from 37°C, spin for 15 seconds, grade the results for agglutination and haemolysis and record the results
5.3 AHG/COOMBS PHASE

1. Wash three times with 0.9% saline
2. Add two drops of anti-human globulin, spin for 15 seconds at 3400 rpm
3. Take out the tubes gently and observe macroscopically for haemolysis and agglutination by gentle shaking
4. Grade and record results

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
- Never issue blood which is found incompatible at any phase of cross-match

**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.

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### Annex 1

<table>
<thead>
<tr>
<th><strong>CELL WASHING PROCEDURE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dispense 0.5 ml of whole blood or packed red cells in a 4 cc clean test tube.</td>
</tr>
<tr>
<td>2. Fill the tube ¾ full with 0.9% saline to re-suspend the cells.</td>
</tr>
<tr>
<td>3. Ensure proper mixing of cells with saline</td>
</tr>
<tr>
<td>4. Centrifuge the tubes for 45 seconds at 3400 rpm.</td>
</tr>
<tr>
<td>5. Discard maximum supernatant fluid/saline by a plastic dropper.</td>
</tr>
<tr>
<td>6. Repeat this washing procedure three times, every time save red cells sediment.</td>
</tr>
<tr>
<td>7. Sediment at the bottom of the tube is washed cells.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>5% RED CELL SUSPENSION PROCEDURE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Take 100 micro-liters of “Washed Red Cells” in a clean labeled test tube.</td>
</tr>
<tr>
<td>2. Add 1900µl of 0.9% saline (1:20 ratio) to make 5% red cell suspension.</td>
</tr>
<tr>
<td>3. Mix thoroughly and this suspension can be used for 12 hours.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>3% RED CELL SUSPENSION PROCEDURE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Take 60 micro-liters of “Washed Red Cells” in a clean labeled test tube.</td>
</tr>
<tr>
<td>2. Add 1940µl of 0.9% saline (1:20 ratio) to make 3% red cell suspension.</td>
</tr>
<tr>
<td>3. Mix thoroughly and this suspension can be used for 12 hours.</td>
</tr>
<tr>
<td>4. Store at 2-8°C when not in use.</td>
</tr>
</tbody>
</table>
Annex 2

READING AND GRADING TUBE AGGLUTINATION

1. Gently shake or tilt the tube to re-suspend 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

INTERPRETATION OF AGGLUTINATION REACTION

<table>
<thead>
<tr>
<th>Macroscopically Observed Findings</th>
<th>Designation(USA)</th>
<th>Score(UK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One solid agglutinate, background clear and no free cells</td>
<td>4+</td>
<td>12</td>
</tr>
<tr>
<td>Several large agglutinates, background clear and no or few free cells</td>
<td>3+</td>
<td>10</td>
</tr>
<tr>
<td>Medium-size agglutinates, background turbid and many free cells</td>
<td>2+</td>
<td>8</td>
</tr>
<tr>
<td>Small agglutinates, background turbid and too many free cells</td>
<td>1+</td>
<td>5</td>
</tr>
<tr>
<td>Few tiny agglutinates, turbid background and all free cells</td>
<td>1+w</td>
<td>4</td>
</tr>
<tr>
<td>Barely visible agglutination, turbid background and almost all free cells</td>
<td>W+ or +/-</td>
<td>2</td>
</tr>
<tr>
<td>No agglutination</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mixtures of agglutinated and agglutinated red cells

<table>
<thead>
<tr>
<th>Haemolysis Grading</th>
<th>Plasma/Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Haemolysis</td>
<td>Haemolysis</td>
</tr>
<tr>
<td>Partial Haemolysis</td>
<td>Partial Haemolysis</td>
</tr>
</tbody>
</table>
PREPARATION OF 6% BOVINE ALBUMIN

**SCOPE:**
6% Bovine Albumin is isotonic to serum and contains no antibodies. It is used in Rh Control and weak D Control

**Requirement:**
1. 22% Bovine Albumin (stock)
2. 0.9% Saline
3. Adjustable Jester (100-1000µL)
4. Tips
5. Sterilized dropper vials
6. Labels and pen

**Calculation: For making 6% Bovine albumin**

**Formula:**
\[ C_1V_1 = C_2V_2 \]

\[ V_1 = \frac{C_2V_2}{C_1} \]

\[ V_1 = \frac{6 \times 5}{22} \]

\[ V_1 = \frac{30}{22} \]

\[ V_1 = 1.364 \text{ ml OR } 1364 \mu\text{L} \]

Volume of diluent can be calculated as follows:

**Volume of diluent = V2 – V1**

\[ \text{Diluent volume (V2)} = 5000 \mu\text{L – } 1364 \mu\text{L} \]
\[ \text{Diluent volume (V2)} = 3636 \mu\text{L} \]

**Preparation:**
1. Take sterilized dropper vial.
2. Using jester, pour 3636 µL OR 3.6 ml (round figure) 0.9% Saline in it.
3. Add 1364 µL OR 1.4 ml (round figure) 22% Bovine Albumin.
4. Mix Well.
5. Label it with 6% Albumin, Manufacturing and Expiry Dates and initials of the technician who made this.

**Note:**
Store at 2-8°C when not in use.
### Annex 5

#### PREPARATION OF CHECK CELLS

| PRINCIPLE | IgG coated check cells are used to validate all tests using antihuman globulin reagent (AHG). Check cells ensures;  
|           | • AHG reagent was added  
|           | • AHG reagent was active  
|           | • Washing was complete (all unbound proteins removed). |

| REAGENTS AND EQUIPMENT | • 12 x 75 mm test tubes  
|                        | • Anti-D human IgG type  
|                        | • 0.9% Saline  
|                        | • Centrifuge  
|                        | • Water bath  
|                        | • Alsever’s Solution  
|                        | • Adjustable Jester 10-100ul  
|                        | • Tips  
|                        | • Labels and pen  
|                        | • Aliquots from known O Rh Positive donor units (segments) from 3-5 donors. |

| PROCEDURE | 1 Label a clean glass tube.  
|           | 2 Dispense 0.5 ml/500µl of whole blood from five different O Positive blood bags to make a pool of 5.  
|           | 3 Wash 3 times with normal saline.  
|           | 4 Add 500µl normal saline.  
|           | 5 Add 0.5ml/500µl of Anti-D human IgG type to the pool cells.  
|           | 6 Mix the contents and incubate at 37°C for 30 minutes, during incubation gently shake the tube after every 5 minutes.  
|           | 7 After incubation, wash the tube 4-6 times with normal saline.  
|           | 8 Make 5% in Alsever’s solution (500 µL packed sensitized red cells to 9500 µL Alsever’s solution in a sterilized dropper bottle) OR 0.9% Saline can be used instead of Alsever’s Solution.  
|           | 9 Label the vial with check cells; manufacturing and expiry dates and initials of the technician who prepared them |

<p>| STORAGE TEMPERATURE | 2-6°C when not in use. |</p>
<table>
<thead>
<tr>
<th>STORAGE PERIOD</th>
<th>If prepared in Alsever’s Solution, shelf life is 2-3 weeks. If prepared in 0.9% saline, shelf life is 12-24 hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUALITY CONTROL</td>
<td>1. Label two tubes one as positive control and the other as negative control.</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>3. Centrifuge both the tubes in a calibrated centrifuge at 3400 rpm for 15 seconds.</td>
</tr>
<tr>
<td></td>
<td>4. Positive control should give 3+ to 4+ results and negative control should be negative.</td>
</tr>
<tr>
<td></td>
<td>5. Results should be documented on the daily QC sheet.</td>
</tr>
</tbody>
</table>
### Annex 6

**ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING**

<table>
<thead>
<tr>
<th>Forward Grouping</th>
<th>Reverse Grouping</th>
<th>Possible Causes</th>
<th>Resolution Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-AB</td>
<td>A1 Cells</td>
</tr>
<tr>
<td>1.</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>2.</td>
<td>4+</td>
<td>NEG</td>
<td>4+</td>
</tr>
<tr>
<td>3.</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
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</table>
# ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

<table>
<thead>
<tr>
<th>Patient</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A, B</th>
<th>A_1 Cells</th>
<th>B Cells</th>
<th>O Cells</th>
<th>Auto Control</th>
<th>POSSIBLE CAUSES</th>
<th>RESOLUTION STEPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>1+</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Subgroup of AB, probable A_2B with anti-A_1 antigen</td>
<td>Use anti-A_1 lectin Antibody Sc &amp; cd</td>
</tr>
<tr>
<td>5</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>NEG</td>
<td>O_2 Bombay</td>
<td>Test with anti-H lectin</td>
</tr>
<tr>
<td>6</td>
<td>NEG</td>
<td>NEG</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>NEG</td>
<td>NEG</td>
<td>Subgroup of A_1, probable A_1 with anti-A_1</td>
<td>Perform saliva studies Or absorption / elution</td>
</tr>
<tr>
<td>7</td>
<td>4+</td>
<td>2+</td>
<td>4+</td>
<td>NEG</td>
<td>4+</td>
<td>NEG</td>
<td>NEG</td>
<td>Group A with an &quot;acquired B&quot; antigen</td>
<td>Check patient history for lower gastrointestinal problem or sepsisemia; 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 antiserum, and measure with a pH meter (this acidified anti-B antiserum would agglutinate only true B antigens and not acquire B antigens)</td>
</tr>
<tr>
<td>8</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>NEG</td>
<td>2+</td>
<td>NEG</td>
<td>Allantoibody (like anti Lea, anti P1, anti M &amp; anti N)</td>
<td>Perform antibody screen and panel Selection of antigen negative reverse grouping cells for A &amp; O</td>
</tr>
</tbody>
</table>

---
Annex 7

ABO Grouping & Rh Typing by Tube Method

Patient or Donor Samples

3x washed red cells 3-5%
serum

1 drop 3-5% red cells
2 drops serum

1 drop reagent

mix gently

3100 rpm
15 seconds

Conclude

if donor and Rh-D negative

perform "weak D" test (SOP/TS/24)
### Annex 8

**ABO and Rh-D BLOOD GROUPING**

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>Anti-D-</th>
<th>A-</th>
<th>B-</th>
<th>D-</th>
<th>conclusion</th>
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</tbody>
</table>

Analysed by: ___________________________  Checked by: ___________________________
Blood Processing Laboratory
Standard Operating Procedures

BLOOD PROCESSING LABORATORY

COMPONENT PRODUCTION

1. Preparation of Red Blood Cells Concentrate (SOP/WP/27) 115
2. Preparation of Fresh Frozen Plasma/Cryoprecipitate (SOP/WP/28) 117
3. Preparation of Platelets (SOP/WP/29) 120
4. Labeling of Blood Bags and Blood Components (SOP/WP/30) 122

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Annex 1. Formula for Calculating RCF 124
STANDARD OPERATING PROCEDURE

BLOOD PROCESSING LABORATORY

PREPARATION OF RED BLOOD CELL CONCENTRATES (RCC)

<table>
<thead>
<tr>
<th>BTS/SOP/WP/27</th>
<th>REGIONAL BLOOD CENTER</th>
<th>Version: 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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
- Freshly collected whole blood in triple bags of average 450 ml (± 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 Label and check the satellite bag with the same donor unit number as that on the primary bag.
5.3 Balance the two sets of triple bags system in the plastic inserts on a two pan weighing scale.
5.4 Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge.
5.5 Position the bags in buckets parallel to the direction of the spin. Centrifuge/soft spin the bags at 2000 rpm for 10 minutes at 20° C.4
5.6 Keep the primary bags containing centrifuged blood on plasma expresser. Release the spring, allowing the plate of the expresser to extract the plasma into the satellite bag.
5.7 Break the seal of the tubing connecting to the satellite bag; the plasma will now flow into the satellite bag.
5.8 Leave 50-60 ml plasma along with the red cells in the primary bag.
5.9 Seal the tubing between the primary bag and the satellite bag at three places; remove the bag by breaking the middle seal.
5.10 Mix the contents thoroughly.
5.11 Label the primary bag as Red Blood Cell Concentrates (RCC) 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.

<table>
<thead>
<tr>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written or revised by</td>
<td>Reviewed or approved by</td>
<td>Authorized by</td>
</tr>
</tbody>
</table>

Date: Date: Date:

4 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/CYROPRECIPITATE

BTS/SOP/WP/28  REGIONAL BLOOD CENTER  Version: 2.0

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°C and it is thawed at 2-6 °C then after a process Cryoglobulin remains as a precipitate which is called Cryoprecipitate. It contains mainly Factor-VIII, Von Willebrand Factor, Factor XII, Fibronectin 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 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 2°C to 6°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.
- Electronic weighing scale/Double pan weighing balance.
- FFP Thawing Bath (+2°C to +6°C).
- Triple bags of average 450ml (± 10%) capacity with CPDA-1 as anticoagulant.
- Plasma deep freezer upright below -30°C.
- Manuals of all equipment for reference regarding use and maintenance of each equipment item.
5. PROCEDURE

5.1 Preparation of FFP & Cryoprecipitate using quadruple 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. Label and check the satellite bag with the same donor unit number as that on the primary bag.
3. Balance the two sets of satellite bags in the plastic inserts.
4. Keep equally balanced buckets diagonally opposite each other in the refrigerated centrifuge.
5. Position the bags in buckets parallel to the direction of the spin. Hard spin the bags at 20°C in pre-cool refrigerated centrifuge at 3500 rpm for 6 minutes\(^5\).
6. Place the centrifuged bag on to the plasma expresser stand.
7. Express the plasma into second empty bag leaving 50-60 ml plasma along with the platelets.
8. Seal the tubing three times and cut the tubing on the middle seal of the plasma bag short (1") to avoid breakage during freezing state.
9. Keep the plasma bag in the quarantine storage in the plasma deep freezer at a temperature below – 30°C.
10. Transfer to plasma deep freezer in issue area when the TTI tests results are available.

5.2 Preparation of Cryoprecipitate using quadruple bags:

1. The basic material is platelet poor fresh frozen plasma prepared according to 5.1, but with a 4\(^{th}\) satellite bag still connected. 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 frozen plasma bags in the cryobath/refrigerator at 2 to 6°C. When the plasma is thawed, place the bags in centrifuge inserts and balance the inserts on weighing scale.
3. Keep the position of the bags in inserts parallel.
4. Hard spin the buckets at 3500 rpm for 6 minutes at 4°C.
5. 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).
6. Seal the tubing and separate the cryoprecipitate and the cryo poor plasma bags.
7. Weigh the cryo and plasma bags and record.
8. Refreeze the cryoprecipitate immediately, i.e. within 1 hour of thawing.
9. The plasma separated is F-VIII deficient plasma. Both the bags are kept in quarantine till the tests are completed.
10. Label and enter in the inventory and place them in deep freezer at below – 300C in issue area after test results are available.

\(^5\) 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).
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.

<table>
<thead>
<tr>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written or revised by</td>
<td>Reviewed or approved by</td>
<td>Authorized by</td>
</tr>
<tr>
<td>Date:</td>
<td>Date:</td>
<td>Date:</td>
</tr>
</tbody>
</table>
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.
- 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.
4 Label and check the satellite bag with the same donor unit number as that on the primary bag.
5 Express the PRP into the satellite bag intended for platelet storage leaving 50-60 ml plasma along with the red cells.
6 Mix the contents thoroughly and seal the tubing between the primary bag and the satellite bag at three places; break the middle seal.
7 Keep the primary bags containing packed cells in quarantine storage in the blood bank refrigerator at 2 to 6°C.
8 Centrifuge the platelet rich plasma (PRP) and satellite bag at 20°C using hard Spin at 5000 rpm for 10 minutes after balancing the inserts.6
9 Express the platelet-poor plasma into the 2nd satellite bag leaving 50-60 ml plasma along with the platelets (use scale).
10 Seal the tubing at three spots; break the middle seal.
11 Leave the platelet concentrates at room temperature for an hour, keeping the label side down.
12 Mix the contents of the bag manually to achieve uniform re-suspension.
13 Place the units for quarantine storage in the incubator at 20°C to 24°C on the lower shelf with continuous gentle agitation.
14 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.

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6 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).
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 in the inventory for use, these 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:
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.

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Annex 1

Formula for calculating relative centrifugal force (RCF)

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<th>RCF</th>
<th>RPM</th>
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<tr>
<td>$28.38 \times R \times (RPM/1000)^2$</td>
<td>$\sqrt{RCF/(28.38 \times R)} \times 1000$</td>
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Where:
- **RCF** = relative centrifugal force ($\times g$)
- **R** = radius in inches
- **RPM** = revolutions per minute
Storage of Blood Components
Standard Operating Procedures

STORAGE AND DISTRIBUTION OF BLOOD COMPONENTS

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STORAGE OF BLOOD COMPONENTS

STORAGE OF RED CELL CONCENTRATES

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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 to 6°C in a blood centre refrigerator.

4.5 Arrange the blood units inside the fridge properly to allow easy circulation of cold wave (air).

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.

5. DOCUMENTATION

Record all whole blood and RCC released for use as well as the unsuitable units to be discarded in the disposal register.
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°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 BLOOD 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 -30°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.

5. DOCUMENTATION

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

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STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS

STORAGE OF PLATELETS

BTS/SOP/WP/33  REGIONAL BLOOD CENTER  Version: 2.0

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°C to +24°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.
5. DOCUMENTATION

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

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Distribution to HBB
STANDARD OPERATING PROCEDURE

DISTRIBUTION TO HBB

RECEPTION AND DOCUMENTATION OF REQUESTS FROM HBB

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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

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Date: Date: Date:
1. **SCOPE AND APPLICATION**

This procedure applies to all blood and blood components that need 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 on the outer side 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 it in 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°C ± 2°C for at least 24 hours on top of the red cells in the transport container (avoid touching of frozen gel with the component bags).
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 designated hospital blood bank staff 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°C ± 2°C or lower for at least 24 hours.
9. Close the container and transport immediately 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.
13. FFP cannot be issued without grouping.

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 20-24°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.
10. Transportation between facilities should not exceed the validated transport time for the transport containers used.
11. Platelets transportation can be in incubator/agitator.

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

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### Formula for calculating relative centrifugal force (RCF)

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<th>RPM = \sqrt{\left(\frac{RCF}{28.38 \times R}\right) \times 1000}</th>
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<tr>
<td>[ RCF = 28.38 \times R \times (\text{RPM}/1000)^2 ]</td>
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Where:
- **RCF** = relative centrifugal force (× g)
- **R** = radius in inches
- **RPM** = revolutions per minute
PREPARATION OF RED BLOOD CELLS CONCENTRATES (RCC)

1. Weigh primary bag and record
2. Erect for 30-45 minutes
3. 2 whole blood units
4. Weigh (including inserts)
5. Fresh Plasma
6. Red Cell Concentrate
7. Mix gently
8. Seal tube (3x)
9. Break middle seal
10. Extract plasma
11. Centrifuge 2000 RPM, 10 minutes, Break 6, 20-22 °C
Standard Operating Procedures

RECEPTION OF BLOOD COMPONENTS

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Reception of Blood Components
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 dis-colouration 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 R BC 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**
Storage of Blood Components
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 compatibility 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 to 6°C.

   4.6 Arrange the blood unit inside the blood bank refrigerator 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.

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STANDARD OPERATING PROCEDURE

STORAGE OF BLOOD COMPONENTS

STORAGE OF FRESH FROZEN PLASMA/CYROPRECIPITATE

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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 (≤-30°C) and place them in the horizontal position.

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 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 (≤-30°C) 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 after the period of 2-3 months.
5. DOCUMENTATION

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

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</table>
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. **MATERIALS REQUIRED**

- 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) on 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 agitation 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.

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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 37°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: (a) 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 (b) immediately change the water if found any contamination in the water bath.
4.8 Hold the issuance (a) if any kind of leakage is observed, (b) if plasma overwrap found fill with water during thawing.

5. **DOCUMENTATION**

Record all FFPs thawed and issued for use.

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Issuance of Blood Components
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 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., RCC, FFP/Cryoprecipitate, Platelet Concentrates, 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 3 ml red top (clot) tube and 2 ml purple top (K3 EDTA) tube. In some cases small quantity of 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, sex and preferably the hospital admission number/medical registration number (MR No).*
         *This must be done by blood drawer (phlebotomist) before leaving patient’s bedside*
         *NOTE: Name, age, sex, MR No. 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 and must not be returned back to the ward 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 lipaemic plasma  

6. Age of sample:  
When samples are intended for use in cross-matching, they must not be 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 at least 72 hours following transfusion.  
b. Keeping patient’s and donor’s samples allow 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.  

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STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS

ABO GROUPING AND Rh D TYPING BY TUBE TEST

<table>
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<tr>
<th>BTS/SOP/TP/45a</th>
<th>HOSPITAL BLOOD BANKS</th>
<th>Version: 2.0</th>
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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 patient/recipient and ensuring the reliability of the results. This procedure describes the method of detection 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 of the new born.

2. RESPONSIBILTY

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

Trained Technician is responsible to perform the ABO grouping and RhD typing of patient/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) react 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, one EDTA (purple tube) and one clotted (red tube) up to the mark
- 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
- Rh control: 6% Bovine Albumin OR commercially available Rh control (cf. Annex 4)
- Fresh 0.9% Saline in washing bottle.
- Prepare 3-5% red cell suspension of donor/patient red cells (cf. Annex 1) a n d for reverse grouping A₁, B & O cells.
- All A/B/O reverse blood grouping cells should be Rh “D" negative (i.e. A Negative, B Negative and O 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.

---

6 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. )
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!

1. Prepare cell suspension for cells being tested (cf. Annex 1)
2. Label 4 clean test tubes with A, B, auto and D along donor ID (auto=auto control)
3. Arrange the test tubes in a row
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.
10. Grade and record test results. (cf. Annex 3)

5.2 SERUM TESTING / REVERSE GROUP TESTING

1. Centrifuge patient/donor blood specimen to get clear serum / plasma for reverse grouping.
2. Label 3 clean test tubes with A1, B, and O along with unique patient/donor ID
3. Arrange the test tubes in a row
4. Add 2 drops of patient/donor serum in all tubes in the corresponding tube
5. Add one drop of known 3 - 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.

**Note:** Run auto-control ONLY in case of ABO discrepancy.
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. In case of ‘Positive Result’, the case is referred to the shift incharge/head of the Hospital Blood Bank to solve the 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:

<table>
<thead>
<tr>
<th>Reaction of Red Cells with Antisera/ Forward Group Typing</th>
<th>Reaction of Serum with reagent Red Cells/Reverse Group Typing</th>
<th>Interpretation of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A Anti-B Rh D auto</td>
<td>A1 Cells B Cells O Cells</td>
<td>ABO Rh D</td>
</tr>
<tr>
<td>+++ - +++ auto</td>
<td>+++ -</td>
<td>A Pos</td>
</tr>
<tr>
<td>- +++ +++ -</td>
<td>+++ -</td>
<td>B Pos</td>
</tr>
<tr>
<td>+++ +++ +++</td>
<td>- -</td>
<td>AB Pos</td>
</tr>
<tr>
<td>- - +++ -</td>
<td>+++ -</td>
<td>O Pos</td>
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<tr>
<td>+++ - - -</td>
<td>+++ -</td>
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<td>+++ -</td>
<td>B Neg*</td>
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<td>+++ +++ -</td>
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<td>AB Neg*</td>
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<tr>
<td>- - - -</td>
<td>+++ -</td>
<td>O Neg*</td>
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</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>False Negative</th>
<th>False Positive</th>
</tr>
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<tbody>
<tr>
<td>1. Centrifugation time too short</td>
<td>Over centrifugation</td>
</tr>
<tr>
<td>2. Reagent or Serum not added</td>
<td>Incorrect Interpretation</td>
</tr>
<tr>
<td>3. Inappropriate ratio of serum / reagent to cells</td>
<td>Used dirty glass ware</td>
</tr>
<tr>
<td>4. Wrong technique, not following manufacturer advice</td>
<td>Used contaminated reagents, cells, normal saline</td>
</tr>
<tr>
<td>5. Haemolysis not identified as positive reaction</td>
<td>Cells contaminated with Wharton’s jelly</td>
</tr>
<tr>
<td>6. Incorrect interpretation</td>
<td>Incorrect interpretation</td>
</tr>
<tr>
<td>7. Weak D Test not performed</td>
<td>False Positive weak D test, due to positive DAT</td>
</tr>
<tr>
<td>8. QC failure of antisera</td>
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</table>

**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 titre 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. 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.

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Page 161
1. **SCOPE AND APPLICATION**

This procedure is applied for compatibility testing of all patients/recepients 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 (properly mixed during blood collection) from blood units to be cross matched.
2 Take a test tube (preferably clean glass tube) and label with the unit number of the blood bag/donor segments to be used.
3 Empty the donor segments in the corresponding glass tube.
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” to 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 patient’s 3-5% cell suspension in the second tube marked “auto”.
10 Centrifuge at 3000 rpm (1000xg) for 15 seconds.
11 Take out the tube gently.
12 Examine the tubes for agglutination (haemolysis) against a good light source macroscopically.
13 If there is no agglutination then proceed to albumin phase or LISS 37°C.

5.2 Albumin Phase or LISS 37°C Phase
1 Add two drops of 22% bovine albumin or LISS in both tubes and incubate for 30-45 minutes and 10-15 minutes for LISS at 37°C.
2 Take out the tubes from 37°C
3 Centrifuge both tubes at 3000 rpm (1000xg) for 20 seconds.
4 Examine for agglutination (haemolysis) in a good light source.
5 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) in both tubes and mix.
3 Centrifuge for 15 seconds to 1 minute at 3000 rpm (1000g).
4 Take out the tubes gently. Observe macroscopically for the agglutination (haemolysis) by gentle shaking.
5 If there is no agglutination add one drop of Coombs Control Cells.
6 Centrifuge for 15 seconds to 1 minute at 3000 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 cross-match 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 at least 72 hours after crossmatch/transfusion.

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

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Date:
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°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 (1ml Diluent/LISS and 10 µl packed red cells or 25 µl 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 aluminium 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 patient’s 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.

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Page 167
1. **SCOPE AND APPLICATION**

This procedure applies to all testing that requires antibody screening, for patient's pre-transfusion incompatibility 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°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
- Aluminium racks to hold serum and coombs’ tubes

5. PROEDURE
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 Centrifuge for 15 seconds at 3000 rpm (1000g).
5.6 Note the agglutination (haemolysis) macroscopically against a good light source.
5.7 According to:
   - **Bovine-albumin procedure**: Add 2 drops 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.
5.8 Incubate the tubes 30 minutes in a water bath of 37°C.
5.9 Mix the cells and serum gently, Centrifuge for 15 seconds at 3000 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 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 at 3000 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°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 initialled by the technician who has performed the test and by the Technologist who has checked the results.
STANDARD OPERATING PROCEDURE

ISSUANCE OF BLOOD COMPONENTS

DAT/DIRECT COOMB’S TEST BY TUBE TECHNIQUE

<table>
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<th>BTS/SOP WP/47b</th>
<th>HOSPITAL BLOOD BANK</th>
<th>Version: 2.0</th>
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</thead>
<tbody>
<tr>
<td>Valid from:</td>
<td>Effective Date:</td>
<td>Review Period: 1 Year</td>
</tr>
</tbody>
</table>

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$_2$EDTA sample, it should not be haemolysed
- 12 x 75 mm clean glass tubes
- Pipettes
- Tips
- Marker
- Test Tube Holder
- Fresh 0.9% Saline
- Calibrated table top centrifuge
- Tissue or gauze piece
- Polyspecific AHG, Monospecific Anti-IgG and Anti-C$\alpha$3d
- 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 labelled tube
5.6 Add 2 drops of Polyspecific AHG
5.7 Mix and centrifuge at 3000 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-C3d 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)!
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°- 6°C
- Table top centrifuge
- Automated cell washer
- Water bath 37°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
• Aluminium 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 Mix well and Centrifuge at 3000 rpm (1000xg) for 15 seconds.
5.5 Examine the tubes for haemolysis.
5.6 Gently re-suspend red cell buttons and examine for agglutination.
5.7 Grade and record test results immediately.
5.8 Add 2 drop of 22% bovine albumin and mix well.
5.9 Incubate at 37°C for minimum 30 minutes.
5.10 Examine the tubes for haemolysis.
5.11 Mix well and Centrifuge at 3000 rpm (1000xg) for 15 seconds.
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 45 seconds.
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.

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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)
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°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°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

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Issuance for Clinical Transfusion
1. SCOPE AND APPLICATION

Before these blood bags are release for use in ward, they are labelled with relevant information about recipient's/patient's unique identity number, ABO and Rh D 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

Pre-printed 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 compatibility testing, blood units are shifted to the section of cross-matched components inventory ready for use.

4.2 Ensure that the compatible units have been labelled 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 labelled, 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*

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Discarding of Blood Components
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.

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

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### CELL WASHING PROCEDURE

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<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>7.</td>
<td>Dispense 0.5 ml of whole blood or packed red cells in a 4 cc clean test tube.</td>
</tr>
<tr>
<td>8.</td>
<td>Fill the tube ¾ full with 0.9% saline to resuspend the cells.</td>
</tr>
<tr>
<td>9.</td>
<td>Centrifuge the tubes for 45 seconds at 3400 rpm.</td>
</tr>
<tr>
<td>10.</td>
<td>Discard maximum supernatant fluid/saline by a plastic dropper.</td>
</tr>
<tr>
<td>11.</td>
<td>Repeat this washing procedure three times, every time save red cells sediment.</td>
</tr>
<tr>
<td>12.</td>
<td>Sediment the bottom of the tube is washed cells.</td>
</tr>
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### 5% RED CELL SUSPENSION PROCEDURE

<table>
<thead>
<tr>
<th>Step</th>
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<tbody>
<tr>
<td>4.</td>
<td>Take 100 micro-liters of “Washed Red Cells” in a clean labeled test tube.</td>
</tr>
<tr>
<td>5.</td>
<td>Add 1900 µl of 0.9% saline (1:20 ratio) to make 5% red cell suspension.</td>
</tr>
<tr>
<td>6.</td>
<td>Mix thoroughly and this suspension can be used for 12 hours.</td>
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### 3% RED CELL SUSPENSION PROCEDURE

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Take 60 micro-liters of “Washed Red Cells” in a clean labeled test tube.</td>
</tr>
<tr>
<td>2.</td>
<td>Add 1940 µl of 0.9% saline (1:20 ratio) to make 3% red cell suspension.</td>
</tr>
<tr>
<td>3.</td>
<td>Mix thoroughly and this suspension can be used for 12 hours.</td>
</tr>
<tr>
<td>4.</td>
<td>Store at 2-8°C when not in use.</td>
</tr>
</tbody>
</table>
Annex 2

**READING AND GRADING TUBE AGGLUTINATION**

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

**INTERPRETATION OF AGGLUTINATION REACTION**

<table>
<thead>
<tr>
<th>Agglutination Grading/Score</th>
<th>Macroscopically Observed Findings</th>
<th>Designation(USA)</th>
<th>Score(UK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One solid agglutinate, background clear and no free cells</td>
<td>4+</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Several large agglutinates, background clear and no/or few free cells</td>
<td>3+</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Medium-size agglutinates, background turbid and many free cells</td>
<td>2+</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Small agglutinates, background turbid and too many free cells</td>
<td>1+</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Few tiny agglutinates, turbid background and all free cells</td>
<td>1+w</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Barely visible agglutination, turbid background and almost all free cells</td>
<td>W+ or +/-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No agglutination</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mixtures of agglutinated and unagglutinated red cells</td>
<td>Mixed field</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Haemolysis Grading</th>
<th>Plasma/Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Haemolysis</td>
<td>Haemolysis</td>
</tr>
<tr>
<td>Partial Haemolysis</td>
<td>Partial Haemolysis</td>
</tr>
</tbody>
</table>
Annex 4

## PREPARATION OF 6% BOVINE ALBUMIN

**SCOPE**

6% Bovine Albumin is isotonic to serum and contains no antibodies. It is used in R₇, Control and weak D Control.

**Requirement:**

7. 22% Bovine Albumin (stock)
8. 0.9% Saline
9. Adjustable Jester (100-1000µL)
10. Tips
11. Sterilized dropper vials
12. Labels and pen

**Calculation:** For making 6% Bovine albumin

Formula: \( C_1V_1 = C_2V_2 \)

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_1 = \text{Initial Concentration} = 22% )</td>
<td>6. Take sterilized dropper vial.</td>
</tr>
<tr>
<td>( V_1 = \text{Initial Volume} = ? )</td>
<td>7. Using jester, pour 3636 µL OR 3.6 ml (round figure) 0.9% Saline in it.</td>
</tr>
<tr>
<td>( C_2 = \text{Final Concentration} = 6% )</td>
<td>8. Add 1364 µL OR 1.4 ml (round figure) 22% Bovine Albumin.</td>
</tr>
<tr>
<td>( V_2 = \text{Final Volume} 5 \text{ ml OR 5000 }\mu\text{L} )</td>
<td>9. Mix Well.</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
V_1 &= \frac{C_1V_1}{C_2} \\
&= \frac{6 \times 5}{22} \\
&= \frac{30}{22} \\
&= 1.364 \text{ ml OR 1364 }\mu\text{L}
\end{align*}
\]

Volume of diluent can be calculated as follows:

Volume of diluent = \( V_2 - V_1 \)

**Diluent volume (V2) = 5000 µL – 1364 µL**

**Diluent volume (V2) = 3636 µL**

Note: Store at 2-8°C when not in use.
## PREPARATION OF CHECK CELLS

### PRINCIPLE
- IgG coated check cells are used to validate all tests using antihuman globulin reagent (AHG). Check cells ensures:
  - AHG reagent was added
  - AHG reagent was active
  - Washing was complete (all unbound proteins removed).

### REAGENTS AND EQUIPMENT
- 12 x 75 mm test tubes
- Anti-D human IgG type
- 0.9% Saline
- Centrifuge
- Water bath
- Alsever’s Solution
- Adjustable Jester 10-100ul
- Tips
- Labels and pen
- Aliquots from known O Rh Positive donor units (segments) from 3-5 donors.

### PROCEDURE
1. Label a clean glass tube.
2. Dispense 0.5 mL/500µL of whole blood from five different O Positive blood bags to make a pool of 5.
3. Wash 3 times with normal saline.
4. Add 500µl normal saline.
5. Add 0.5 mL/500 µL of Anti-D human IgG type to the pool cells.
6. Mix the contents and incubate at 37°C for 30 minutes, during incubation gently shake the tube after every 5 minutes.
7. After incubation, wash the tube 4-6 times with normal saline.
8. 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.
9. Label the vial with check cells; manufacturing and expiry dates and initials of the technician who prepared them.

### STORAGE TEMPERATURE
- 2-6°C when not in use.

### STORAGE PERIOD
- If prepared in Alsever’s Solution, shelf life is 2-3 weeks. If prepared in normal saline, shelf life is 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 3+ to 4+ 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

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-AB</th>
<th>A1 Cells</th>
<th>B Cells</th>
<th>O Cells</th>
<th>Auto Control</th>
<th>POSSIBLE CAUSES</th>
<th>RESOLUTION STEPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Newborn with group &quot;O&quot; or elderly patient  Patient may have hypogammaglobulinemia, or agammaglobulinemia  May be taking immunosuppressive drugs</td>
<td>Check age of patient  Incubate at RT for 30 min or at 4°C for 15 min for weak antigens  Immunoglobulin levels  Drugs History</td>
</tr>
<tr>
<td>2</td>
<td>4+</td>
<td>NEG</td>
<td>4+</td>
<td>1+</td>
<td>4+</td>
<td>NEG</td>
<td>NEG</td>
<td>Subgroup of A1: probable A2 group with Anti-A1  Allo antibody</td>
<td>Use anti A1 Lectin  Antibody screening and identification</td>
</tr>
<tr>
<td>3</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>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-Pk, anti-M, anti-Le(a))</td>
<td>1) Wash red cells; use saline replacement technique  2) Perform cold auto absorption technique or use rabbit erythrocyte stroma (REST) absorbs.  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</td>
</tr>
</tbody>
</table>
### ABO DISCREPANCIES BETWEEN FORWARD AND REVERSE GROUPING

<table>
<thead>
<tr>
<th>Patient</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A, B</th>
<th>A&lt;sub&gt;r&lt;/sub&gt; Cells</th>
<th>B Cells</th>
<th>O Cells</th>
<th>Auto Control</th>
<th>POSSIBLE CAUSES</th>
<th>RESOLUTION STEPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
<td>1+</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Subgroup of AB; probable A&lt;sub&gt;B&lt;/sub&gt; with anti-A&lt;sub&gt;r&lt;/sub&gt;</td>
<td>Use anti-A&lt;sub&gt;r&lt;/sub&gt; lectin Antibody Sc &amp;d</td>
</tr>
<tr>
<td>5</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>NEG</td>
<td>O&lt;sub&gt;B&lt;/sub&gt; Bombay</td>
<td>Test with anti-H lectin;</td>
</tr>
<tr>
<td>6</td>
<td>NEG</td>
<td>NEG</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
<td>NEG</td>
<td>NEG</td>
<td>Subgroup of A&lt;sub&gt;r&lt;/sub&gt; probable A&lt;sub&gt;r&lt;/sub&gt; with anti-A&lt;sub&gt;r&lt;/sub&gt;</td>
<td>Perform saline studies Or absorption / elution</td>
</tr>
<tr>
<td>7</td>
<td>4+</td>
<td>2+</td>
<td>4+</td>
<td>NEG</td>
<td>4+</td>
<td>NEG</td>
<td>NEG</td>
<td>Group A with an &quot;acquired B&quot; antigen</td>
<td>Check patient history for lower gastrointestinal problem or sepsisemia; use modified BS-I lectin if available; or acidify anti-B typing reagent to pH 0.0 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)</td>
</tr>
<tr>
<td>8</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>NEG</td>
<td>2+</td>
<td>NEG</td>
<td>Anti-antibody (like anti-Lea, anti-P&lt;sub&gt;1&lt;/sub&gt;, anti-M &amp; anti-N)</td>
<td>Perform antibody screen and panel Selection of antigen negative reverse grouping cells for A &amp; O</td>
</tr>
</tbody>
</table>
Annex 7

Blood Grouping Flow Chart

ABO Grouping & Rh Typing by Tube Method

1. Patient or Donor Samples
   - Ix washed red cells 3.5%
   - Serum

2. 1 drop 3.5% red cells
3. 2 drops serum
4. 1 drop reagent
5. Mix gently
6. 3400 rpm 15 seconds

<table>
<thead>
<tr>
<th>Color of Reaction</th>
<th>Forward Grouping with Antiserum</th>
<th>Reverse Grouping with Antiglobulin Blood Cells</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| 1                 | +                              |      | A | +
| 2                 | +                              |      | B | +
| 3                 | +                              |      | AB| +
| 4                 | +                              |      | 0 | +
| 5                 | +                              |      | A | +
| 6                 | +                              |      | B | +
| 7                 | +                              |      | AB| +
| 8                 | +                              |      | 0 | +

7. Grade and record results
8. If donor and Rh-D negative, perform "weak D" test (SOP/TR24)
## Annex 8

**BLOOD GROUPING FORM**

**ABO and Rh-D BLOOD GROUPING**

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>Anti-D</th>
<th>A1</th>
<th>B</th>
<th>O</th>
<th>Conclusion</th>
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</tbody>
</table>

Analysed by: [Name]  
Checked by: [Name]
Annex 9

COMPONENT ISSUANCE REGISTER

<table>
<thead>
<tr>
<th>Blood Bag No.</th>
<th>Blood Group</th>
<th>Patient's unique hospital ID</th>
<th>Patient's Name/Age/Sex</th>
<th>Patient's Blood Group</th>
<th>Type of component issued</th>
<th>Date and Time of issuance</th>
<th>Cross match Result</th>
<th>Donor's Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Saline/Immediate spin phase</th>
<th>Albumin/LUSS 37°C</th>
<th>Coomb's/Anti Human Globulin</th>
<th>HBs Ag</th>
<th>HCV Ab</th>
<th>HIV Ab</th>
<th>Syphilis</th>
<th>Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Emergency</th>
<th>No. of Units Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planned Surgery/Transfusion</td>
<td></td>
</tr>
</tbody>
</table>

No. of Units Required

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Type of Desired Blood Component

<table>
<thead>
<tr>
<th>Whole Blood</th>
<th>Un-crossmatch “O” Negative Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell Concentrate</td>
<td>Group Specific un-crossmatched Blood</td>
</tr>
<tr>
<td>Platelet Concentrate</td>
<td>Gamma Irradiated</td>
</tr>
<tr>
<td>Fresh Frozen Plasma</td>
<td></td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td></td>
</tr>
</tbody>
</table>

Clinical Diagnosis (Indication for Transfusion)

______________________________

Baseline Investigations of Patient

| Haemoglobin                  |                                           |
| Platelet Count               |                                           |
| Prothrombin Time             |                                           |
| 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 labelled) 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

<table>
<thead>
<tr>
<th>Patient Blood Group</th>
<th>Donor Blood Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Saline Phase/Immediate spin phase</td>
<td>□ Compatible</td>
</tr>
<tr>
<td>□ Albumin Phase/LISS 37°C</td>
<td>□ Compatible</td>
</tr>
<tr>
<td>□ Coombs Phase/Anti Human Globulin phase</td>
<td>□ Compatible</td>
</tr>
</tbody>
</table>

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 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Temp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Pulse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ B.P mm/Hg</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>□ Duty MO</td>
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</tr>
<tr>
<td>□ Duty RN</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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____________________

<table>
<thead>
<tr>
<th>Patient Blood Group</th>
<th>Donor Blood Group</th>
</tr>
</thead>
</table>

Date / Time of Issue___________________________________________
Issuing Technologist ________________________________
Verifying Technologist ________________________________

Blood Bag(s) Received By

**TRANSFUSION RECORD (To be filled by Duty MO/RN)**

<table>
<thead>
<tr>
<th>Vital Signs</th>
<th>Pre-Transfusion</th>
<th>Time</th>
<th>During Transfusion</th>
<th>Time</th>
<th>Post-Transfusion</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Temp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pulse</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>☐ B.P mm/Hg</td>
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<td></td>
</tr>
<tr>
<td>☐ Duty MO</td>
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</tr>
<tr>
<td>☐ Duty RN</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

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 13

EMERGENCY ABO GROUPING AND Rh D TYPING BY TILE METHOD

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 being 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 Rh D 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 (A1, B and O cells)
- Anti-D
- 0.9% 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 A1, 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 A1, B and O cells to A1, 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°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.

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CLINICAL TRANSFUSION CHAIN
Standard Operating Procedures

PRE-TRANSFUSION PROTOCOLS

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CLINICAL TRANSFUSION PROTOCOLS

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Annex 8. Patient Haemovigilance Forms (ARs Reporting Forms) 231
Annex 9. Result entry format for the patient file/record 235
Pre-transfusion Protocols
1. **SCOPE AND APPLICATION**

This procedure applies to all prescribers who are responsible to take decision for transfusion of blood and blood components. It explains what type of information is required to put forward the requisition to blood bank for arranging the blood in both emergency and non-emergency cases.

2. **RESPONSIBILITY**

It is the responsibility of the duty medical officer to fill in the form as instructed by the prescriber and get it signed. Duty nurse is responsible to collect the sample and send it to the blood blank with the requisition form. Duty nurse is also responsible to check vitals before sending issuance slip to blood bank.

3. **PRINCIPLE**

Blood request form is introduced to see the compliance with clinical use of blood guidelines. Prescriber should have a thorough knowledge of the indications for the blood components and knowledge to answer patient’s queries. Through this procedure unnecessary transfusions can be avoided.

4. **MATERIAL**
   
a) Blood Request Form  
b) Issuance Form

5. **PROCEDURE**

   5.1 Assess the clinical condition of the patient  
   5.2 If transfusion is indicated, decide which component and number of units are required  
   5.3 Check the patient past transfusion history  
   5.4 Check the baseline investigations of patient  
   5.5 Fill in ‘Blood Request Form’ completely and correctly (cf. Annex 1)  
   5.6 Send the form to the Blood Bank with properly labeled sample for cross match  
   5.7 When blood is required,
a. Complete the issuance slip or blood release form
b. Check vitals (B.P, Pulse & Temperature) and take pre-transfusion blood sample of patient in EDTA and plain tube
c. Send the issuance slip to the Blood Bank signed by duty medical officer

6. DOCUMENTATION

Keep the record of sending request to blood bank in patient's file

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1. **SCOPE AND APPLICATION**

This procedure includes detailed instructions on the procedure for pre-transfusion identification of both the intended recipient and the blood component(s) to be transfused. It ensures that the products received from Hospital Blood Bank are properly checked for integrity of the bags (visually inspected for signs of damage, contamination, spoilage and haemolysis), expiry date and the results of compatibility and screening tests attached.

2. **RESPONSIBILITY**

Two persons are responsible to perform pre-transfusion identification include: a registered nurse & doctor (Senior OT Technician & Anaesthetist/Surgeon in case of transfusion in OR). They are responsible to receive and inspect the blood component(s) before transfusion and to confirm that the blood bag is correctly labeled and data on the cross-match form is identical with that of the patient for whom the request is generated. They are also responsible to check the screening results and expiry date of the bag.

3. **PRINCIPLE**

The majority of the transfusion errors are of a clerical nature. Checking the blood bags labeling and patient identification empower the high degree of precision needed for safe transfusion process. Different blood components have different storage conditions with varying shelf life, clear expiry date identification also guarantees patients receive only safe products. The bedside check is a vital step in preventing transfusion error.

4. **MATERIAL**

- Blood Component(s) received
- Cross match result slip/card and pasted label of screening results
- Transfusion record register
- Patients file
5. PROCEDURE

5.1 Inspects the blood units visually in well-lit area (cf. Annex 3) for
   • Leakage, haemolysis or unusual color (Lipaemic/Icterus)
   • Clumping or unusual cloudy appearance in platelets
   If any discrepancy/defect found then blood components must not be accepted/transfused and returned to the blood bank with reasons documented (cf. Annex 4)

5.2 Checking labeling and patient identification prior to transfusion
   (a) Check the donation and expiry date on the blood bag label
   (b) Check the blood component unique number on the compatibility slip or cross match slip against the blood unit number on the blood bag label
   (c) Now check the ABO & Rh blood group type of the donor and recipient on the blood label which are documented on compatibility slip.
   (d) This checking process must be established by both nursing staff and duty medical officer
   (e) If you find any discrepancy during the labeling confirmation process then send the component back to blood bank.
   (f) If labeling is correct then ask the patient about his/her full name and date of birth. It is essential that an open question is used, for example: "what is your name please?" not "are you Mr. /Mrs. ABC?"
   (g) Check the information received from patient with is against the ID or patient’s identity band, if the patient is confused or unconscious a responsible visitor/ attendant or a staff member who knows the patient can be asked to verify the patient’s identity.
   (h) Match the patient’s names and date of birth against the patient’s details written on wristband, blood bag and compatibility slip.
   (i) The information provided by the patient must be identical with wrist band and cross match slip.
   (j) If there is any discrepancy found during the above mentioned checking procedure, the blood component must not be transfused until the discrepancy is resolved.

6. DOCUMENTATION

   (a) Blood unit number
   (b) Type of blood components receive
   (c) Receiving date and time
Full and accurate documentation of every step of the pre-transfusion observation of bags is vital. Document the entire detail in transfusion record register.

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Checklist at the time of reception of blood bags

**TO BE FILLED BY MEDICAL STAFF**

Patient’s Name_____________________________ Sex____________________________

Hospital Registration No.____________________ Location_________________________

Blood/Blood Component:

<table>
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<tr>
<th>Whole Blood</th>
<th>Red Cell Concentrates</th>
<th>Platelets</th>
<th>FFP</th>
<th>Cryoprecipitate</th>
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Tick mark the box if find OK

1. Match recipient’s name with hospital admission record [ ]
2. Match recipient’s name with cross match form [ ]
3. Check recipient’s ABO group and Rh type on the form [ ]
4. The donor’s unit number(s) [ ]
   (In case of more than 1 unit write all the Unit numbers)
5. Check donor’s ABO and Rh type (bag label/on the form) [ ]
6. Cross-match Results (compatible) [ ]
7. Screening results (negative) [ ]
8. Acceptable date of expiry of the component [ ]
9. Visual inspection of bag [ ]
10. Intact all seals of the blood bag [ ]

The date_____________________________ and time of receiving____________________

Blood received by

Name: ____________________________Employee#: ____________________________

Initials of the staff receiving the blood/blood components: _______________________
1. **SCOPE AND APPLICATION**

This procedure applies to obtain the consent for the transfusion from the patients or their attendants after making them aware about the need of blood under emergency and non-emergency condition.

2. **RESPONSIBILITY**

It is the responsibility of prescribers/duty medical officer to inform the patient and obtain consent for the transfusion.

3. **PRINCIPLE**

Although the risks associated with receiving a transfusion are small in the context of overall risks of hospital care. However, as part of an effective quality system, patients who are able to communicate must be informed in good time about their treatment. Formal consent for transfusion is a pre-requisite. Regardless of any legal requirement, prescriber has a professional duty to make sure the patient knows if and why a transfusion is required. The discussion should include the reasons why transfusion may be needed and the risks and benefits of receiving blood.

4. **MATERIAL**

- Pre-transfusion Counselling Educational Material (cf. Annex 5)
- Consent Form (cf. Annex 6)

5. **PROCEDURE**

5.1 Provide information about transfusion as part of the information given to the patient about the whole process of care

5.2 Undertake the discussion with patient on risks and benefits of transfusion

5.3 Provide information leaflets

5.4 Inform the patient about the baseline and periodic observations of temperature, pulse, respiration and blood pressure

5.5 Record indication for transfusion and the discussion with patient on patient’s file
7. DOCUMENTATION

Keep the consent form in patient file

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Transfusion Protocols
1. SCOPE AND APPLICATION
This SOP will cover all the re-prerequisites required for the preparation of a recipient preceding the transfusion including identification of the patient, taking an informed consent for the transfusion from the patient or his/her legal guardian, checking of vitals, selection of blood infusion set, preparation of venipuncture site and the start of transfusion. This procedure applies to all the clinical setups/locations specified for transfusion such as hospital ward/Emergency Room/Operation Room/Intensive care unit, etc.

2. RESPONSIBILITY
It is the responsibility of the duty medical officer/house officer to supervise the procedures related to vitals assessment and preparation of venepuncture site and make sure that all details should be documented on the Haemovigilance form/patients file.
A registered nurse is responsible for taking vitals and preparing venipuncture site prior to transfusion.

3. PRINCIPLE
After completion of the first step for pre-transfusion verification of labeling of blood components with unit numbers and expiry date, donor and patient ABO & Rh type, results of cross-match and TTI screening and identification of intended recipient/patient (especially in case of similar names to avoid any fatal mistakes), the second step is to prepare the patient for transfusion of blood components. Blood/blood component transfusion must be within 30 minutes of issuance from bank.

4. MATERIAL
- B.P apparatus
- Thermometer
- Stethoscope
- Blood Infusion Set
- I.V Cannula (Optional)
- Blood Transfusion Form (ARs Form)
5. **PROCEDURE**

5.1 **Patient Identification at the time of Venipuncture**

(a) Identify the patient before blood is transfused.

(b) Perform a two-person check to confirm the patient identity before each unit of blood component is transfused. This will include:

- Check full name and age on the (1) blood unit (2) cross-match form and (3) patients file
- Check the medical record number/patient registration number on the (1) blood unit (2) cross-match form (3) patient file

5.2 **Checking Vitals**

(a) Record the vital signs (B.P, Pulse, R/R & Temperature) just before the transfusion, at the onset of the transfusion, at 15 minutes from starting time, midway for RCC and on completion of the transfusion (The vitals should be taken before sending the request for issuance of blood/blood components to ensure all vitals are appropriate for blood transfusion to avoid any unnecessary delays after issuance from BB)

(b) Document each set of vitals with date and time on blood transfusion form/ARs reporting form and on patients file

5.3 **Selection of Infusion Set**

(a) For whole blood, red cell concentrates, platelets, plasma and cryoprecipitate, use an infusion set containing an integral filter (170-200 microns) with 18G needle of normal set type 5. However #20 guage I/V catheter/cannula/needle is acceptable to transfuse Red Cell Concentrates

(b) Change the infusion set after a maximum of 6 hours or used for 2 units of Blood or Red Cell Concentrates

(c) In case of the massive transfusions, the blood infusion set may be changed if the set becomes blocked or have been used to transfuse 2 units of blood or Red Cell Concentrates

(d) In case of multiple blood components transfusion, use a new infusion set to transfuse platelets after the red cell concentrates or plasma transfusion

(e) Do not use the same infusion set for the post transfusion subsequent fluid infusions

(f) Do not administer any other infusion solutions or drugs through the same infusion set

5.4 **Preparation of Venipuncture Site**

(a) Make the recipient lie down with a pillow under the head or recline in a comfortable couch.

(b) Identify the patient again by name.

(c) Enter the details of unit to be transfused in patients file.

(d) Identify venipuncture site suitable for blood transfusion free from scar or skin lesions.

(e) 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.

(f) 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.

(g) Starting at the intended site of venipuncture and moving outward in a concentric spiral, apply spirit swab; let stand for 30 seconds.

(h) Allow the disinfected venipuncture site to air dry completely. Do not blow on it.

(i) Do not touch the prepared area before the needle has been inserted.

(j) Do not re-palpate the vein at the intended venipuncture site.

(k) Cover the area with dry, sterile gauze until the time of venipuncture.

(l) Dispose off used swab(s) into a waste bin meant for bio-hazardous material.

5.5 Transfusion Process

(a) Mix components thoroughly by inversion and transfuse through an intravenous line approved for blood administration, incorporating a standard 170 to 200 μm filter to remove clots and aggregates. Note: Use blood filters in accordance with the manufacturers’ instructions.

(b) Start transfusion within 30 minutes of issuance from blood bank.

(c) Observe the patient receiving a blood transfusion for signs of potential complications of transfusion and monitor vital signs for recognition of unusual reaction or any unusual symptom.

(d) Do not prime, add or infuse medications or solutions through the same tubing with blood components, except 0.9% Sodium Chloride Injection. ABO-compatible plasma, 4% Albumin or other suitable plasma expanders may be used only upon approval of the patient’s physician.

(e) Proceed with the transfusion not faster than 5 mL/min (10 to 15 drops per minute) for the first 15 minutes, unless otherwise indicated by the patient’s clinical condition. The rate of the transfusion will depend on the clinical context, age and cardiac status of the patient.

(f) Blood of the same component type can be administered sequentially without flushing between units. Flushing with a small amount of normal saline between component packs may help improve venous access flow rate or keep access open if the next units is not readily available.

(g) Complete transfusion of RCC unit within 4 hours (maximum). The rate for routine transfusion is 2-3ml/min for RCC & 7-10ml for plasma and platelets, faster rate of 20 ml/min can be applied in emergency cases. Discard remaining blood product if time of transfusion exceeds 4 hours. In case of neonates or elderly with cardiac disease, aliquoting is preferred.

(h) Transfuse one unit at a time. Use separate transfusion set if transfusion time is more than 4 hours. Administering two different types of blood components concurrently (such as platelets and plasma) is not recommended in routine practice because in the event of an adverse reaction it is difficult to ascertain which component was responsible. However, this may be unavoidable in an urgent massive transfusion episode.

(i) On completion of transfusion, discontinue the I/V line or place stopper if there is a cannula, take the patient’s vital signs, document and do initial in ARs form.

(j) Enter transfusion notes (concerning the safe transfusion or any reaction) in the patient’s file and sign.
(k) After completion of transfusion, return the empty blood bag or partially transfused bag (in case of reaction) with filled Blood Transfusion Report/ARs form to the Blood Bank. Follow the same in case of no transfusion reaction observed.

(l) If the transfusion of blood components cannot be initiated within 30 minutes of the receipt, components must be returned back to the Blood Bank. The reason for returning the unit shall be documented.

6. DOCUMENTATION

- Enter all set of vitals as mentioned in the procedure on Blood Transfusion Form/Patient file.
- In case of returned blood mention the reason on the file.
- Enter the date and time of transfusion when started and completed.
- Keep the record of informed consent form in the patient’s chart.
- Complete the Transfusion Notes in the nursing progress sheet of the ICU / Ward
  a) Signature with the name and designation
  b) Blood product and its identification number, starting date and time, completion time and total amount transfused
  b) Record change of infusion set as used
  b) Reaction should be reported if any has occurred
  c) Document the type of blood components on the parenteral therapy section of the ICU / Nursing flow sheet
- Intake and output record is to be updated.

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1. SCOPE AND APPLICATION

This procedure covers all the steps necessary to observe and monitor the transfusion process wherever (Ward/OR/ER/ICU) it is conducted. It also encompasses steps required to identify, investigate and manage the transfusion reaction if any take place. It applies to all members of hospital transfusion committee to follow them as deem necessary for the safety of the patients.

2. RESPONSIBILITY

It is the responsibility of duty medical officer and nursing staff to observe and monitor the transfusion process, to evaluate adverse reaction and take necessary actions whenever it takes place. Haematologist/duty pathologist is responsible for the investigation of reaction.

3. PRINCIPLE

In order to improve patient safety as well as ameliorating transfusion practices, hospital transfusion committee should play a key role in dealing with haemovigilance and management of adverse reactions in coordination with all multidisciplinary teams with the protocols developed. A Blood Bank Incharge should be consulted regarding the evaluation of patients with reactions, as well as selection of appropriate blood components for future transfusion.

4. MATERIAL

- B.P Apparatus
- Stethoscope
- Thermometer
- Blood collection tubes
- Adverse reaction investigation form

5. PROCEDURE

Transfusion Process Observation
(c) Record the vital signs (B.P, Pulse, R/R & Temperature) just before the transfusion, at the onset of the transfusion, at 15 minutes from starting time and at the completion of the transfusion.

(d) Do not leave the patient unattended during first 15 minutes of initiating transfusion.

(e) Document each set of vitals with date and time on blood transfusion/ARs reporting form and on patients file.

(f) Take pre-transfusion blood samples of patient in EDTA and plain tube

**Adverse Transfusion Reactions Management**

Educate the patient prior to staring transfusion to report any untoward effect experienced in transfusion to the attending doctor/nurse immediately.

Recognize transfusion reaction as mild/moderate/severe (*cf. Annex 7*)

**For mild reactions (first time)**

**Symptoms (Mild Febrile Reaction)**
- Temperature increase <1.5°F from baseline
- Stable haemodynamics
- No respiratory distress and no other symptoms

**Symptoms (Mild Allergic Reaction)**
Occasional urticarial spots and no other symptoms

**Actions**
1. Stop the transfusion immediately
2. Check the blood bag label and recipient identity
3. Check the vitals and call for medical assessment
4. Medical staff may consider the need to prescribe Paracetamol for pyrexia or antihistamines for urticaria
5. Continue transfusion at a slower rate with increased monitoring, e.g. TPR/B.P at 15-30 minutes intervals only if misidentification has been ruled out and BP is stable.
6. Do not re-start transfusion if patient is complaining of fever, breathlessness, backaches or any other severe problem
7. Send one sample for group and antibody screening (EDTA & Clotted) tubes to the blood bank and the filled ARs form

**Subsequent transfusion and recurrence of mild febrile reactions OR recurrence of mild allergic reactions**

**Actions**
1. Slow down transfusion rate
2. Check and monitor vital signs
3. Febrile reaction: Consider giving pre-medication of an antipyretic (e.g. Paracetamol)
4. Urticarial/allergic reaction: Consider giving premedication of an antihistamine (e.g. oral Phenergan)

**For moderate and severe reactions**

**Symptoms**
- Fever >1.5°F from baseline with or without rigors/chills
- Unexpected tachycardia or change in blood pressure
- Acute breathlessness, desaturation, wheeze, stridor or cyanosis
- Facial oedema +/- pharyngeal or laryngeal oedema
- Extensive erythematous or urticarial rash
- Acute pain up transfusion arm
- Chest or loin pain
- Severe apprehension
- JVP acutely elevated, onset of crepitation in lung
- Haemoglobinuria

**Actions**
1. Stop transfusion
2. Check the blood bag label and recipient identity
3. Check the vitals and call for medical assessment
4. Comfort and keep patient informed
5. Replace infusion set and administer saline to maintain the I/V line or blood pressure
6. Treat and stabilize patient as per medical instruction
7. Collect the blood sample for group and antibody screening (away from the site of cannula)
8. Send the pre-transfusion and post-transfusion samples, infusion set and attached blood bag along with properly filled ARs investigation form to the blood bank as soon as possible.
9. In case of suspected Haemolysis: Send sample for full blood count, blood film, coagulation screen to Haematology; Urea & Electrolytes, bilirubin to biochemistry and urine sample for urinalysis to check haemogolbinuria
10. In case of suspected Sepsis: Send blood cultures from patient and blood bag to microbiology
11. If respiratory distress presents: Send blood gases to biochemistry
12. Notify the blood bank promptly by phone
13. For all severe transfusion reactions: Inform the Transfusion Medicine Specialist or Clinical Haematologist immediately for further clinical advice and support.

**Adjuvant Treatment**
- Depends on cause, clinical state, test results and Transfusion Medicine Specialist or Clinical Haematologist Consultation:
  - Sepsis likely: broad spectrum injectable antibiotics
  - Anaphylaxis/anaphylactoid reaction: Depending on severity can include adrenaline IM & antihistamine IV
  - Transfusion associated circulatory overload (TACO): Diuretics and oxygen, positive airway pressure
  - Transfusion related acute lung injury (TRALI): respiratory support, RBC will initiate blood donor investigation
  - Recurrent severe allergic reactions: Use of washed cellular components may be required
  - Acute Haemolysis: Maintain blood pressure, force diuresis and alkaline urine

**Note:**
- Notify the Blood Bank immediately about the occurrence of adverse reaction and briefly describe the nature
- For any intervention, take advice of Transfusion Medicine Specialist or Clinical Haematologist.
- Delay the transfusion of additional units until the possibility of serological incompatibility has been investigated.
- Do not transport IV line with insertion spike (sharp end) exposed
6. **DOCUMENTATION**

1. Document the vitals both on ARs form and patient file
2. Complete the adverse reaction reporting form (*cf. Annex 8*) which will serve as a written request for investigation of the reaction
3. Document the final results in patient file

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Post-transfusion Protocols
1. **SCOPE AND APPLICATION**

These specifications provide guidance to the Ward Nurses, Doctor In charge, Blood Bank In charge and Blood Bank Technicians, as how to report transfusion reactions. All type of transfusion reactions (mild to life threatening) and transfusion related errors/near miss must be reported to the hospital blood transfusion service (HBB). The transfusion service will investigate, assess and report the event to the ‘Hospital Transfusion Committee’.

2. **RESPONSIBILITY**

Nurse/doctor transfusing blood is responsible for reporting the reaction. Blood bank technologist is responsible for investigating it. Conclusion should be made by the duty pathologist/haematologist.

3. **PRINCIPLE**

Any untoward effect during or following blood transfusion is considered as blood transfusion reaction. It needs to be reported, investigated promptly and defined as per SOP. Reactions related to the quality of the product must be reported to the distributing Regional Blood Centre.

4. **MATERIAL**

Blood transfusion bag with tubing (for performing re-cross match)
- Fresh sample for CBC (post transfusion Haemoglobin)
- Fresh sample for serum bilirubin
- Urine sample for R/E (free Haemoglobin & red cells)

5. **PROCEDURE**

Following samples should be sent to blood bank: spiked blood unit, 5 ml clotted or EDTA blood tube and any voided urine sample.
Following is done by blood bank technologist
   a) Exclude clerical error; recheck paper work, bag label and patient sample
   b) Repeat blood group on bag/segment, pre transfusion and post transfusion samples
c) Visual check of pre and post transfusion sample to rule out haemolysis

d) Repeat cross match of bag with pre and post transfusion sample

e) Perform Direct Coombs on post transfusion sample

f) Check haemoglobin in urine (post-transfusion) sample

g) Post-transfusion Haemoglobin and Bilirubin

h) Perform Gram Stain and Blood Culture on spiked blood unit and recipient samples

i) Type of reaction should be concluded by pathologist

6. DOCUMENTATION

6.1 All documentation should be done regarding:

a) Sample sent for re-examination

b) Haematologist report

c) Filing of transfusion reaction report to the patient record

6.2 Authorized doctor in charge of blood bank will document & dispatch the report to physician incharge of the patient and must file transfusion report to patient personal file.

6.3 The adverse events should be discussed at the ‘Hospital Transfusion Committee’ meetings and the hemovigilance data must be submitted to the respective blood transfusion authority.

<table>
<thead>
<tr>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
<th>Name &amp; signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written or revised by</td>
<td>Reviewed or approved by</td>
<td>Authorized by</td>
</tr>
</tbody>
</table>

Date: 

Written or revised by: 

Date: 

Reviewed or approved by: 

Date: 

Authorized by: 

Date:
ANNEXES
Annex 1

BLOOD REQUEST FORM

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

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
- Emergency
- Planned Surgery/Transfusion

Type and No. of Desired Blood Component(s) / Units:

<table>
<thead>
<tr>
<th>Type</th>
<th>Required No.</th>
<th>Date &amp; Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell Concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Frozen Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Un-crossmatch &quot;O&quot; Negative Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group Specific un-crossmatched Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma Irradiated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clinical Diagnosis (Indication for Transfusion)

____________________________________________________________________________________

Baseline Investigations of Patient

- Haemoglobin
- Platelet Count
- Prothrombin Time
- Partial Thr

Past Transfusion History

Previously Identified Blood Group __________________________ Irregular Antibodies____________________

Last Transfusion Date____________________________

Previous Adverse Reaction Type __________________________ Date________________________

Requesting Physician Name and Sign: __________________________ Receiving Technician Name and Sign:

Date/Time: __________________________ Date/Time: __________________________
Annex 2

ISSUANCE SLIP

BLOOD RELEASE FORM

Name of Patient ____________________________________________________________

Age_____________________________Sex________________________________________

Patient Registration No. ____________________________________________________

Location (ICU/OT/Clinical Ward) ____________________________________________

PLEASE RELEASE __________________________ UNIT(S) OF RCC/FFP/PLATELET

Signature ________________________________________________________________

Date____________________________

Received By ____________________ Date ________________ Time____________________
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Red Cell Concentrates</th>
<th>Platelets</th>
<th>Fresh Frozen Plasma</th>
<th>Cryoprecipitates</th>
<th>Red Cell Concentrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>• Loss of intact red cells results in lower hematocrit and a brighter cherry red color. • Free hemoglobin imparts a light pink tinge to a dark red almost purple color to the supernatant. • Occurs in expired bags.</td>
<td>• Red cells present in plasma</td>
<td>• Free hemoglobin imparts a light pink tinge to a dark red almost purple color to the supernatant.</td>
<td>• Red cells present in plasma. When associated with hemolysis, a pink to red discoloration may be seen.</td>
<td>• Large dark red or purple color in supernatant.</td>
</tr>
<tr>
<td>Red Cell Contamination</td>
<td>• Grey discoloration</td>
<td>• Increased opacity</td>
<td>• Grey discoloration</td>
<td>• Increased opacity</td>
<td>• Grey discoloration</td>
</tr>
<tr>
<td></td>
<td>• Excessive and unusual air</td>
<td>• Bubbles</td>
<td>• Excessive and unusual air</td>
<td>• Bubbles</td>
<td>• Excessive and unusual air</td>
</tr>
<tr>
<td></td>
<td>Bright yellow to brown</td>
<td>• Milky white appearance</td>
<td>Bright yellow to brown</td>
<td>• Milky white appearance</td>
<td>Bright yellow to brown</td>
</tr>
<tr>
<td></td>
<td>Sandy color, light pink to a dark pink / light tan</td>
<td>Increased opacity</td>
<td>Sandy color, light pink to a dark pink / light tan</td>
<td>Increased opacity</td>
<td>Sandy color, light pink to a dark pink / light tan</td>
</tr>
<tr>
<td></td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
</tr>
<tr>
<td></td>
<td>Milky debriot, similar to a strawberry</td>
<td>Increased opacity</td>
<td>Milky debriot, similar to a strawberry</td>
<td>Increased opacity</td>
<td>Milky debriot, similar to a strawberry</td>
</tr>
<tr>
<td>Lipemia</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
</tr>
<tr>
<td></td>
<td>• A lighter shade of red and white</td>
<td>• Increased opacity</td>
<td>• A lighter shade of red and white</td>
<td>• Increased opacity</td>
<td>• A lighter shade of red and white</td>
</tr>
<tr>
<td></td>
<td>Bright yellow to brown</td>
<td>• Milky white appearance</td>
<td>Bright yellow to brown</td>
<td>• Milky white appearance</td>
<td>Bright yellow to brown</td>
</tr>
<tr>
<td></td>
<td>Sandy color, light pink to a dark pink / light tan</td>
<td>Increased opacity</td>
<td>Sandy color, light pink to a dark pink / light tan</td>
<td>Increased opacity</td>
<td>Sandy color, light pink to a dark pink / light tan</td>
</tr>
<tr>
<td></td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
</tr>
<tr>
<td></td>
<td>Milky debriot, similar to a strawberry</td>
<td>Increased opacity</td>
<td>Milky debriot, similar to a strawberry</td>
<td>Increased opacity</td>
<td>Milky debriot, similar to a strawberry</td>
</tr>
<tr>
<td>Particulate Material</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
<td>• Clots and fibrin strands</td>
<td>• Excessive and unusual air</td>
</tr>
<tr>
<td></td>
<td>• A dark purple to black</td>
<td>• Increased opacity</td>
<td>• A dark purple to black</td>
<td>• Increased opacity</td>
<td>• A dark purple to black</td>
</tr>
</tbody>
</table>

**VISUAL APPEARANCE OF AFFECTED BLOOD COMPONENTS**

---

**Annex 3**
masses that do not dissipate with gentle manipulation in red cells.

- Cellular aggregates appear as white and opaque masses that do not dissipate with gentle manipulation.
- White particulate matter varies from flattened specks to a greasy film and may dissipate with a change in temperature.
- Cold agglutinins form large red blood cell masses that do not dissipate with gentle manipulation.

The clotting process and may appear as white/opaque masses or whitish thread like strands that do not dissipate with gentle manipulation

- Cellular aggregates may appear as white and opaque masses that do not dissipate with gentle manipulation.
- Particulate matter may vary considerably in size.

Discoloration

- See hemolysis, lipaemia, bacterial contamination

| Brown | Brown | Brown | Particulate matter may not dissipate with gentle manipulation of red blood cell masses that do not appear as white and opaque masses or whitish thread like strands that do not dissipate with gentle manipulation.
|---|---|---|---|---|
| Very considerably in size. | Very considerably in size. | Very considerably in size. | Cellulose aggregates may not dissipate with gentle manipulation of red blood cell masses that do not appear as white and opaque masses or whitish thread like strands that do not dissipate with gentle manipulation.
| Particulate matter may not dissipate with gentle manipulation of red blood cell masses that do not appear as white and opaque masses or whitish thread like strands that do not dissipate with gentle manipulation.
| Particulate matter may not dissipate with gentle manipulation of red blood cell masses that do not appear as white and opaque masses or whitish thread like strands that do not dissipate with gentle manipulation.
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### Conditions

<table>
<thead>
<tr>
<th>Blood Components</th>
<th>Hemolysis</th>
<th>Lipaemia</th>
<th>Icterus</th>
<th>Bacterial contamination</th>
<th>Particulate matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Cell Concentrates</td>
<td>Some degree of hemolysis is acceptable and expected. The CSA standard will define acceptable levels as ≤ 0.8% at expiry.</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Not Acceptable</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Platelets</td>
<td>Some degree of hemolysis is possible depending on the number of red cells in the plasma.</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Not Acceptable</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Fresh Frozen Plasma</td>
<td>Some degree of hemolysis is possible depending on the number of red cells in the plasma.</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Not Acceptable</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>Some degree of hemolysis is possible depending on the number of red cells in the plasma.</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Not Acceptable</td>
<td>Not Acceptable</td>
</tr>
<tr>
<td>Red Cell Concentrates</td>
<td>Blood components containing clots and/or fibrin strands should not be transfused.</td>
<td>Blood components containing clots and/or fibrin strands should not be transfused.</td>
<td>Blood components containing clots and/or fibrin strands should not be transfused.</td>
<td>Blood components containing clots and/or fibrin strands should not be transfused.</td>
<td>Blood components containing clots and/or fibrin strands should not be transfused.</td>
</tr>
</tbody>
</table>

**Annex 4**

**BLOOD COMPONENTS ACCEPTABILITY CRITERIA**
Why do you receive a blood transfusion?

During management of disease, a patient may need a blood or blood product transfusion. Blood products include red blood cells, platelets, plasma and cryoprecipitate. The goal of transfusion therapy is to provide the most appropriate blood product for the patient.

**Red cell transfusion** is necessary in patients with aplastic anaemia, thalassemia, leukaemia, cancer and sometimes during and after surgery.

**Platelets** are small particles which help in clot formation. Platelet transfusion controls bleeding in patients with leukaemia, cancer and aplastic anaemia.

**Plasma** helps blood clot in patients having chronic liver disease, severe infections, inherited clotting factor deficiency and cancer.

**Cryoprecipitate** is used in patients with factor VIII deficiency as an alternative to factor VIII concentrate.

In case of haemorrhage, most people cope well with losing a moderate amount of blood. This loss can be replaced with intravenous fluid. Over the next few weeks, body will make new red blood cells to replace this loss. Medicines such as iron supplements can also help compensate for blood loss. However, if larger amounts are lost, a blood transfusion is the best way of replacing the blood rapidly. Some medical treatments or operations cannot be carried out safely without using blood products.

The decision to give a blood product is made only after careful consideration. In making this decision, the doctor will have had to balance the risk of having blood transfusion against the risk of not having one. If a blood transfusion is needed, the doctor should explain why it is necessary.

**Risks of Blood Transfusions**

The beneficial effect of each unit of blood or blood component transfused is accompanied by the possibility that the patient may experience an adverse reaction to the transfusion.

Transfusion reactions may be acute or delayed. In **acute transfusion reactions**, clinical signs and symptoms occur during or within 1 to 2 hours after the completion of the transfusion. A **delayed transfusion reaction** may not be evident for days, weeks, months, or even years after the transfusion.

**Why a prescriber should always prefer blood component transfusion instead of whole blood transfusion?**

In component therapy, one unit of whole blood can be divided into a number of components so that many patients can benefit from a single blood donation.

Blood transfusions are given to replace blood loss in surgery and after accidents. It is always preferable to transfuse a blood component required for the particular problem e.g. Red Cell Transfusions to treat severe anaemia (lack of red blood cells), Platelet Transfusion to treat thrombocytopenia (low or functionally defective platelets) or Plasma or Cryoprecipitate Transfusion to treat coagulopathies (decrease in clotting factors). With this practice the patient can be saved from the risks of the blood components which are not needed for the patient.
Annex 6

INFORMED CONSENT FORM FOR TRANSFUSION OF BLOOD COMPONENTS

I. PATIENT STATEMENT: I, the undersigned CONSENT to undergo the procedure of transfusion of blood or blood components with full knowledge of the need, the benefits, possible risks, side effects and the alternatives to a transfusion. I have also been informed about the risks and consequences of not receiving this therapy and been given an opportunity to ask questions regarding transfusion and have received answers to my questions and concerns in a language understandable to me.

(Signature/Thumb Imprint & Name)

II. PATIENT REPRESENTATIVE / INTERPRETER'S STATEMENT:

1. The patient is unable to consent because (where applicable):

   ____________________________________________________________
   ____________________________________________________________

2. I, therefore, consent for the patient:

   (Signature and Name) (Relationship to Patient)

3. Interpreter's attestation (where applicable): The translation has been provided by me:

   ________________________________
   (Signature and Name)

III. DOCTOR'S AFFIRMATION: I declare that I have personally explained the above information in detail to the patient and/or the patient's representative and have answered the entire patient's questions to the best of my knowledge.

   ________________________________
   (Signature and Name)
Annex 7

LIST OF ADVERSE REACTIONS, SIGNS AND SYMPTOMS

List of Adverse Reactions

1. Immunological haemolysis due to ABO incompatibility
2. Immunological haemolysis due to allo-antibody
3. Post-transfusion Purpura
4. Allergic Reaction
5. Anaphylactic/hypersensitivity reaction
6. Transfusion related acute lung injury (TRALI)
7. Graft versus host disease
8. Transfusion associated HIV-1/2 infection
9. Transfusion associated HBV infection
10. Transfusion associated HCV infection
11. Other transfusion associated viral infection
12. Sepsis due to bacterial contamination of the donor unit
13. Transfusion associated malaria infection
14. Other transfusion associated parasitical infection
15. Transfusion associated circulatory overload

Clinical Symptoms

1. Discomfort
2. Chills/rigors/flushing
3. Itching
4. Urticaria Isolated/Extensive
5. Redness
6. Rash
7. Jaundice
8. Low back pain
9. Chest/abdominal pain
10. Nausea/vomiting
11. Dyspnoea/Wheze/stridor/Pulmonary Oedema/Cough/Hypxaemia
12. Acute renal failure
13. Shock
14. Loss of consciousness

Biological Signs

1. Positive DAT/Direct Coombs
2. Hyperbilirubinemia
3. ALT>2N
4. Transfusion refractoriness
5. Haemoglobinuria
6. Haematuria
7. Fever
8. Raised JVP
9. Hypertension/Hypotension
10. Arrhythmia and 11. Respiratory Rate
### Annex 8

**RECIPIENT ADVERSE REACTION REPORTING FORM**

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Sex:  M [ ]  F [ ]  Age: ———— years  File number/MR No. ————</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Location (Ward/ICU/OT)</strong></td>
<td>————</td>
</tr>
<tr>
<td><strong>Date of transfusion (dd/mm/yy):</strong></td>
<td>————  / ————  / ————</td>
</tr>
<tr>
<td><strong>Date and Time of Appearance of Transfusion Reaction:</strong></td>
<td>———— min / ———— hour(s) / ———— day(s) / ———— year(s)</td>
</tr>
</tbody>
</table>

*(all other information is confidential and appears only on the hospital forms/patient record)*

<table>
<thead>
<tr>
<th>Patient’s Primary Diagnosis</th>
<th>Surgical [ ]  Medical [ ]  Obstetric [ ]  Oncologic [ ]  Haemotologic [ ]  Other: ————</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Indication for Blood Transfusion:</th>
<th>Specify:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Type of Blood Component (BC) Transfused</th>
<th>Type of BC: Whole Blood [ ]  Red cells [ ]  Platelets [ ]  Plasma/FFP [ ]  Additional modification of BC: Leucocyte-poor [ ]  Leucocyte-depleted/filtered [ ]  CMV negative [ ]  Irradiated [ ]  Plasma-depleted/washed [ ]  Other (specify): ————</th>
</tr>
</thead>
</table>

| Place of BC issuance | ———— |
|---------------------|———|

---
### Signs and Symptoms of Transfusion Reaction Observed

<table>
<thead>
<tr>
<th>Signs</th>
<th>Before tx</th>
<th>After tx</th>
<th>Clinical Symptoms (1)</th>
<th>Clinical Symptoms (2)</th>
<th>Biological Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td>...........</td>
<td>...........</td>
<td>□ Discomfort</td>
<td>□ Lower back pain</td>
<td>□ Positive DAT /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Chills</td>
<td>□ Chest/abdominal</td>
<td>Direct Coombs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Itching</td>
<td>□ pain</td>
<td>□ Hyperbilirubin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Urticaria</td>
<td>□ Nausea/vomiting</td>
<td>□ ALT &gt; 2N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Redness</td>
<td>□ Dyspnea</td>
<td>□ Transfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Rash</td>
<td>□ Oliguria/Anuria</td>
<td>refractoriness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Jaundice</td>
<td>□ Shock</td>
<td></td>
</tr>
<tr>
<td>Others:</td>
<td></td>
<td></td>
<td></td>
<td>□ Loss of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>consciousness</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Others:</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

#### Investigations Performed

<table>
<thead>
<tr>
<th>Name of Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Blood Group Reconfirmed</td>
<td></td>
</tr>
<tr>
<td>2 Re-Cross Match</td>
<td></td>
</tr>
<tr>
<td>3 Complete Blood Count</td>
<td></td>
</tr>
<tr>
<td>4 ALT</td>
<td></td>
</tr>
<tr>
<td>5 Bilirubin</td>
<td></td>
</tr>
<tr>
<td>6 Hemoglobinemia/Hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>7 DAT</td>
<td></td>
</tr>
<tr>
<td>8 Antibody Screening</td>
<td></td>
</tr>
<tr>
<td>9 Antibody Identification</td>
<td></td>
</tr>
<tr>
<td>10 Gram Stain</td>
<td></td>
</tr>
<tr>
<td>11 Blood Culture</td>
<td></td>
</tr>
<tr>
<td>12 Viral Serology</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions on RECIPIENT AR (Adverse Reaction to Transfusion) (only one for each report):

**Immunological**
- Haemolysis due to ABO incompatibility
- Haemolysis due to irregular antibody
  Specify: .............................................
- Immunisation to:
  - Red cells  
  - Platelets
  - HLA  
  - IgA
- PTP (post-transfusion purpura)
- Allergic reaction (mild)
- Anaphylactic reaction (severe)
- TRALI (tx related acute lung injury)
- TACO (tx associated circulatory overload)

**Infectious**
- Blood component with bacterial contamination
  Microorganism(s): .........................
- HIV
- HBV
- HCV
- CMV
- Malaria
- Other infectious agent: .................

**Others**
- NHFTR (Non haemolytic febrile transfusion reaction)
- TA-GVHD (tx associated graft versus host disease)
- Pulmonary oedema (due to cardiac failure, circulatory overload)
- Haemosiderosis
- Unspecified: ..............................................................

**Severity**
- 0. no effect
- 1. immediate, no vital
- 2. immediate, vital
- 3. long term morbidity
- 4. death

**Imputability**
- 0. excluded
- 1. possible, dubious
- 2. likely, probable
- 3. certain, proven

Other relevant clinical information on the transfused patient:
(e.g. prior condition of the recipient, medication, ....)

Patient outcome:

---

<table>
<thead>
<tr>
<th>Transfusion process</th>
<th>Location:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐ Operation Theatre</td>
<td>☐ Intensive Care Unit</td>
<td>☐ Medical</td>
<td>☐ Paediatric</td>
</tr>
<tr>
<td></td>
<td>☐ Outpatient Clinic</td>
<td>☐ Other unit/ward:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time:</td>
<td>☐ Working hours</td>
<td>☐ Night shift</td>
<td>☐ Weekend</td>
<td></td>
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</table>

Incorrect blood component transfused (IBCT):  Yes ☐ No ☐

Where in the process did the error occur?
- producing blood centre  
- hospital blood bank
- clinical unit/ward
- other: ..............................................................
<table>
<thead>
<tr>
<th>At production of blood component</th>
<th>At cross-matching</th>
</tr>
</thead>
<tbody>
<tr>
<td>At distribution/issuing</td>
<td>At transfusion (administration of BC to patient)</td>
</tr>
<tr>
<td>Other:</td>
<td>Describe the error:</td>
</tr>
</tbody>
</table>

Describe:...............................................................................................

### Associated involvement

- Materiovigilance / medical devices failure
- Reactovigilance / laboratory reagents failure
- Pharmacovigilance / medical products, medicines failure

Describe:..............................................................................................
Annex 9

TRANSFUSION REPORT FOR THE PATIENT FILE/RECORD

(to be filled for each transfusion)

Name of Patient: _________________________________ Age / Sex: ________________
Hospital Registration No: ___________________________ Ward: ________________
Patient's Blood Bank Ref No: _________________________
Patient’s Blood Group: ____________________ Unit No: _____________
Blood Group of the Unit: ________________ Compatibility Label Checked: Y / N (circle)
Type of component (circle one): RCC / WB / FFP / Platelet Concentrate / Pooled Platelets
Volume of the unit: __________ml
Name of the Prescriber (doctor): ____________________________________
Details of the nursing staff/duty medical officer performing the checks and starting the
transfusion:
Name: __________________________              Signature: ____________________
Date / time of starting the transfusion: ________________________________
Any IV Fluid joined? Y/N ___________ Any pre-medication given? Y/N ____________
Vitals to be noted as below:

<table>
<thead>
<tr>
<th>Time</th>
<th>Vitals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
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</tbody>
</table>

Outcome of the transfusion: □ Completed / □ Transfusion reaction occurred / (tick the
appropriate)
If reaction occurred, is it reported and are blood samples sent to the Blood Bank? Y / N
____________
Is the blood bank in-charge or blood bank staff informed? Y / N ________________
Details of the nursing staff / duty medical officer completing the transfusion / reporting the
reaction:
Name: ______________________________ Signature: ______________________

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References

5. Donor history questionnaire documents (Version No 1.1, dated June 2005), prepared by the AABB Donor History Task Force.
6. FDA’s guidance for industry: Implementation of acceptable full length donor history methodology.
12. Online WILEY Library, 14th July 2015
13. Operating Instructions for Blood Cell Separator COM.TEC ®. Fresenius HaemoCare Deutschland GmbH, Produktionsbereich Geräte, Hafenstrasse 9, D-97424 Schweinfurt, Germany
15. Package insert for qualitative determination of plasma reagins. Spinreact, SA, Ctra. Santa Coloma, 7 E-17176 Sant Esteve De Bas (GI) Spain.
17. Procedural Manual, Haemocue Haemoglobinometer. Territory Surgical Supplies Unit 7/9 Delatour Street, Coconut Grove NT 0801, Australia
20. WHO 2012 Donor Deferral Criteria

Appendix 1

SOP Flyer with Critical Control Points
### Standard Operating Procedures

#### Blood Center

<table>
<thead>
<tr>
<th>Donor Management</th>
</tr>
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<tbody>
<tr>
<td>Reception of Donor</td>
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<tr>
<td>Haemoglobin Screening</td>
</tr>
<tr>
<td>Pre-donation Counseling</td>
</tr>
<tr>
<td>Medical Interview</td>
</tr>
<tr>
<td>Physical Examination</td>
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<table>
<thead>
<tr>
<th>Collection of Donation</th>
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</thead>
<tbody>
<tr>
<td>Inspection of Blood Bags and Labeling</td>
</tr>
<tr>
<td>Preparation of Venipuncture Site</td>
</tr>
<tr>
<td>Proper sealing of tubes and labeling</td>
</tr>
<tr>
<td>Phlebotomy and Collection of WB</td>
</tr>
<tr>
<td>Apheresis Collection of BC</td>
</tr>
<tr>
<td>Collection of Blood Samples</td>
</tr>
<tr>
<td>Post Donation Care/Refreshments</td>
</tr>
<tr>
<td>Management of Adverse Reactions</td>
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<tr>
<td>Documentation of Adverse Reactions</td>
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<tr>
<td>Reception of Blood Samples</td>
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<tr>
<td>Testing for HBsAg</td>
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<tr>
<td>Testing for HCV Antibodies</td>
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<tr>
<td>Testing for HIV Antibodies</td>
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<tr>
<td>Syphilis Screening</td>
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<tr>
<td>Malarial Parasite Detection</td>
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<td>Reporting of Results</td>
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<td>Identification of Weak ABO Types</td>
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<tr>
<td>RHD Typing</td>
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<td>Identification of Weak Rh Types</td>
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<td>Red Cell Concentrates</td>
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<td>FFP/ Cryoprecipitate</td>
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<td>Dispatching Blood Components</td>
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<tr>
<td>Transporting Blood Components</td>
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### CCP Inspection

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<td>Donor ID</td>
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<td>HB + ABO/Rh(D)</td>
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<td>Informed Consent</td>
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<td>Check Questionnaire</td>
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<tr>
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<td>QC internal, external Devices Check</td>
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<td>Delivery</td>
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<td>Transport Standards</td>
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## Appendix 2

Pictorial View of WG Meetings

### Reception of BC
- Documented Reception of Blood Bags
- Inspection of Blood Bags
- Data Registration
- Temperature Monitoring

### Storage
- Red Cell Concentrates
- Fresh frozen plasma/Cryoprecipitate
- Platelets
- Thawing of FFP
- ID’s
- Documents
- Wrapping
- Storage
- Equipment

### Issuance of BC
- Reception of Clinical Request / Blood Samples
- Blood Grouping
- Cross Matching
- Antibody Screening
- Antibody Identification
- Filtration
- Washing
- Forms
- Pre-transfusion Testing
- Validation of Reagents and procedures
- QC internal, external
- Result Validation
- Centrifugation

### Distribution to Ward
- Labeling
- Handling returned / expired BC
- ID Check
- Documentation
Appendix 2

Pictorial View of WG Meetings
Appendix 3

SOP as Training Modules 1 & 2
STANDARD OPERATING PROCEDURE
HAEMOGLOBIN SCREENING BY HAEMOGLOBINOMETER

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. RESPONSIBILITIES

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

3. PRINCIPLES

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

Donor Management Department

<table>
<thead>
<tr>
<th>BTS/SOP/TP/02b</th>
<th>Regional Blood Center</th>
<th>Version 5.0</th>
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</thead>
<tbody>
<tr>
<td>Valid from:</td>
<td>Effective Date:</td>
<td>Review Period: 1 year</td>
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</table>
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.

**STEP 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.

**STEP 2:**
Give the donor a hand roller/squeezer to hold.

**STEP 3:**
Prepare the venepuncture site. *(SOP/WP/07)*

**STEP 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)*

**STEP 5:**
Inspect and label the bag. *(SOP/WP/06)*

**STEP 6:**
Position bag below level of donor’s arm
STEP 7: 
Apply a haemostat to tubing before needle is uncapped to prevent air from entering line.

STEP 8: 
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.

STEP 9: 
Reapply tourniquet or inflate blood pressure cuff. Have donor open and close hand until previously selected vein is again prominent.

STEP 10: 
Uncover sterile needle and keep the bevel of the needle facing upward and the shaft at an angle of 15° to the arm.

STEP 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.

STEP 12: 
Secure the needle in place by applying adhesive tape on the tubing to the donor's arm and cover site with sterile gauze.

STEP 13: 
Advise the donor open and close hand or squeeze the tennis ball every 10-12 seconds during collection to improve the blood flow.

STEP 14: 
Once blood enters the bag tubing, press the blood mixer 'start' switch to allow the blood to flow into the bag.
STEP 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).

STEP 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.)

STEP 17: Keep the donor under observation throughout the donation process. The donor never should be left unattended during or immediately after the donation.

STEP 18: When blood draw is complete, clamp tubing near venepuncture site using a haemostat or other temporary clamp.

STEP 19: Deflate cuff; remove tourniquet; and remove needle gently from the donor’s arm, pressing the phlebotomy site with a sterilize gauze.

STEP 20: Apply pressure over gauze and, with one hand, help donor raise arm straight up, holding firmly over phlebotomy site with other hand.

STEP 21: Take the test samples directly from the bleed line or from sample Pouch (deviation bag) of the collecting system.

STEP 22: Discard needle assembly into biohazard container designed to prevent accidental needle-sticks.
STEP 23: Invert bag several times to mix thoroughly.

STEP 24: Seal the blood bag tubing with the tube sealer.

STEP 25: Allow blood collecting tubing to refill with anti-coagulated blood from the bag.

STEP 26: Using the hand sealer, seal tubing attached to collection bag into segments, leaving each segment number clearly and completely readable.

STEP 27: Recheck donor identification number, donation number on bag, processing laboratory sample tubes, donation record, and retention segment—Make certain they all match.

STEP 28: Place the blood bags into controlled temperature storage and transport to the processing site under temperature conditions appropriate for the component.

STEP 29: For instructions on attending the donor (cf. Post-donation Care SOP-11)
1. MATERIAL

1) Povidine-iodine solution

2) Blood collecting triple bag

3) Hand Sealer

4) Blood Mixer with Automatic Balance system to monitor volume of blood drawn

5) Sterile gauze and haemostats and forceps

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

7) Blood tubing stripping device

8) Adhesive Tape

9) Sharp Container

10) Blood pressure cuff or Tourniquet
11) Comfortable donor couch or chair

12) Tennis ball

13) SOP for preparation of venipuncture site

14) SOP for Inspection and labeling of bag
6. DOCUMENTATION

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
Safe Blood Transfusion Programme Pakistan

Ministry of National Health Services, Regulation & Coordination, Government of Pakistan
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