**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Antibody Screening**

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| **SOP no.** | **Effective Date** | **Pages** | **Prepared by** | **Authorised by** |
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| **LOCATION** : Red Cell Serology Laboratory | | | | |
| **SUBJECT** : Antibody Screen | | | | |
| **FUNCTION :** Detection of Unexpected Blood group Antibodies. | | | | |
| **DISTRIBUTION**: Red Cell Serology Laboratory  Master File | | | | |

1. **SCOPE & APPLICATION:**

Detection of Unexpected Blood group Antibodies. This procedure applies to all testing that requires antibody screening, including donor units, patient's pre-transfusion blood grouping and prenatal specimens.

1. **RESPONSIBILITY:**

It is the responsibility of the technician/supervisor in the red cell serology laboratory to perform the antibody screen using proper cell concentrations. One technician performs all tests and another checks it. If any unexpected blood group antibody is detected, inform the medical officer for Advanced Red Cell Serology investigations.

1. **MATERIAL REQUIRED:**

**Equipment:**

* Refrigerator to store samples & reagents at 2-6 degree Centigrade.
* Deep Freezer to store enzyme papaine cystein in frozen state.
* Tabletop centrifuge.
* Automated cell washer (for patient pre-transfusion and prenatal testing).
* Microscope.
* Incubator

**Specimen:**

Clotted blood sample of donors /patients.

**Reagents:**

* Group O polled cells/Antibody-screening reagent red blood cells (two or three cells).
* Papain cystein.
* 22% Bovine albumin.
* Antihuman globulin reagent(anti-IgG+anti-C3d)
* IgG sensitised control cells.
* 0.9% saline
* Distilled water

**Glassware**:

* Serum tubes.
* Coombs' tubes(for patient pre-transfusion & prenatal testing).
* Micro tubes.
* Pasteur pipettes.
* Glass slides.

**Miscellaneous**:

* Rubber teats.
* Disposal box.
* 2 plastic beakers.
* Aluminium racks to hold serum and coombs' tubes.

1. **PROCEDURE**

**Principle:** The antibody screen test is used in the detection of unexpected blood group antibodies. In this test, pooled O cells or the antibody-screening reagent red blood cells are combined with 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.

**Antibody Screen:**

1. Label tubes with donor/patient and test identification.
2. Add two drops of test serum to each tube.
3. Add 1 drop of papain cystein to all tubes labelled 'enzyme' (if enzyme method is being followed).\*
4. To each of the tubes labelled 'saline' or 'enzyme/albumin', add 1 drop of 2% pooled O red cell suspension (or 2% suspension of the antibody-screening reagent red cells).
5. Add 1 drop of 22% abovine albumin to tubes labelled 'albumin' (if albumin method is being followed).\*
6. Add 1 drop of 5% pooled O red cell suspension (or 5% suspension of antibody-screening reagent red cells) to tubes labelled 'IAT', followed by 2 drops of 22% bovine albumin.
7. Mix the contents of the tubes gently and incubate for minimum 15 minutes.

\* Either enzyme or albumin method may be followed for detection of incomplete

antibodies.

**Results:**

1. Centrifuge saline, enzyme and albumin tests at 1000rpm for 1 minute.
2. Examine for haemolysis.
3. Gently resuspend the red cell button and examine for agglutination.
4. Examine all visually negative tests microscopically.
5. Grade and record test results immediately.
6. Proceed to perform antiglobulin phase of the indirect antiglobulin test on tubes labelled 'IAT'.
7. Wash the cells 3 times with saline. Decant completely after last wash. (washing can be done manually or using automated cell washer).
8. Add 2 drops antihuman globulin reagent to the dry cell button.
9. Mix well and centrifuge at 1000 rpm for 1 minute.
10. Read and record results.
11. Add drop IgG sensitised cells to all negative results. This shows a positive agglutination.

**Interpretation:**

1. Hemolysis or agglutination in any test may indicate the presence of an unexpected antibody.
2. The absence of agglutination and hemolysis in all tests is a negative test result.
3. After addition of IgG-sensitized 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.

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| **TEST** | **INCUBATION**  **TEMPERATURE** | **IDEAL INCUBATION**  **TIME** |
| Saline | Room Temperature | 1 hour |
| Enzyme | 370 C | 45 minutes |
| Albumin | 370 C | 45 minutes |
| IAT | 370 C | 1 hour |

* Follow manufacturer’s directions when using commercial reagents.
* Either enzyme or albumin method may be followed for detection of incomplete Antibodies

**Limitations:**

If tests with all reagent red cells are reactive, the possibility of spontaneous agglutination should be considered. A control of cells washed three to four times added to two drops of saline must be non-reactive.

1. **Documentation:**

* Results of donor unit antibody screen are entered in the relevant registers.
* Results of patients antibody screen are entered in the patient grouping register, blood group requisition form, serial case number register and computer. All records are initialled by the technician who has performed the test and by the technician who has checked the results.

1. **REFERENCES:**
2. Technical Manual of the American Association of Blood Banks – 15th Edition, 2005.
3. Procedures in Blood Banking & Immunohaematology – H.M. Bhatia, 1977.
4. Introduction to Transfusion Medicine – Zarin Bharucha & D.M. Chouhan, 1st Edition 1990.
5. **END OF DOCUMENT**