**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Rh D Typing**

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| **SOP no.**  | **Effective Date** | **Pages** | **Prepared by**  | **Authorised by**  |
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| **LOCATION** : Red Cell Serology Laboratory |
| **SUBJECT** : Rh D Typing |
| **FUNCTION** : Tube Test for Rh Testing.  |
| **DISTRIBUTION**: Red Cell Serology Laboratory Master File |

1. **SCOPE & APPLICATION:**

This Standard Operating Procedure (SOP) provides the method to be followed to determine the Rh D type of an individual and ensure the reliability of the result. This procedure describes the method for detection of D antigen on the red Cells. It provides guidance for the use of anti D blood grouping reagent.

1. **RESPONSIBILITY:**

It is the responsibility of the technician/supervisor in the red cell serology laboratory to perform the D typing of donors and patients using one monoclonal & one bioclonal reagent. If a discrepancy is encountered between the two batches of anti D, the test should be repeated by the same technician. If the discrepancy persists, the sample should be handed over to the advanced red cell serology laboratory for further work – up. If results of D typing of a blood donor are negative, the technician should proceed with D typing procedure. It is the responsibility of all staff performing the D typing to ensure that quality controlled reagents and proper cell concentration are used.

1. **MATERIAL REQUIRED:**

 **Equipment:**

* Refrigerator to store samples and reagents at 2 – 60C
* Table top centrifuge.
* Microscope
* Incubator/dri bath.

 **Specimen:**

* Clotted or anti-coagulated blood samples of donors.
* Clotted blood sample of patients.
* Test red cells suspended in native serum/plasma or saline.

 **Reagents:**

* Anti D bioclone.
* Anti D monoclonal (IgM/IgG blend)
* 0.9% saline.
* Distilled water.

 **Glassware:**

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

 **Miscellaneous:**

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

1. **Procedure:**

**Principle:** After ABO next clinically significant blood group system is Rhesus. Though no naturally occurring antibodies are there typing is done, because antigens are very immunogenic and Transfusion into Rh negative person can lead to promotion of clinically significant antibodies which can cause problems of hemolytic disease of newborn and homolytic transfusion reactions, of all Rh antigens. D is most immunogenic, Hence routinely only Rh D Typing is done. ABO & Rh (D) Typing is done simultaneously.

1. Label 2 test tubes with Pt./Donor name/D1, Pt./Donor name/D2.
2. Add 1 drop of respective anti D in the test tubes.
3. Add 2-5% washed test cell suspension.
4. Mix well, centrifuge at 1000 rpm x 1 min.
5. Resuspend cell button & look for agglutination
6. Confirm all negative reactions under microscope.
7. Control: +ve control: Known Rh-D +sample & Anti D
8. Negative control; Known Rh -D Neg. sample & Anti D

**RESULTS:**

1. Centrifuge all tubes at 1000 rpm for 1 minute (or as specified by manufacturer).
2. Gently resuspend the red cell button & examine for agglutination.
3. Grade and record test results.

**Interpretation:**

1. Agglutination of the red blood cells in the presence of reagent is a positive test result and indicates the presence of the D antigen.
2. A smooth suspension of RBCs after resuspension of RBC button is a negative test result. All negative results must be verified under microscope. Cells should appear separate without ant agglutination.
3. The interpretation of Rh D type is as follows:

|  |  |  |
| --- | --- | --- |
| **Cell Typing Anti – D Bioclone** | **Cell Typing Anti – D Monoclonal** | **Interpretation of Rh D type** |
| V | V | Positive |
| - | - | Negative\* |
| V | - | ?\* |
| - | V | ?\* |
| V= Visible Clumps |

1. Proceed with weak D (Du) TYPING using indirect anti-globulin technique in case of **blood donor sample**.

\* Hand over sample to Advanced Red Cell Serology Laboratory for further work-up.

**N.B.:**

1. Invalid test results may be obtained with this reagent if the blood tested is from a person with autoantibodies or abnormal serum proteins. Concurrent testing for the ABO blood group serves as a routine simultaneous control. The simultaneous use of an Rh – hr control is required only when the cells under test are found to be reactive with anti-A, anti-B and anti-D. If the use of an additional control is necessary, isotonic saline or 6% to 8% albumin in isotonic saline or a patient auto control may be used. If the control gives a positive result, a valid interpretation cannot be made.
2. Cord red blood cells heavily sensitised with anti-D may demonstrate a false negative test result.
3. **DOCUMENTATION:**

Enter the result of donor grouping in the donor grouping register and computer. Enter the results of patients grouping in the patient grouping register, blood group requisition form, serial case number register and computer.

1. **REFERENCES:**
2. Technical manual of the American Association of Blood Banks – 13th Edition, 1999. Pages 150-151, 307-312, 657-658.
3. Introduction to Transfusion Medicine; Zarin Bharucha & D.M. Chouhan, 1st edition, 1990. Pages 47-48.
4. Procedures in Blood banking and Immunohaematology; H.M. Bhatia, 1977. Page 37.
5. **END OF DOCUMENT**