**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Quality Control of Reagents**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SOP no.** | **Effective Date** | **Pages** | **Prepared by** | **Authorised by** |
| 6.1.1 | 27-11-2000 | 9 |  |  |
| **Version** | **Review Period** | **Date of Review** | **Reviewed by** | **Number of copies** |
| VI | 2 years | 01-01-2015 |  | 10 |
| **LOCATION** : Blood Group Laboratory (Serology) | | | | |
| **SUBJECT** : Quality Control - To ensure reliability and reproducibility of blood group  results | | | | |
| **FUNCTION** : Daily Quality Control of ABO & Rh Blood group reagents. | | | | |
| **DISTRIBUTION**: Supervisor Incharge Red Cell Serology Laboratory  Master File | | | | |

1. **SCOPE & APPLICATION:**

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

**2. RESPONSIBILITY:**

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

**4. MATERIALS REQUIRED:**

***Equipment:***

* Refrigerator to store samples and reagents at 2 – 60 C.
* Table top Centrifuge.
* Automated Cell Washer.
* Microscope.

***Reagents:***

* Anti-A, Anti-B, Anti-AB, Anti – D(Monoclonal and Bioclone) Antisera.
* Clotted or anticoagulated blood samples of random blood donors.
* Group A, B and O pooled Cells.
* 0.9% saline.

***Glassware:***

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

***Miscellaneous:***

* Rubber teats.
* Disposal box.
* 2 plastic beakers.
* Aluminium racks.

**5. PROCEDURE:**

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

***Quality Control Checks.***

|  |  |  |
| --- | --- | --- |
|  | Red Cells for Testing | |
| **Positive Reactors** | **Negative Reactors** |
| Anti – A | Pooled A cells | Pooled B, Pooled O Cells |
| Anti – B | Pooled B Cells | Pooled A, Pooled O Cells |
| Anti – AB | Pooled A, Pooled B Cells | Pooled O cells. |
| Anti – D Bioclone | Rh D – positive cells  (any ABO group) | Rh D – negative cells  (any ABO group) |
| Anti – D monoclonal | Rh D – positive  (any ABO group) | Rh – D – negative  (any ABO group) |

Reagents are to be assessed for:

**REACTIVITY**: Ability of a reagent to react with the corresponding antigen.

**Specificity:** Test reagent against known positive and negative cells.

**AVIDITY:** Time interval between first rapid mixing of the 2 drops and beginning of   
macroscopic agglutination.

**SENSITIVITY:** Titration of antisera denotes sensitivity.

***IMPORTANT GENERAL GUIDELINES***

1. Use the oldest reagent first
2. Reconstitute use and store according to manufacturer’s instructions at recommended   
   temperature only.
3. Allow potency contaminated reagents should be discarded.
4. Every new lot should be evaluated for potency and efficiency.
5. All results of quality control should be recorded and records are to be kept   
   appropriately.
6. If the results are different than the limits set by the manufacturer, report it to the manufacturer immediately.
7. Reagents records.
   1. Name
   2. Lot number
   3. Batch number
   4. Expiry date
   5. Name of manufacturer
   6. Licence No.
   7. Standard colour
   8. Free from HIV, HbsAg information.

**QUALITY CONTROL OF THE A, B, O REAGENTS CELLS**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PARAMETER** | **QUALITY** | |  |  | **FREQUENCY** |
|  | **REQUIREMENT** | | |  | **OF TESTING** |
| GROSS | NO TURUIDITY | | |  |  |
| (VISUAL | NO HEMOLYSIS | | |  | DAlLY |
| INSPECTION) | NO CHANGE IN COLOUR | | | |  |
|  | NO PARCILES | | |  |  |
| SENSITIVITY | UNEQUIVOCAL | | |  |  |
| AND | REACTION | | OF | KNOWN | DAILY |
| SPECIFICITY | S ERA | AGAINST | | RED |  |
|  | CELLS ANTIGENS | | |  |  |

1. Prepare the cells daily
2. Test daily for above requirements
3. Refrigerate cells when not in use
4. A void contamination and lysis
5. If Hemolysis suspected, wash cells till clear supernatant appears and then use   
    cells.

**QUALITY CONTROL ABO SERA.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| PARAMETER |  | QUALITY REQUIREMENT | | | | | | | | | | | FREQUENCY | | | OF | | |  |
|  |  |  |  |  | |  |  | | |  | | | TESTING | |  |  | | |  |
| GROSS |  | NO TURBIDITY | | | | |  | | |  | | |  | |  |  | | |  |
| (VISUAL |  | NO FOUL SMELL | | | | |  | | |  | | | DAILY | |  |  | | |  |
| INSPECTION) |  | NO PARTICLES OR GEL | | | | | | | |  | | |  | |  |  | | |  |
|  |  | STANDARD COLOUR | | | | | | | |  | | |  | |  |  | | |  |
| SENSITIVITY |  | TITRATION OF | |  | | ANTISERA | | | | | | | DAILY | |  |  | | |  |
|  |  | USING | CORRESPONDING | | | | | | | | | | ( WITH | | EVERY | | | |  |
|  |  | CELLS. |  | UNDILUTED | | | | | | | | | NEW LOT NO. OR | | | | | |  |
|  |  | ANTISERA | | $5% | | | | | | CELL | | | EVERY | | SEVEN | | | |  |
|  |  | SUSPENSION | |  | |  |  | | |  | | | DAYS | |  |  | | |  |
|  |  |  |  |  | |  |  | | | -----.- | | |  | |  |  | | |  |
| SPECIFICITY |  | CLEAR | REACTIONS | | | | | | | WITH | | |  | |  |  | | |  |
|  |  | RED | CELLS | | | | HAVING | | | | | |  | |  |  | | |  |
|  |  | CORRESPONDING | | | | | | | |  | | | DAIL Y | |  |  | | |  |
|  |  | ANTIGENS | |  | |  |  | | |  | | |  | |  |  | | |  |
|  |  |  |  |  | |  |  | | |  | | |  | |  |  | | |  |
| AVIDITY |  | SPEED | OF | MACROSCOPIC | | | | | | | | | DAILY | | AND | | | |  |
|  |  | AGGLUTINATION WITH 50% | | | | | | | | | | | WITH | | EVERY | | | |  |
|  |  | RBC | SUSPENSION | | | | | | | IN | | | NEW LOT NO. | | |  | | |  |
|  |  | NORMAL | SALINE | | | | IN | | | SLIDE | | |  | |  |  | | |  |
|  |  | TEST |  |  | |  |  | | |  | | |  | |  |  | | |  |
|  | \_. -------- | |  |  | |  |  | | |  | | |  | |  |  | | | U |
|  |  |  | | |  | | |  |  | | , |  | |  | | |  |  | | |  |

**AVIDITY AND SPECIFICITY OF ANTISERA**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ANTISERA | MINIMUM  TITRE  (2-3% RED  CELL  SUSPENSION) | MINIMUM  TITRE  (5 MIN  INCUBATION AND SPIN) | AVIDITY | REACTION GRADE A MINUTES |
| ANTI A | AI CELLS  A2 CELLS -  A2B CELLS | 1:256  I :128 1:32 | 10 SECONDS  15-18 SEC.  15-18 SEC | +++  ++ To ++  ++ |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | -- |  |  |  |  |
| ANTI B | 13 CELLS | 1:256 | 10 SECONDS | +++ |  |
|  | A1 B CELLS | 1:256 | 10 SECONDS | +++ |  |
| ANTI AB | A1 CELLS | 1:256 | 10 SECONDS | +++ |  |
|  | A2 CELLS | 1 :256 | 15 SECONDS | +++ |  |
|  | B CELLS | 1: 128 | 10 SECONDS | ++ To +++ |  |

***TEST FOR AVIDITY***

1- Prepare 50% saline suspension of test cells

2- Place 2 drops of blood grouping antiserum to be checked on glass slide

3- Place net to it 1 drop if cell suspension

4- Mix the 2 drops and spread out in a circle about 15 mm in diameter.

5- Rotate the slide slowly from side to side.

6- Record the time in seconds required for clumps (1 mm) to appear.

7- Perform each test twice and ensure similar results with in a limit of 3 seconds.

***TEST FOR SENSITIVITY:-***To standardize and quality check the antisera.

**DOUBLING DILUTION METHOD TITRATION**

*Principle:* Titration is semi quantitative technique of measuring the concentration of an   
antibody in a serum which is usually determined by testing two fold serial dilution of the serum in saline against selected red cells.

***Method:***

* 1. Arrange & label ten tubes in a tube rack label to 10 (serum dilutions 1: 1 of 1:512).
  2. Put I volume ( 2 drops saline) in all tubes except the first.
  3. In the first & second tube put 2 drops serum (I volume)
  4. Use clean pipette, mix contents of tube 2.
  5. Transfer I volume ( 2 drops ) to tube 3 to make dilution 1:4 from tube 2
  6. Continue the process till tube 10.
  7. Remove I volume from the last tube. Do not discard. Save it for use if further dilutions needed.
  8. Add I volume ( 2-5% saline suspended) red cells according to antisera in each tube (e.g. A cells when anti A is used)
  9. Mix well & incubate at room temperature for 30-45 minutes.

Or

Centrifuge all tubes at 1000 rpm for 1 minute

* 1. Record results. Gently dislodge the button
  2. Grade the agglutination reaction.
  3. Last positive reaction is titer of antibody for that antisera.
  4. For incomplete antibodies use AHG or Enzyme technique.

|  |  |  |
| --- | --- | --- |
| PARAMETER | QUALITY CHECK | FREQUENCY OF TESTING |
| GROSS  (VISUAL  INSPECTION | NO CONTAMINATION  NO CIIANGE IN COLOUR NO TURBIDITY  NO GEL FORMATION  NO PARTICLES | DAILY |
| SPECIFICITY | CLEAR REACTION WITH O POSITIVE CELLS AND NO REACTION WITH O NEGATIVE CELLS | DAILY AND WITH EVERY NEW LOT |
| AVIDITY | TIME OF START OF VISUAL AGGLUTINATION EVERY NEW LOT  IN SLIDE TEST WITH 40%  RED CELLS AT 370C | DAILY AND WITH EVERY NEW LOT |
| POTENCY | TITER OF ANTISERA ANTI C, ANTI-D, ANTI-E AND ANTI-E WITH CORRESPONDING CELLS 3 + OR 4 + REACTION IN UNDILUTED SERUM | DAILY AND WITH EVERY NEW LOT |

***AVIDITY OF ANI1 -D***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| TYPE OF ANTISERA | 40 % SLINE SUSPENSION | REACTIVITY | 50% SUSPENSION | TITER 15 MIN (370 C) | REMARKS |
| SALINE IgM(MONOCLONAL) | 15-20 SECONDS | +++/++++ AT END OF 2 MIN | +++ | 1:128-256 | Du Not Detected |
| IgG (POLYCLONAL | 30-60 SEC | +++ | +++ | 1:32 | - |
| IgG + IgM BLEND | 15-20 SEC | +++/++++ AT END OF 2 MIN | +++ | 1:128-256 | Du Not Detected |

***IMPORTANT POINTS TO REMEMBER:***

1. Use two different anti D from two different manufacturers

Put adequate positive (O+RH) and negative ( O-rr) control with auto controls.

**5. DOCUMENTATION:**

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

**6. REFERENCES:**

1. Technical Manual of the American Association of Blood Banks – 15th edition 2005.
2. Introduction to Transfusion Medicine – Zarin Bharucha and D.M. Chouhan; 1st Edition, 1990.
3. Training module for laboratory technologists. National AIDS Control Organization, Ministry of Health and Family Welfare, Govt. of India publication, 1995.

**7. END OF DOCUMENT.**