1. **Principle**

To differentiate rouleaux from true agglutination.

Rouleaux is a phenomenon that mimics agglutination. Red cells manifesting rouleaux appear microscopically like aggregates of stacked coins. It is caused by abnormalities in the patient’s plasma, not in the red cells. When the plasma is replaced with saline the rouleaux is dispersed but true agglutination remains.9.1

Rouleaux is seen in diseases associated with abnormal serum protein concentrations.9.1

1. **Scope and Related Policies**
   1. Normal saline replacement is usually done when all red cells tested at room temperature or 37°C show positive reactions with the patient’s plasma.
   2. Saline replacement is not done when all red cells tested by antiglobulin testing show positive reactions.
2. **Specimens**

EDTA anticoagulated whole blood

1. **Materials**

**Equipment:** Serological centrifuge

Block for test tubes

Microscope

Vacuum aspiration (optional)

**Supplies:** Test tubes – 10 x 75 mm, 12 x 75 mm

Serological pipettes

**Reagents:** Normal saline

1. **Quality Control – N/A**
2. **Procedure**

|  |  |
| --- | --- |
| **STEP** | **ACTION** |
| * 1. Read tubes microscopically | 1. Check agglutination for the characteristic “stacked coins” appearance |
| * 1. Centrifuge the tubes | 1. Centrifuge all tubes at 3400 rpm for 10-15 seconds. |
| 1. Using a pipette or vacuum aspiration equipment, remove all the supernatant from the centrifuged tubes leaving the cell button undisturbed |
| * 1. Re-Suspend and Read | 1. Add 2 drops of normal saline to each tube |
| 1. Re-suspend gently, read by initial reading method (i.e., macroscopically or microscopically). |
| * 1. Grade and record results. | 1. See RT. 001 – Reading and Recording Hemagglutination Reactions. |
| 1. Indicate that the reading is after normal saline replacement by writing “SR” above the column where the results are being recorded.  |  |  | | --- | --- | | ***If*** | ***Then*** | | entering results in a computer | Footnote “reading performed after normal saline replacement.” | |
| * 1. Interpret the results | 1. See 7.0 – Reporting. |
| * 1. Perform a clerical check. | * + 1. When the procedure is complete, perform a clerical check that: * The patient name and identification number are identical on all specimens and the request form or computer screen * The patient name is the same on all the test tubes and the request form or computer screen * Verify that the test results have been interpreted correctly |
| * 1. Initial | * 1. Initial or sign and record the completion time and date on the request form or in the computer. |
| * 1. Verification of results must be recorded. See 7.0 Reporting. |

1. **Reporting**
   1. No agglutination after the normal saline replacement procedure usually indicates that rouleaux was present and has been dispersed by the normal saline. Report the reaction as negative.
   2. Agglutination after the normal saline replacement procedure usually indicates true agglutination. Interpret the reaction as positive and continue with appropriate follow up (e.g., NRT.003 – ABO Group Problem Solving or antibody investigation if indicated).
2. **Procedural Notes – N/A**
3. **References**
   1. Roback JD, ed. AABBTechnical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: 488,901-902.
   2. Issitt PD, Anstee DJ. Applied Blood Group Serology, 4th ed. Durham, NC: Montgomery Scientific Publications, 1998: 1135.
4. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| March 1, 2014 | * Revised name of manual * Section 6.4.1- PA. 006 changed to RT.001 * Updated the list of references to include the most recent editions |