**Purpose:** This procedure provides instructions for how to determine the presence of unexpected antibodies by the tube Indirect Antiglobulin Test (IAT) method using Low Ionic Strength Solution (LISS).

* ***Note:*** *A patient antibody screen test may be performed at the same time as the patient ABO/D test, per established procedure.*

**Procedure:**

|  |  |  |
| --- | --- | --- |
|  | **Action** | **Related Documents**  |
| **1** | * Confirm sample acceptability.
 | * Evaluating Patient Samples and Request Forms
 |
| **2** | * Label tubes for 3 test cell antibody screen panel.
* Arrange the tubes in the rack.
* Ensure reagents are well mixed and at room temperature before use.
 | * Labeling Tubes Procedure
* Rack Set-Up Procedure
 |
| **3** | * Add **2** drops of patient plasma/serum to each tube.
 |  |
| **4** | * Add **1** drop of reagent screening cells to respective labelled tubes.
* Mix gently.
 |  |
| **5** | * Add **2** drops of LISS reagent to each tube.
* Mix gently.
* Inspect each tube for comparable appearance and volume.
 |  |
| **6** | * Incubate at 37°C incubation per manufacturer’s package insert instructions. (Minimum of 10 min at 37°C; can be extended to 30 min.)
 |  |
| **7** | * Centrifuge for the posted time in a calibrated serologic centrifuge.
 |  |
| **8** | * Read and record macroscopic readings.
 | * Reading and Grading Tube Hemagglutination Reactions
 |
| **9** | * Wash the tubes four times with saline.
 | * Washing Red Cell Samples (Manual or Automated Procedure)
 |
| **10** | * Add **2 drops** of anti-IgG.
 |  |
|  | **Action** | **Related Documents**  |
| **11** | * Mix the tubes **immediately.**
* Centrifuge for the posted time in a calibrated serologic centrifuge.
 |  |
| **12** | * Immediately after centrifugation:
* Resuspend the cells, and observe macroscopic agglutination.
* Record results.
 | * Reading and Grading Tube Hemagglutination Reactions
 |
| **13** | * Validate all weak and negative antiglobulin results:
* Add **1** drop of IgG-coated control cells to each tube with a weak or negative antiglobulin result.
* Centrifuge for the posted time in a calibrated serologic centrifuge.
* Resuspend the cells, and observe macroscopic agglutination.
* Record results.

***Valid control results****: Agglutination of at least grade 2+ must be present or the test results are invalid and the test must be repeated*. |  |
| **14** | * Analyze the reactions of the IgG-coated RBCs as follows:
 |
| **If agglutination is…** | **Then…** |
| * Present
 | * Test is complete.
 |
| * Absent
 | * Test is invalid:
* Repeat Steps 1-13.
* Consider cell washer problem or inactive AHG.
 |

|  |  |  |
| --- | --- | --- |
| **15**  | Consult the following table to interpret the antibody screen results. | * Antibody Identification Process
 |
| If the IAT results show | Then | Report Antibody screen as |
| * No hemolysis (at 37°C) and no agglutination
 | * Antibodies were not present or were undetected.
 | Negative |
| * Hemolysis (at 37°C) or agglutination (any strength)
 | * An antibody is present.
* Proceed to antibody identification procedures.
 | Positive |
| 16 | * Check that the record is complete:
* Date and time of completion,
* Technologist identification, and
* Final clerical check
 |  |

Reference:

Current version of reagent manufacturer’s package insert instructions.

AABB Technical Manual, Current Edition