**Purpose:**

This procedure provides instructions for how to perform an identification panel for unexpected antibodies by the tube Indirect Antiglobulin Test (IAT) method.

**Procedure:**

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| **Step** | **Action** | **Related Documents** |
| 1 | Confirm sample acceptability and check for previous records. | Sample Acceptance Evaluation  SQ Using Blood Bank Inquiry |
| 2 | Quality Control   * Daily QC performed * Visually inspect reagent panel red cells for evidence of deterioration   *The reactivity of the red cells may be checked periodically by testing antigens likely to deteriorate (ex. Lea) with a weakly reactive antibody of the same specificity. If red blood cells are nonreactive then do not use the panel cells.* |  |
| 3 | Label tubes   * Add patient serum/plasma and reagents according to Step 4 * Set up autocontrol using patient red cells and plasma. * Compare each tube for comparable appearance and volume. | Labeling Tubes for Manuel Bench Testing  Preparation of 3-5% Red Cell Suspension |

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| **Step**4 | **Tube Method Summary Table** | | | |
| **Saline IAT method** | **LISS IAT method** | **PEG IAT method** | |
| Add **2** drops of patient plasma/serum. | Add **2** drops of patient plasma/serum. | Add **2** drops of patient plasma/serum.  Add **1** drop of reagent panel cells to respective tubes. | |
| Add **1** drop of reagent panel cells to respective tubes.  Mix gently | Add **1** drop of reagent panel cells to respective tubes.  Mix gently | Mix well and centrifuge.  Examine for hemolysis or agglutination and record if present. | |
| N/A | Add **2** drops of LISS reagent.  Mix gently. | Add 2 drops of PEG reagent.  Mix gently. | |
| Incubate:   * + **30-60 minutes** at   + 37° +/-1°C incubation | Incubate   * **10-30 minutes** at * 37° +/-1°C incubation | Incubate:   * **10-30 minutes** at * 37° +/-1°C incubation | |
| Centrifuge for the posted time in a calibrated serologic centrifuge. | Centrifuge for the posted time in a calibrated serologic centrifuge. | Do **Not** Centrifuge. | |
| Read and record macroscopic readings | Read and record macroscopic readings | Examine for gross hemolysis and record if present. | |
| 5 | After 37°C reading (except for PEG), wash the tubes four times with saline. | | | Washing Patient Red Cells |
| 6 | Add 2 drops of anti-IgG. | | |  |
| 7 | Mix the tubes **immediately** and centrifuge for the posted time in a calibrated serologic centrifuge. | | |  |
| 8 | Immediately after centrifugation:   * Resuspend the cells, and * Read macroscopically and record results, per established procedure. | | | Reading and Grading Tube Hemagglutination |
| 9 | Validate all weak and negative antiglobulin results:   * Add 1 drop of IgG-coated control cells to each tube with a negative antiglobulin result. * Centrifuge for the posted time in a calibrated serologic centrifuge. * Resuspend the cells. * Read macroscopically and record the 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*. | | | Reading and Grading Tube Hemagglutination |

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| **Step** | **Action** | **Related Documents** |
| 10 | Analyze the reactions of the IgG-coated RBCs as follows:   |  |  | | --- | --- | | **If agglutination is…** | **Then…** | | Present | Test is complete. | | Absent | Test is invalid:   * + Repeat Steps 1-9.   + Consider inadequate cell washing. | | |
| 11 | Check that the record is complete:   * Date and time of completion, * Technologist identification, and * Final clerical check. * Record that the check has been done. |  |
| 12 | Proceed to rule out and antibody interpretation. | Guidelines for Antibody Identification |

Reference:

AABB Technical Manual, Current Edition

Current version of Mfg. package insert