**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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|  | **Action** | **Related Documents** |
| **1** | * Confirm sample acceptability, per established procedure. * Check for previous records per established procedure. | * Sample Acceptance Evaluation * Blood Order Processing Overview |
| **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, per established procedure. * Arrange the tubes in the rack per established procedure. * Add patient serum/plasma and reagents according to the Tube Method Summary Table for the order in which to add cells and reagents. * Set up autocontrol using patient red cells and plasma. * Compare each tube for comparable appearance and volume. | * Labeling Tubes for Manuel Bench Testing * Manual Bench Set-Up |

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|  | **Tube Method Summary Table** | | |
| **Saline IAT method** | **LISS IAT method** | **PEG IAT method** |
| * Add **4** drops of patient plasma/serum. | * Add **2** drops of patient plasma/serum. | * Add **2** drops of patient plasma/serum. * Mix well and centrifuge. * Examine for hemolysis or agglutination and record if present. * Add **1** drop of reagent panel cells to respective tubes. * Mix gently. |
| * Add **1** drop of reagent panel cells to respective tubes. * Mix gently. | * Add **1** drop of reagent panel cells to respective tubes. * Mix gently. | * Add **2** drops of PEG. * Mix gently. |
| * N/A | * Add **2** drops of LISS reagent. * Mix gently. | * N/A |
| * Incubate:   + **30-60 minutes** at   + 37°C incubation | * Incubate * **15 minutes** at * 37°C incubation | * Incubate: * **15 minutes** at * 37°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. |
| * Record macroscopic readings, per established procedure * Related document: Reading and Grading Tube Hemagglutination Reactions | * Record macroscopic readings, per established procedure * Related document: Reading and Grading Tube Hemagglutination Reactions | * Examine for gross hemolysis and record if present. |
|  | **Action** | | **Related Documents** |
| **4** | * After 37°C reading (except for PEG), wash the tubes four times with saline, per established procedure. | | * Washing Red Cell Samples (Manual or Automated Procedure) |
| **5** | * Add 2 drops of anti-IgG. | |  |
| **6** | * Mix the tubes **immediately.** * Centrifuge for the posted time in a calibrated serologic centrifuge. | |  |
| **7** | * Immediately after centrifugation: * Resuspend the cells, and * Read macroscopically and record results, per established procedure. | | * Reading and Grading Tube Hemagglutination Reactions |

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| **8** | 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. * 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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| 9 | Analyze the reactions of the IgG-coated RBCs as follows: | |
| **If agglutination is…** | **Then…** |
| * Present | * Test is complete. |
| * Absent | * Test is invalid:   + Repeat Steps 1-7.   + Consider inadequate cell washing. |

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| **10** | * Check that the record is complete: * Date and time of completion, * Technologist identification, and * Final clerical check. * Record that the check has been done. |  |
| **11** | * Proceed to rule out and antibody interpretation. | * + - Guidelines for Antibody Identification |

Reference:

AABB Technical Manual, Current Edition

Current version of Mfg. package insert