**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 EvaluationSQ 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 TestingPreparation 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.
 |

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| 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