**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.

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|  | **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 |

|  |  |
| --- | --- |
| 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
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Reference:

AABB Technical Manual, Current Edition

Current version of Mfg. package insert