**Purpose:**

This procedure provides instructions for how to determine the presence of unexpected antibodies by the tube Indirect Antiglobulin Test (IAT)..

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

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| --- | --- | --- |
|  | **Action** | **Related Documents Title**  |
| **1** | * Confirm sample acceptability.
 | * Sample Acceptance Evaluation
 |
| **2** | * Prewarm saline by placing squeeze bottle in Helmer Plasma Thawer using overwrap bag to protect bottle.
 |  |
| **3** | * Label tubes.
* Arrange the tubes in the rack.
 | * Labeling Tubes for Manual Bench Testing
* Manual Bench Set-Up
 |
| **4** | * Add **sufficient** drops of patient plasma/serum to complete tests to a labelled tube
 |  |
|  | * Prepare a 3% to 5% cell suspension of each donor red cell unit per established procedure.
 | * Preparation of 3-5% Suspension of Red Cells
 |
| **5** | * Add **1** drop of each donor cell suspension to a labelled testing tube.
 |  |
| **6** | * Incubate all tubes (patient plasma, each donor cell testing tube):
* **10 to 15 minutes** at
* **37 C** incubation
 |  |
| **7** | * + Add 2 drops warm plasma to each warm reaction tube.
	+ Mix gently.
	+ Incubate 30 to 60 minutes at 37 C.
 |  |
| **8** | * + Wash the tubes four times with warm saline.
		- ***NOTE:*** *37C reading is omitted for pre-warm methods*
 | * + Washing Red Cell Samples (Manual or Automated Procedure)
 |
|  | **Action** | **Related Documents** |
| **9** | * Add 2 drops of anti-IgG.
	+ ***Note:*** *Anti-IgG is necessary for the pre-warm method.*
 |  |
| **10** | * Mix the tubes immediately.
* Centrifuge for the posted time in a calibrated serologic centrifuge.
 |  |

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| **12** | * Validate 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*.
 |  |

|  |  |
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| **13** | * + Analyze the reactions of the IgG-coated RBCs as follows:
 |
| **If agglutination is…** | **Then…** |
| present | test is complete. |
| absent | test is invalid:* Repeat Steps 1-11.
* Consider cell washer problem or inactive AHG.
 |

|  |  |
| --- | --- |
| **14** | * + Consult the following table to interpret the X-match results
 |
| If the IAT results show | Then | Report antibody XM as |
| * No agglutination
 | * Antibodies were not present or were undetected.
 | * Negative/Compatible
 |
| * Agglutination (any strength)
 | * An antibody is present.
 | * Positive/Incompatible
 |

**References:**

AABB Technical Manual, Current Edition

Judd’s Methods in Immunohematology, Current Edition

Current version of reagent manufacturer’s package insert instructions