**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. |

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| **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