**Purpose:** This procedure provides instructions for how to perform ABO/D testing by

tube technique for a patient older than four months of age.

**Procedure:**

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| --- | --- | --- | --- |
|  | | **Action** | **Related Documents** |
| **1** | Confirm sample acceptability, per established procedures. | Sample Acceptance Evaluation |
| **2** | * Label tubes, per established procedure. * Add patient serum/plasma **and** reagents. * Perform a visual check to ensure that the reagent volume is correct. * Do NOT save extra plasma in a secondary aliquot tube. Instead, return the unused plasma from pipette back into original blood tube before sampling RBCs to make cell suspension. The tube must be centrifuged again if more plasma is needed for advanced testing. | Labeling Tubes for Manual Bench Testing  LIS Downtime Manual Bench Testing Form |
| **3** | * Prepare a 3% to 5% cell suspension of patient red cells per established procedure. * Add 3% to 5% suspension of the patient red cells to forward testing tubes. * Mix all tubes. | Preparation of a 3-5% Suspension of Red Cells |
| **4** | * Centrifuge for the posted optimal time in a calibrated serologic centrifuge. |  |
| **5** | * Remove the tubes from the centrifuge. * Check that the identifier on each tube is comparable with the name identifier on the request and on the computer screen or worksheet. |  |

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|  | | **Action** | | | | | | **Related Documents Title** | |
| **6** | * Gently resuspend the cell button. * Examine macroscopically for hemolysis and agglutination including mixed field agglutination per established procedure. * Grade and record the results. | | | | | | | Reading and Grading Tube Hemagglutination Reactions  BOP in Sunquest |
| **7** | If anti-A, anti-B and anti-D are POS, a control is required:   * Utilize Seraclone Control ABO + Rh reagent * Repeat steps 2 through 6. | | | | | | | |
| **8** | Use the following table to interpret the results: | | | | | | | |
|  | **If the forward grouping reaction of patient cells with** | | | **And the reverse grouping reaction of patient serum or plasma tested with** | | **And the reaction of patient cells with** | | **Then interpret the ABO/D type as** |
| **Anti-A is** | | **Anti-B is** | **A1 cells is** | **B cells is** | **Anti-D is** | **Control**  **is** |
| 0 | | 0 | ≥ 2  H | ≥ 2  H | ≥ 2 | ND | OPOS |
| 0 | | 0 | ≥ 2  H | ≥ 2  H | 0 | ND | ONEG |
| ≥ 2 | | 0 | 0 | ≥ 2  H | ≥ 2 | ND | APOS |
| ≥ 2 | | 0 | 0 | ≥ 2  H | 0 | ND | ANEG |
| 0 | | ≥2 | ≥ 2  H | 0 | ≥ 2 | ND | BPOS |
| 0 | | ≥ 2 | ≥ 2  H | 0 | 0 | ND | BNEG |
| ≥ 2 | | ≥ 2 | 0 | 0 | ≥ 2 | 0 | ABPOS |
| ≥ 2 | | ≥ 2 | 0 | 0 | 0 | ND | ABNEG |
| **H = Hemolysis, ND = Not Done**  **NOTE: If mixed field agglutination is observed, results cannot be interpreted until investigation and resolution have been completed.** | | | | | | | |

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|  | | **Action** | | | | | **Related Documents Title** |
| **9** | | Evaluate the test results. | | | | | * ABO Discrepancy Resolution * Discrepant Result Resolution Process * Weak D Test by Tube |
| **If** | | | **Then** | |
| Test results could be interpreted | | | Go to step 10 | |
| There are any discrepancies in results when compared to the table in Step 8 | | | * Resolve the discrepancy, per established procedure, and * Proceed to Step 10 once the discrepancy is resolved. | |
| **10** | | Compare the current ABO/D results with any previous ABO/D results. | | | |  | |
| **If a previous record** | **And the current and previous results** | | **Then** | * SQ Blood Order Processing * ABO D Type by Tube Method * Discrepant Result Resolution Process | |
| exists | agree | | record the interpretation in the computer or on the worksheet |
| do not agree | | Resolve the discrepancy, per established procedure. |
| does not exist | N/A | | Perform a second ABO/D examination. |
| **11** | | Confirm that the computer record or the worksheet contains the:   * date and time of completion, * technologist identification, and * Final clerical check of samples, labels, and request. | | | |  | |
| **12** | | Dispose of all tubes and pipettes in a biohazard waste container, per established procedure. | | | | Laboratory Biohazardous Waste Procedure | |

**References:**

AABB Technical Manual, Current Edition.

Judd’s Methods in Immunohematology, Current Edition

Current manufacturer’s package insert instructions.