**Purpose**:

To identify and determine cold reacting antibodies detected at the antibody screen or at ABO Rh typing.

**Background:**

Investigation into a possible cold antibody is done typically if patient has history of cold agglutinin disease or there are discrepancies in the patients ABO/Rh type.

A Cold Panel may be indicated when any of the following occurs:

* ABO discrepancy is detected during ABO testing.
* The panel for the warm reactive antibodies is inconclusive with a stronger reaction at 370C than the antiglobulin phase.
* Immediate spin crossmatch is positive when the antibody screen was negative.

**Procedure:**

|  |  |  |
| --- | --- | --- |
| **Step** | **Action** | **Related Documents** |
| 1 | Prepare a 3-5% patient red cell suspension using saline. | Preparation of 3-5% Red Cell Suspension |
| 2 | Label tubes for each reagent cell. Label one tube for autocontrol. | Labeling Tubes for Manual Bench Testing |
| 3 | Add 2 drops patient plasma and 1 drop patient 3-5% red cells suspension to the tube labeled autocontrol.Add 2 drops of patient plasma to each tube, and then add 1 drop of each reagent cell to their respective tubes. |  |
| 4 | Mix well and centrifuge per established guidelines.Dislodge the cell buttons gently.* Examine macroscopically for agglutination and hemolysis.
* Grade and record the results.
* If reactivity is < 2+, continue to next phase
* If reactivity is ≥ 2+, testing complete. Interpret results.
 | Antibody Identification WorksheetReading and Grading Tube Hemagglutination |
| 5 | Mix and incubate test tubes at Room Temperature (RT) 15-30 minutes:* + Centrifuge per established guidelines.
	+ Dislodge the cell buttons gently.
	+ Examine macroscopically for agglutination and hemolysis.
	+ Grade and record the results.
* If reactivity is < 2+, continue to next phase
* If reactivity is ≥ 2+, testing complete. Interpret results.
 | Antibody Identification WorksheetReading and Grading Tube Hemagglutination |
| 6 | Mix and incubate test tubes at 4 C for 30-60 minutes |  |
| **Step** | **Action** | **Related Documents** |
| 7 | Centrifuge per established guidelines.* Dislodge the cell buttons gently.
* Examine macroscopically for agglutination and hemolysis.
* Grade and record the results.

***Note:*** *Do not allow 4 C tests to warm during centrifugation. If a cold room in which a centrifuge may be placed is not available, return the centrifuged tubes to the refrigerator for 5 minutes before reading.* | Antibody Identification WorksheetReading and Grading Tube Hemagglutination |
| 8 | Determine the antibody specificity and/or perform additional studies as necessary per Flowchart A. |  |

**References:**

AABB Technical Manual, Current Edition

Judd’s Methods in Immunohematology, Current Edition

**Flowchart A: Process Flow for Cold Antibody Panel**

Test Results at IS, RT (20-250C), and at 40C (if required)

POS NEG

Positive Autocontrol Negative Autocontrol

All cells POS Some or all cells POS

Probable Cold Autoantibody Not a Cold antibody

 Probable Cold Alloantibody

Perform Patient antibody screen using Prewarm IAT procedure. If negative, crossmatch can be done using this method.

**Note:**

Cold antibodies typically exhibit stronger reactions at cold temperatures and weaker reactions at warm temperatures.