**PURPOSE:**

To provide instructions for performing cold panels for ABO discrepancy resolution and cold reactive antibody identification in the following situations:

* ABO discrepancy during ABO/Rh testing
* Inconclusive panel for warm reactive antibodies with a stronger reaction at 37°C than the antiglobulin phase
* Unexplained incompatible crossmatch at Immediate Spin phase

**PRINCIPLE & CLINICAL SIGNIFICANCE:**

Cold antibodies can interfere with routine blood group and antibody screen testing making it difficult to interpret the results and potentially masking clinically significant alloantibodies that have the potential to cause hemolysis if antigen negative units are not selected for transfusion.

**POLICIES:**

NA

**SPECIMEN REQUIREMENTS:**

EDTA is preferred and if not tested soon after collection, should be stored at 1-6°C.

Red top tubes are acceptable**.**

See SOP *Specimen Acceptability*

**REAGENTS/SUPPLIES/EQUIPMENT:**

|  |  |  |
| --- | --- | --- |
| **Reagents:** | **Supplies:** | **Equipment:** |
| * A1 cells\* * A2 cells\* * B cells\* * Group O or type specific cord cells <7 days old * Group O antibody screening cells * LISS * Anti-IgG * IgG coated control cells * Blood Bank Saline | * 12 × 75 glass tubes * Blood bank transfer pipettes | * Calibrated serologic centrifuge * Calibrated cell washer * 37°C Heat block * Agglutination viewer * Refrigerator |

**\*NOTE**: Expired reagents are acceptable to use as a secondary source once appropriate QC testing is performed

**QUALITY CONTROL:**

Quality Control is performed each day of use.

**INSTRUCTIONS:**

**TABLE OF CONTENTS**

Cold Panel Testing

Appendix A: Cold Panel Interpretation of Results

**Cold Panel Testing**

| **STEP** | **ACTION** |
| --- | --- |
| 1 | Wash the group O cord blood cells 3 times and prepare a 3-4% suspension using blood bank saline |
| 2 | Prepare a 3-4% red cell suspension of the patient cells using blood bank saline |
| 3 | Label test tubes for each of the following:   * Three cell antibody screen * Auto-control * A1 cells * A2 cells * B cells   Group O or type specific cord cells  **NOTE:** Testing may be modified, if necessary due to limited sample availability |
| 4 | Add 2 drops of patient plasma/serum to all tubes |
| 5 | Add 1 drop of the appropriate 3-4% reagent cell suspension to appropriate labeled tubes |
| 6 | Mix and centrifuge according to centrifuge calibration for saline phase |
| 7 | Resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 8 | Read, grade, and record results on the *Cold Panel Worksheet*   |  |  | | --- | --- | | **If reaction pattern** | **Then** | | Matches any pattern shown in Appendix 1: Table 1 | * Confirm antibody presence by testing additional cells to complete the rule of three * Go to *Interpreting Results* section | | Does not match any pattern shown in Appendix 1: Table 1 | Go to next step | |
| 9 | Incubate tubes at room temperature (RT) for 15-30 minutes |
| 10 | Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 11 | Read, grade, and record results on the *Cold Panel Worksheet*   |  |  | | --- | --- | | **If reaction pattern** | **Then** | | Matches any pattern shown in Appendix 1: Table 1 | * Confirm antibody presence by testing additional cells to complete the rule of three * Go to *Interpreting Results* section | | Does not match any pattern shown in Appendix 1: Table 1 | Go to next step | |
| 12 | Incubate tubes in refrigerator at 1-6°C for 15-30 minutes |
| 13 | Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 14 | Read, grade, and record results on the *Cold Panel Worksheet*   |  |  | | --- | --- | | **If reaction pattern** | **Then** | | Matches any pattern shown in Appendix 1: Table 1 | * Confirm antibody presence by testing additional cells to complete the rule of three * Go to *Interpreting Results* section | | Does not match any pattern shown in Appendix 1: Table 1 | Go to next step | |
| 15 | Add 2 drops of LISS to each tube ( refer to SOP *LISS Indirect Antiglobulin Technique*) |
| 16 | Incubate at 37°C ±1 for 10-30 minutes |
| 17 | Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 18 | Read, grade, and record results on the *Cold Panel Worksheet* |
| 19 | Resuspend cells and wash 3 times with saline |
| 20 | Add 2 drops of Anti-IgG |
| 21 | Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 22 | Read, grade, and record results on the *Cold Panel Worksheet*   |  |  | | --- | --- | | **If reaction pattern** | **Then** | | Matches any pattern shown in Appendix 1: Table 1 | * Confirm antibody presence by testing additional cells to complete the rule of three * Go to *Interpreting Results* section | | Does not match any pattern shown in Appendix 1: Table 1 | Go to next step | |
| 23 | Add 1 drop of IgG coated control cells to each tube with a negative antiglobulin test and mix well |
| 24 | Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis |
| 25 | Read, grade and record reactions on the *Cold Panel Worksheet*  **NOTE:** Negative AHG tests must show reactivity following the addition of IgG coated control cells to be considered valid |

**CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL**

**VALUES/CRITICAL VALUES**

**Interpretation**

Refer to SOP *Antibody Identification*

**Results Reporting in Sunquest**

Refer to the appropriate SOP:

SOP *Antibody Identification*

SOP *ABO/Rh Discrepancy*

**CALIBRATION:**

NA

**NOTES AND LIMITATIONS:**

* Rule of three is commonly used in antibody identification to ensure with 95% confidence the results obtained are not due to chance. If a minimum of three antigen positive cells react and three antigen negative cells fail to react, the resulting p-value obtained is < 0.05 (95% confidence that results are not due to chance)
* Antigen negative cells may be necessary to resolve blood group discrepancies in the presence of a cold reactive alloantibody

**REFERENCES:**

* Technical Manual. Bethesda, MD: AABB Press, current edition
* Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition
* Reagent Manufacturer’s Inserts; current version

**RELATED DOCUMENTS:**

SOP *Labeling for Manual Testing*

SOP *Grading Reactions*

SOP *ABO/Rh Discrepancy*

SOP *Antibody Identification*

SOP *LISS Indirect Antiglobulin Technique*

**APPENDICES:**

**Table 1: Cold Panel Results Interpretation**\*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reagent Cell** | **Antibodies** | | | | |
| H | IH | I | i | A1 |
| A1 | + | 0 | + | 0\↓ | + |
| A2 | ↑ | + | + | 0\↓ | 0 |
| B | + | 0 | + | 0\↓ | 0 |
| O Cord | ↑↑ | 0 | 0↓ | + | 0 |
| O Adult (screening cells) | ↑↑ | + | + | 0\↓ | 0 |
| Auto | \*+/0 | \*+/0 | + | 0\↓ | 0 |

* May vary with blood group of patient

0\↓ Negative or weaker reaction

↑ Stronger reaction

|  |  |  |  |
| --- | --- | --- | --- |
| **UWMC SOP Approval:** | | | |
|  |  |  |  |
| **UWMC CLIA Medical Director** |  |  |  |
|  | Mark H. Wener, MD | Date |  |
|  |  |  |  |
| **Transfusion Service Manager** |  | Date |  |
|  | Deanne Stephens |  |  |
|  |  |  |  |
| **Compliance Analyst** |  | Date |  |
|  | Christine Clark |  |  |
| **Transfusion Service**  **Medical Director** |  | Date |  |
|  | John R. Hess, MD |  |  |
|  |  |  |  |
| **UWMC Biennial Review:** | |  |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |
|  |  | Date |  |
|  |  |  |  |