University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 05/25/17 Revision Effective Date:	Number: PC-0062.02

TITLE: **Cold Antibody Panel**

PURPOSE:
To provide instructions for identifying cold reactive agglutinins in the following situations:
- ABO discrepancy during ABO/Rh testing
- Cold reactive antibody with concerns for hemolytic anemia
- Inconclusive panel for warm reactive antibodies with a stronger reaction at immediate spin or 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. In addition, cold reactive antibodies can result in cold agglutinin disease and clinically significant hemolysis.

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 and Order Receipt*

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:

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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 in Appendix: 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 in Appendix: 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 in Appendix: 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 in Appendix: Table 1 / Go to next step

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>12</td><td>Incubate tubes in refrigerator at 1-6°C for 15-30 minutes</td></tr>
<tr><td>13</td><td>Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis</td></tr>
<tr><td>14</td><td>Read, grade, and record results on the Cold Panel Worksheet
<table>
<tr><th>If reaction pattern</th><th>Then</th></tr>
<tr><td>Matches any pattern in Appendix: Table 1</td><td>• Confirm antibody presence by testing additional cells to complete the rule of three
• Go to Interpreting Results section</td></tr>
<tr><td>Does NOT match any pattern in Appendix: Table 1</td><td>Go to next step</td></tr>
</table></td></tr>
<tr><td>15</td><td>Add 2 drops of LISS to each tube (refer to SOP LISS Indirect Antiglobulin Technique)</td></tr>
<tr><td>16</td><td>Incubate at 37°C ±1 for 10-30 minutes</td></tr>
<tr><td>17</td><td>Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis</td></tr>
<tr><td>18</td><td>Read, grade, and record results on the Cold Panel Worksheet</td></tr>
<tr><td>19</td><td>Resuspend cells and wash 3 times with saline</td></tr>
<tr><td>20</td><td>Add 2 drops of Anti-IgG</td></tr>
<tr><td>21</td><td>Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis</td></tr>
<tr><td>22</td><td>Read, grade, and record results on the Cold Panel Worksheet
<table>
<tr><th>If reaction pattern</th><th>Then</th></tr>
<tr><td>Matches any pattern shown in Appendix 1: Table 1</td><td>• Confirm antibody presence by testing additional cells to complete the rule of three
• Go to Interpreting Results section</td></tr>
<tr><td>Does not match any pattern shown in Appendix 1: Table 1</td><td>Go to next step</td></tr>
</table></td></tr>
<tr><td>23</td><td>Add 1 drop of IgG coated control cells to each tube with a negative antiglobulin test and mix well</td></tr>
<tr><td>24</td><td>Centrifuge, resuspend the cells, and observe for macroscopic agglutination and/or hemolysis</td></tr>
<tr><td>25</td><td>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</td></tr>
</table>

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*

APPENDIX:

Table 1: Cold Panel Results Interpretation*

Reagent Cell	**Antibodies**				
	H	IH	I	i	A1
A_1	+	0	+	0\↓	+
A_2	↑	+	+	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	Nina Sen	Date	
Compliance Analyst	Christine Clark	Date	
Transfusion Service Medical Director	Monica B. Pagano, MD	Date	
UWMC Biennial Review:			
		Date	
		Date	

REVISION HISTORY:
01/15/21 – Clarification of situations when a cold panel should be considered and changed title from *Cold Panel* to *Cold Antibody Panel*