University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 02-11-16 Revision Effective Date:	Number: PC-0032.02
TITLE: **PeG Indirect Antiglobulin Technique**		

PURPOSE:
To provide instructions using tube Indirect Antiglobulin Test (IAT) technique with PEG to detect the presence of unexpected antibodies and perform seriological crossmatches

PRINCIPLE & CLINICAL SIGNIFICANCE:
An indirect antiglobulin test (IAT) demonstrates in-vitro reactions between red cells and antibodies, and is used in antibody detection, antibody identification, crossmatching, and blood group phenotyping.

PEG acts as an additive for tests to detect blood group antibodies, enhancing the sensitivity of the antibody detection by creating a low-ionic strength test environment that increases the rate of antibody uptake during incubation

POLICIES:
This is the primary manual method for:
- Detection and identification of unexpected antibodies (antibody screen or panel)
- AHG crossmatching when the patient has a history of unexpected clinically significant antibodies

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:
• Gamma PeG • Anti-IgG • Antibody Screen/Panel Cells • 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

QUALITY CONTROL:
Quality Control is performed daily

INSTRUCTIONS:

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Label 12 X 75 mm tubes for each cell to be tested according to the Labelling for Manual Testing SOP

NOTE: Bio-Rad reagent red blood cells need to be room temperature before using.</td></tr>
<tr><td>2</td><td>Make an approximate 3-4% red cell suspension of patient or donor cells as per the following:
<table>
<tr><th>Test</th><th>3-4% Cell Suspension</th></tr>
<tr><td>Antibody Panel</td><td>Patient cells</td></tr>
<tr><td>Crossmatch</td><td>Donor cells</td></tr>
</table>
NOTE: Reagent red blood cells can be used directly from the vial.</td></tr>
<tr><td>3</td><td>Add 2 drops of patient plasma or serum to each tube.</td></tr>
<tr><td>4</td><td>Add 1 drop of red cells to the respectively labelled tubes and mix gently
<table>
<tr><th>Test</th><th>Red Cells</th></tr>
<tr><td>Antibody screen</td><td>Screening cells</td></tr>
<tr><td>Panel</td><td>Panel cells</td></tr>
<tr><td>Auto control</td><td>Patient 3-4% red cell suspension</td></tr>
<tr><td>Crossmatch</td><td>Donor 3-4% red cell suspension</td></tr>
</table></td></tr>
<tr><td>5</td><td>
<table>
<tr><th>If performing</th><th>Then</th></tr>
<tr><td>Crossmatch</td><td>• Centrifuge and observe for hemolysis
• Shake gently to resuspend the cell button and examine macroscopically for agglutination
• Read, grade (refer to SOP Grading Reactions) and record reactions</td></tr>
<tr><td>Antibody screen/panel</td><td>• Go to next step (immediate spin phase is not required for antibody screen or panel)</td></tr>
</table></td></tr>
<tr><td>6</td><td>Add 2 drops of PeG to each test tube</td></tr>
<tr><td>7</td><td>Mix well and incubate at 37°C ±1 for 10-30 minutes</td></tr>
<tr><td>8</td><td>Examine for hemolysis and document in the INC phase in the Sunquest testing grid</td></tr>
<tr><td>9</td><td>Wash the tubes 3 times with saline</td></tr>
<tr><td>10</td><td>Add 2 drops of Anti-IgG and mix well and centrifuge according to calibration for AHG phase testing</td></tr>
<tr><td>11</td><td>Shake gently to resuspend the cell buttons, and examine macroscopically for agglutination</td></tr>
<tr><td>12</td><td>Read, grade and record reactions</td></tr>
</table>

STEP	ACTION
13	Add 1 drop of IgG coated control cells to each tube with a negative antiglobulin test
14	Mix gently and centrifuge according to calibration for AHG phase testing
15	Shake gently to resuspend the cell buttons, and examine for agglutination
16	Read, grade and record reactions
17	Go to section Interpretation and Results Reporting

CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES

Interpretation

If Agglutination	Interpret as
Present after any phase testing	Positive
Not present	Negative **NOTE:** Results are considered invalid and must be repeated if tube does not agglutinate with IgG coated control cells

If test performed is	Then	
Antibody Screen	Refer to SOP *Antibody Screen Testing*	
Antibody Panel	Refer to SOP *Antibody Identification*	
Crossmatch	**If**	**Then RBC is**
	No hemolysis at 37°C **AND** No agglutination at any phase of testing	Compatible ~~Refer to SOP *Crossmatching*~~
	Hemolysis at 37°C **AND/OR** Agglutination at any phase of testing	Incompatible ~~Refer to SOP *Crossmatching*~~

Results Reporting:

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Record reactions immediately upon reading in on appropriate form or Sunquest</td></tr>
<tr><td>2</td><td>
<table>
<tr><th>If performing</th><th>Then</th></tr>
<tr><td>Antibody screen</td><td>Refer to SOP Antibody Screen <u>Testing</u></td></tr>
<tr><td>Antibody ID</td><td>• Record results on the appropriate antibody ID panel or Extended Testing Worksheet
• Refer to SOP Antibody Identification</td></tr>
<tr><td>Crossmatch</td><td><u>Enter appropriate reaction and interpretation per table below</u>
<table>
<tr><th colspan="4"><u>Reaction Grid</u></th><th rowspan="2"><u>Interpretation</u></th><th rowspan="2"><u>SQ Code</u></th><th rowspan="2"><u>SQ Hot Key</u></th></tr>
<tr><th><u>XIS</u></th><th><u>XINC</u></th><th><u>XAHG</u></th><th><u>XCC</u></th></tr>
<tr><td><u>0</u></td><td><u>ND</u></td><td><u>0</u></td><td><u>+</u></td><td><u>PEG Compatible</u></td><td><u>PCMP</u></td><td><u>!</u></td></tr>
<tr><td><u>0</u></td><td><u>ND</u></td><td><u>+</u></td><td><u>ND</u></td><td><u>PEG Incompatible</u></td><td><u>PICMP</u></td><td><u>%</u></td></tr>
<tr><td><u>+</u></td><td><u>ND</u></td><td><u>+</u></td><td><u>ND</u></td><td><u>PEG Incompatible</u></td><td><u>PICMP</u></td><td><u>%</u></td></tr>
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</table></td></tr>
</table>
</td></tr>
</table>

NOTES AND LIMITATIONS:

- Polyethylene glycol ~~has a tendency to~~<u>tends to</u> precipitate serum globulins. Accordingly, when using Gamma PeG to detect unexpected antibodies, it is especially important to assure that the red blood cells are thoroughly resuspended in each change of saline during the washing phases of the test. When testing samples containing elevated globulin levels, three washes may not be sufficient to remove unbound protein. If precipitated globulin remains enmeshed in the red blood cell button, it may neutralize the Anti-Human Globulin and cause a false negative test result. In the case of specimens having an exceptionally high level of globulin (as in multiple myeloma), the addition of polyethylene glycol may cause a gel to form. This will preclude the use of the polyethylene glycol test procedure when testing these specimens for unexpected antibodies.
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- Precipitation of fibrinogen may be observed when testing plasma samples. In such cases, as with elevated globulin levels, it may be necessary to wash the red blood cells more than three times to remove all unbound human protein.
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- Precipitation of serum proteins when PeG is added appears to be related to elevated serum globulin levels. The problem becomes apparent when the IgG-coated red cells are nonreactive or unexplained weak reactions are detected. At least four manual washes of the red cells at AHG phase, with agitation, will fully resuspend the red cells and usually prevent the problem from occurring.

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- Test method must be followed exactly to avoid changing the ionic strength of the mixture.
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- 3-4% suspension of RBCs can be prepared by adding one drop of packed RBCs and approximately 1-1.5 mL of blood bank saline in an appropriately labeled tube. The suspension can be prepared using volume estimation with comparison to the reagent red cells for visual verification.
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- It may be necessary to switch to an alternate test method such as LISS due to problems with protein precipitation that causes neutralization of the antiglobulin reagent causing false negative test results. In these cases, the IgG coated control cells will not react and the test will be invalid (see SOP *LISS Indirect Antiglobulin Technique*)
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- Warm autoantibodies may react strongly in PeG test method and can mask the presence of underlying alloantibodies. In these cases, it may be desirable to switch to a less sensitive method such as LISS to attempt to detect and identify any underlying alloantibodies. (see SOP *LISS Indirect Antiglobulin Technique*)

REFERENCES:

- Technical Manual. Bethesda, MD: AABB Press, current edition
- Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition
- Gamma Peg - Polyethylene Glycol Additive for Antibody Detection Tests, Immucor, Norcross, GA 10/10.

RELATED DOCUMENTS:

SOP *Labelling Tubes and Gel Cards for Testing*
SOP *Antibody Identification*
SOP *Antibody Screen Testing*
~~SOP *Crossmatching*~~
SOP *Quality Control of Manual Testing Reagents*
SOP *Grading Reactions*
SOP *Specimen Acceptability and Order Receipt*
SOP LISS Indirect Antiglobulin Test

APPENDIX:

NA

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 Pagano, MD	Date	_____
UWMC Biennial Review:			
	_____	Date	_____
	_____	Date	_____

03/30/2021: Instructions added for entering reactions and interpretation for crossmatches