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-0031.02
TITLE: **LISS Indirect Antiglobulin Technique**		

PURPOSE:
To provide instructions using tube Indirect Antiglobulin Test (IAT) technique with Low Ionic Strength Solution (LISS) as a secondary manual method to detect the presence of unexpected antibodies as a secondary method to a PeG Indirect Antiglobulin test

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.

Use of a LISS additive accelerates antibody binding to red cells

POLICIES:
- This method is used as an alternate method (see SOP *PeG Indirect Antiglobulin Technique* for primary 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
Refer to SOP *Specimen Acceptability and Order Receipt*

REAGENTS/SUPPLIES/EQUIPMENT:

Reagents:	Supplies:	Equipment:
• LISS (Ortho AES) • 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 of LISS is performed on DAY OF USE. All other reagents are quality controlled daily

INSTRUCTIONS:

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Verify quality control for LISS was performed
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Performed</td><td>Go to next step</td></tr>
<tr><td>Not performed</td><td>• Perform QC as per SOP Quality Control of Manual Testing Reagents
• Go to next step</td></tr>
</table></td></tr>
<tr><td>2</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>3</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>4</td><td>Add 2 drops of patient plasma or serum to each tube</td></tr>
<tr><td>5</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>6</td><td>Add 2 drops of LISS reagent to each tube</td></tr>
<tr><td>7</td><td>Mix well and incubate all tubes at 37°C ±1 for 10-30 minutes</td></tr>
<tr><td>8</td><td>Centrifuge according to calibration for LISS phase and observe for hemolysis</td></tr>
<tr><td>9</td><td>Shake gently to resuspend the cell buttons and examine macroscopically for agglutination</td></tr>
<tr><td>10</td><td>Read, grade and record reactions</td></tr>
<tr><td>11</td><td>Wash the tubes 3 times with saline</td></tr>
<tr><td>12</td><td>Add 2 drops of Anti-IgG and mix well and centrifuge according to calibration for AHG phase testing</td></tr>
<tr><td>13</td><td>Shake gently to resuspend the cell buttons and examine macroscopically for agglutination</td></tr>
</table>

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

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

Interpretation

If the following is observed	Interpret as
Agglutination of test cells after any phase of testing	Positive
No agglutination of test cells	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*	
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
	Hemolysis at 37°C **AND/OR** Agglutination at any phase of testing.	• Incompatible

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 (SQ)</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 Testing</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>Enter appropriate reaction in SQ Reaction Grid and interpretation fields per table below
<table>
<tr><th>XIS</th><th>XINC</th><th>XAHG</th><th>XCC</th><th>Interpretation</th><th>SQ Code</th><th>SQ Hot Key</th></tr>
<tr><td>ND</td><td>0</td><td>0</td><td>+</td><td>LISS Compatible</td><td>CMP</td><td>C</td></tr>
<tr><td>ND</td><td>0/+</td><td>0/+</td><td>ND</td><td>LISS Incompatible</td><td>ICMP</td><td>I</td></tr>
</table>
</td></tr>
</table>
</td></tr>
</table>

NOTES AND LIMITATIONS:

- Test method must be followed exactly to avoid changing the ionic strength of the mixture
- Weak reactions may be obtained if the tests are incubated less than 10 minutes for LISS
- Complement dependent antibodies may not be detected if plasma is used
- See current version of package insert
- 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.

REFERENCES:

- Technical Manual. Bethesda, MD: AABB Press, current edition
- Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition
- ORTHO® Antibody Enhancement Solution Ortho-Clinical Diagnostics, Inc. Raritan, NJ 2011

RELATED DOCUMENTS:

SOP *Antibody Identification*
SOP *Antibody Screen Testing*
SOP *Specimen Acceptability and Order Receipt*
SOP *Grading Reactions*
SOP *Quality Control for Manual Testing Reagents*
SOP *Labelling Tubes and Gel Cards for Testing*

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

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