for optimal specificity against red blood cells demonstrating the different forms of polyagglutinability.

Dolichos biflorus reacts with red cells that are Group A₁ or A₂B. A MSDS information sheet is available for this product at www.hemobioscience.com.

The reagent contains 0.1% (w/v) sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

The format for the expiration date is expressed as DD-MMM-YYYY (day-month-year).

No US standard of potency.

Storage
The reagent should be stored at 2-8°C. Do not freeze or expose to elevated temperatures. Do not use if turbid. Lectins are clarified by filtration but may become turbid over time due to lipid precipitation. The lectin may also become darker in color. These are not indications of diminished potency or specificity.

Sample Collection
No special preparation of the patient is required prior to specimen collection. Red cells obtained from a fully clotted specimen or anticoagulated plasma can be used. Testing should be performed as soon as possible following collection to minimize the chance that false positive or falsely negative reactions will be encountered due to contamination or improper storage of the specimen. Samples that cannot be tested immediately should be stored at 2-8°C as soon as possible.

Procedure:

Materials Provided
Hemo bioscience Lectin Kit

Materials Required But Not Provided
Test Tubes
Centrifuge (1,000 rcf)
Phosphate Buffered Saline (PBS)
 Pipettes
Timer

Recommended Technique:
1. Label four test tubes for each red blood cell suspension to be tested.
2. Place two drops of each lectin into the appropriately labeled test tubes.
3. Add one drop of an approximate 3-5% suspension of red blood cells to be tested, washed at least once and resuspended in PBS.
4. Mix thoroughly by shaking the tube and incubate for 10 minutes at room temperature (23±3°C).
5. Centrifuge at 1000 rcf (g) for 15 seconds or a time and speed appropriate for the centrifuge used.
6. Resuspend by shaking gently.
7. Read macroscopically for agglutination and record test results. Microscopic reading of test results is not recommended.

NOTE: Final test results must be interpreted immediately upon centrifugation and resuspension.

Interpretation of Results:

Positive Test
Agglutination of the red blood cells.

Negative Test
No agglutination of the red blood cells.

Refer to Table 1 for results interpretation

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Normal Cells</th>
<th>Polyagglutinable Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachis hypogaea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Salvia sclarea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dolichos biflorus*</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Glycine soja</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

*Group O, B or A2 only. Red cells that are Group A₁ will react with Dolichos biflorus

Results of testing with these lectins are intended to allow for a PRELIMINARY classification of polyagglutinable red blood cells. Confirmatory tests should be performed.

Additional testing that may be performed to confirm (Table 2):

1. Confirm the absence of agglutinins specific to the suspected form of polyagglutination from the patient’s serum.
2. Adsorption of a normal adult serum to confirm that the patient’s red blood cells specifically remove agglutinins for the suspected form of polyagglutination.
3. Test polyagglutinable cells to determine whether or not the membrane sialic acid level is diminished. The Glycine soja lectin is used for this purpose. T-transformed red blood cells are agglutinated and T-transformed red blood cells are not agglutinated, since T-transformation is associated with a reduction of sialic acid level. However, the reactivity of Cad red blood cells with Glycine soja can not be explained, since Cad+ cells are not associated with a reduction of sialic acid. This can be confirmed using Polybrene[12]. Normal red blood cells are aggregated.
and sialic acid deficient red blood cells are not when incubated for 5 minutes at room temperature with a 1% solution of Polybrene.

4. Observe the effect of treating the red blood cells with a proteolytic enzyme (i.e. ficin or papain). The Tk receptor is enhanced by enzyme treatment while the T receptor is damaged. Another form of polyagglutination (Th) has been described which, like T and Tk, reacts with Arachis hypogaea, but in Th cells, this reactivity is diminished significantly by enzyme treatment. Th cells react weakly or are nonreactive with Glycine soja.

5. An anti-H reagent, such as HBS Anti-H Lectin (Alexis europeaus, product code H244) can be used to measure H antigen reactivity; Tk polyagglutination is associated with a reduction in H antigen reactivity. Another form of polyagglutination (VA) may also be associated with H antigen depression. VA red blood cells are nonreactive when tested with Arachis hypogaea, Salvia sclarea and Glycine soja.

<table>
<thead>
<tr>
<th></th>
<th>Cad+</th>
<th>Tn+</th>
<th>T+</th>
<th>Tk+</th>
</tr>
</thead>
<tbody>
<tr>
<td>H Antigen</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>↓</td>
</tr>
<tr>
<td>Polybrene solution</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Proteolytic enzymes</td>
<td>Normal</td>
<td>Normal</td>
<td>↓</td>
<td>+</td>
</tr>
<tr>
<td>Most normal sera</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

↓ = decreased reactivity
↑ = increased reactivity

Table 2: Expected results of confirmatory testing

Quality Control

Hemo bioscience performs lot release on each batch of lectin kit reagents. Positive control cells are typically not readily available. If desired, T and Tk cells can be prepared (8). Positive control cells for the Glycine Soja lectin can be prepared by treating normal adult red blood cells with ficin. Normal red blood cells (or commercial reagent red blood cells) may be used as negative controls.

Limitations:

1. Dolichos biflorus will react with cells that are Group A and A,B.
2. Stability of the antigens is of paramount importance and this will only be achieved if storage conditions are optimal.
3. The strength of reactivity with the lectins will depend on the degree to which red blood cells have been transformed.
4. Red blood cells may simultaneously exhibit more than one form of polyagglutinability which could result in a pattern of reactivity with the lectins which does not allow for presumptive identification.
5. Additional forms of polyagglutinability may exist whereby the red blood cells are not agglutinated by these lectins yet are agglutinated when tested with fresh normal adult sera.
6. Since some forms of polyagglutinability are transient, uniform reactivity and patterns may not be observed when testing samples collected from the same patient on different occasions.
7. “Mixed-field” agglutination may be noted and the proportion of agglutinated to unagglutinated cells may be variable, even on samples collected from the same patient on different occasions.
8. Some lectins are known to have anti-bacterial activity, such that they may agglutinate red blood cells that are coated with bacterial polysaccharides as a result of sepsis or in vitro sample contamination (i.e. Salvia sclarea reacts with cells coated with group C streptococci). This is different from the agglutination of red blood cells changed by the action of bacterial enzymes. For this reason, lectins alone should not be used to identify red blood cell polyagglutination. Lectins can give an indication of the likely cause of polyagglutination but confirmatory tests should be performed.
9. False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents or samples and certain disease states.
10. Weakened reactivity may be observed with control cells that have been stored in commercial red cell support solutions. Washing the cells 3 times in isotonic saline before testing may increase reaction strength.
11. Due to the variation in antigen strength among individuals, weak positive or negative reactivity may be seen when testing Cad+ cells using Glycine soja lectin.

Specific Performance Characteristics:

Hemo bioscience Lectin kit is supplied for use in the serological investigation of the major forms of erythrocyte polyagglutination. Each lot is tested to assure appropriate reactivity when used by the recommended test procedure. For technical support, contact Hemo bioscience at 1-866-332-2835.

Bibliography: