EDUCATIONAL COMMENTARY – DIRECT ANTIGLOBULIN TESTING (DAT) UPDATE

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits click on Earn CE Credits under Continuing Education on the left side of the screen.

LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- discuss the principle of the direct antiglobulin test (DAT).
- identify types of reagents used in DAT.
- correlate DAT results with patient disease states.

Introduction

The direct antiglobulin test (DAT) is used to detect antibody or complement that has bound to the patient’s red blood cells (RBCs) in vivo. Polyspecific or monospecific antihuman globulin (AHG) reagents allow this sensitization to be detected. There are many causes of a positive DAT, including hemolytic transfusion reactions, hemolytic disease of the fetus and newborn (HDFN), autoimmune hemolytic anemia (AIHA), and drug-induced antibodies in the patient. The DAT should be performed on patients who exhibit signs of hemolysis to distinguish immune-mediated from non–immune-mediated hemolysis. Positive DATs may occur in up to 15% of hospital patients and 1 in 1000 to 1 in 14000 blood donors with no obvious signs of hemolysis; therefore, the DAT should be used as a diagnostic tool. Interpretation of a positive result requires knowledge of the patient’s history of drug therapy, recent transfusion, or evidence of in vivo hemolysis.

Table I. Some Causes of a Positive DAT

| Hemolytic transfusion reactions |
| Drug-induced antibodies         |
| Hemolytic disease of the fetus and newborn |
| Autoimmune hemolytic anemias    |
| High levels of protein (eg, hypergammaglobulinemia, high-dose IV immune globulin) |

Antihuman Globulin Reagents

Conventionally, AHG reagents are manufactured by injecting rabbits with human globulins, which stimulates the production of antibodies. When using these reagents, the antigen-binding (Fab) sites of the antiglobulin molecule attach to either the Fc portion of the bound antibody or to the complement component on two adjacent RBCs, forming a lattice that allows for visible agglutination. Polyspecific AHG reagents are a blend of anti-immunoglobulin (Ig) G and anti-C3 and are used to detect IgG antibodies.
and/or complement components, such as C3d or C3b. If the DAT is positive, it should be repeated using monospecific reagents to determine the specific protein causing the positive reaction. Anti-IgG reagents detect IgG antibody sensitization only, and anticomplement reagents detect complement sensitization only. IgM and IgA bound to RBCs are not detected with these methods, although IgM immunoglobulins are capable of activating complement, which may lead to a positive result with anti-C3 reagents.

The presence of bound IgG antibody detected by monoclonal anti-IgG AHG can be confirmed by elution. Antiglobulin-reactive typing reagents cannot be used on these cells until the bound IgG antibody is removed. Monoclonal anti-C3 detects activated complement components, which may or may not indicate IgM immunoglobulin involvement. Once complement is activated in vivo, the final degradation of C3b to C3d occurs, and C3d is firmly bound to the RBC membrane. Because of this, anti-C3d is required in AHG reagents, although most reagents may also contain anti-C3b.

**Interpretation of DAT Results**

Evaluation of reactions using polyspecific and monospecific AHG reagents can help characterize the immune process. Test results using all three AHG reagents may be positive in cases of warm autoimmune hemolytic anemia (WAIHA) or mixed-type AIHA involving IgG and/or IgM immunoglobulins. A DAT that is reactive only with anti-C3d (-C3b) may indicate cold agglutinin syndrome or paroxysmal cold hemoglobinuria. Reactions with polyspecific and/or anti-IgG AHG only may indicate a hemolytic transfusion reaction, HDFN, warm AIHA, or drug-induced hemolytic anemia. It is possible for the DAT to be nonreactive if the RBC-bound IgG is below the detectable threshold.

**Table II. Typical Patterns of Reactivity in Autoimmune Hemolytic Anemia**

<table>
<thead>
<tr>
<th>Type of AIHA</th>
<th>Anti-IgG</th>
<th>Anti-C3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm AIHA</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Cold Agglutinin Syndrome</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Paroxysmal Cold Hemoglobinuria</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mixed-type AIHA</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Adherence to the reagent manufacturer instructions for proper testing methods and proper reagent storage is required to ensure valid test results. Neutralization of AHG reagents by elevated serum protein can cause false-negative reactions if the patient’s red blood cells are not thoroughly washed prior to testing. Manufactured reagent IgG-coated or complement-coated RBCs, commonly referred to as check cells, should be used to detect false-negative reactions. These check cells should react with the AHG, causing agglutination and indicating a true-negative test. Interruption of testing or reading can cause
bound IgG to dissociate from the RBCs; therefore, the test must be read immediately after centrifugation.
Fresh specimens collected in EDTA are preferred for DAT testing, because the use of clotted specimens may cause in vitro activation of complement, giving a false-positive result.

**Summary**

The DAT can be useful in assisting with the diagnosis of hemolytic anemias, including hemolytic transfusion reactions, AIHA and HDFN; however, other causes for a positive DAT must be considered.
To determine the condition, the clinical history of the patient or blood donor must be evaluated in conjunction with the DAT results. Polyspecific AHG reagents can be used as a screening test, but must be followed by testing with monospecific AHG reagents to identify the specific cause of the positive DAT.
Several sources of error in the testing procedure can lead to false-positive or false-negative results. To avoid inaccurate test results, the reagent manufacturer’s instructions must be followed.

**References**

