EDUCATIONAL COMMENTARY – ANTIBODY ELUTION TESTING: WHEN TO USE IT AND HOW TO DO IT CORRECTLY

Learning Outcomes
Upon completion of this exercise, the participant should be able to:

- identify the causes of a positive direct antiglobulin test result.
- explain the uses and the interpretation of elution results.
- identify the technical aspects that influence the outcome of the elution procedure.

Antibody elution is an important step in processing a sample with a positive direct antiglobulin test (DAT) result or direct Coombs. By freeing antibody bound to RBCs, the antibody's antigen specificity is identified. This is accomplished by various methodologies affecting the stability of the antigen-antibody complex.

Direct Antiglobulin Test
A DAT may be ordered by a physician who suspects that the patient is experiencing immune-mediated hemolysis (i.e., an antibody is causing the destruction of the patient’s RBCs). If the sample yields a positive DAT with polyspecific antihuman globulin (AHG) reagent, the result may be due to the presence of IgG and/or complement (C3) attached to the patient’s cells. This finding should be followed by another procedure in which the patient’s RBCs are incubated with an antiserum specific for IgG and another for C3. This phase of testing may be called the “split DAT.” The most common result in autoimmune hemolytic anemia (AIHA) is a DAT positive for IgG only; next most common are cases with both IgG and C3 attached to the cells. Either result is characteristic of the warm type of AIHA. In the rarer form of AIHA, cold agglutinin disease (CAD), the autoantibody is an IgM. The DAT in CAD is positive only for C3 because the AHG reagent does not recognize IgM, and this antibody class is very efficient in activating complement and causing intravascular hemolysis.

Another circumstance where a DAT is indicated is when the autocontrol is reactive during the workup of a positive transfusion antibody screen. If the DAT is also positive, it confirms that the antibody that is binding in vitro (positive autocontrol) is also doing so in vivo, and thus, may be clinically significant. The stronger the DAT, the more hemolysis the antibody is expected to cause if the donor unit is transfused. It is very important to read the DAT result under the microscope to assess if all RBCs are agglutinating or if there are clumps as well as free RBCs. The latter picture represents a “mixed-field” positive DAT that is typical of samples with a mixture of patient’s cells and recently transfused RBCs. The positive DAT in this case is due to an antibody to the transfused RBCs (alloantibody) as opposed to the more common cause of a positive DAT, which is an autoantibody.
Elution—Why and When
When a patient has a positive DAT for IgG, performing an elution allows the identification of the antibody specificity by incubating the eluate with reagent RBCs of known phenotype. If the patient has not been transfused in the last three months, a positive DAT is expected to be due to an autoantibody because the only circulating cells are the patient's own RBCs. However, it is possible that a transfusion—of which the patient is not aware—occurred at another facility. On the other hand, while most autoantibodies react with an antigen that is present on all RBCs, some autoantibodies have a relative specificity, such as to the \( e \) (little e) antigen. Such antibodies are suspected when the patient’s serum is only reacting with reagent RBCs that express the specific antigen.

In a patient who has been transfused in the past three months, a positive DAT may be due to an alloantibody attached to the RBCs from the transfused units, and the elution procedure is critical to make the antibody identification. The most common reason for this scenario is when the patient’s pretransfusion testing was unable to detect the alloantibody due to a very low level, but a transfusion acts as a boost in the immune response when the same antigen to which the patient had been previously sensitized is present in the transfused RBCs. This is the mechanism behind a delayed hemolytic transfusion reaction. When suspected, these reactions must be thoroughly investigated with a repeat antibody screen and a DAT, followed by an elution of any amount of IgG that is found on the cells.

Incubating the eluate with the reagent panel cells is imperative to identify the specificity of the eluted alloantibody in order to provide the patient with units for transfusion that lack the corresponding antigen now and in the future. Depending on the strength of the DAT, the eluate may not react with any cell. If the antibody screen is positive, one hopes that the same antibody in the plasma is the one attached to the transfused RBCs. However, a positive DAT due to an alloantibody may be accompanied by a negative antibody screen if the IgG has been completely adsorbed by the transfused cells. In that case, the elution is the only method available for antibody identification.

A specific example of a positive DAT and a negative antibody screen and elution is drug-induced AIHA. Typically in these situations, the autoantibody binds to the patient's RBCs while the culprit drug is present in the plasma. Without the drug present, the antibody screen and the eluate will not react despite even a strong DAT. Penicillin and cephalosporin, among other drugs, are known to be associated with drug-induced AIHA. Eliciting a history of recent exposure to drugs is paramount to explain the reason for the positive DAT in these cases. After the likely drug is identified, using it to reproduce antibody binding from the plasma, serum, or eluate will confirm the diagnosis.
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An elution is also useful when blood specimens from individuals of blood groups A, B, or AB have a positive DAT and a history of recent platelet transfusion. In these patients, the elution can differentiate between a warm autoantibody or an alloantibody, such as anti-A and/or anti-B passively received from the transfused unit. In the latter case, the eluate will not react with any cell in the antibody screen or panel (all group O), and must be tested with reagent groups A and B cells. Because donors of platelets may have strong anti-A and/or anti-B and their units may have to be given to a patient with those antigens, it is safer to only issue units with titers less than 50 in order to avoid clinically significant hemolysis. If that is not possible, the number of transfusions of mismatched platelets should be kept to a minimum, and the risk of hemolysis should be assessed by serial DATs.

A positive DAT in an umbilical cord or neonatal sample should also be subjected to an elution to characterize the type of antibody bound to the child’s RBCs. If the mother has a known auto- or alloantibody, the finding in the child’s sample can be explained. If the mother is of group O and the child is A or B, anti-A,B is the expected antibody and can be identified with the same cells used for reverse typing (A1 and B cells).

Several technical factors affect the success of the elution procedure:
1. RBCs with positive DAT must be thoroughly washed from excess IgG from the plasma that is specifically bound to the membrane, and the last wash must be negative for antibody reactivity.
2. Washed RBCs must be placed in a clean test tube prior to elution to avoid contamination of the eluate with antibody bound to the test tube wall during the previous phase.
3. A negative elution may be due to antibodies that have already dissociated during washing. This is particularly possible if the DAT is due to anti-A or anti-M and can be avoided by washing the cells with cold saline.
4. If organic solvents are used for elution or if the tonicity or pH of the eluate is not corrected prior to testing, the eluate can cause hemolysis or nonspecific clumping of the reagent RBCs.
5. Because eluates are not stable, they should be tested immediately after preparation. If that is not possible, the eluate may be frozen in 6% weight/volume of bovine albumin.
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Summary
The elution procedure is a valuable tool for evaluating patients in a variety of situations. Each laboratory must be proficient with at least one technique and follow it consistently to yield reliable results. The AABB Technical Manual is an excellent resource and should be available in every laboratory that performs elutions. The proper interpretation of the elution must take into account the patient’s clinical history (including recent transfusions) as well as the results of the antibody screen, panel, and DAT. The Table summarizes the most likely explanations for common elution results.

TABLE. Summary of Elution Results.

<table>
<thead>
<tr>
<th>Eluate Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate reacts with all cells in the panel.</td>
<td>Autoantibody</td>
</tr>
<tr>
<td>Eluate only reacts with a few cells.</td>
<td>Alloantibody bound to recently transfused cells</td>
</tr>
<tr>
<td>Eluate does not react with any panel cell.</td>
<td>Low concentration of IgG, drug-dependent autoantibody, anti-A or anti-B, antibody dissociated prior to elution, long delay between elution and testing</td>
</tr>
<tr>
<td>Hemolysis of RBCs occurs after incubation with eluate.</td>
<td>Improper preparation of the eluate (tonicity, pH)</td>
</tr>
</tbody>
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Suggested Reading


Reardon JE, Marques MB. Laboratory evaluation and transfusion support of patients with autoimmune hemolytic anemia. Am J Clin Pathol. 2006;125(suppl):S71-S77.

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