

EDUCATIONAL COMMENTARY – DIRECT ANTIGLOBULIN TESTING

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Learning Outcomes

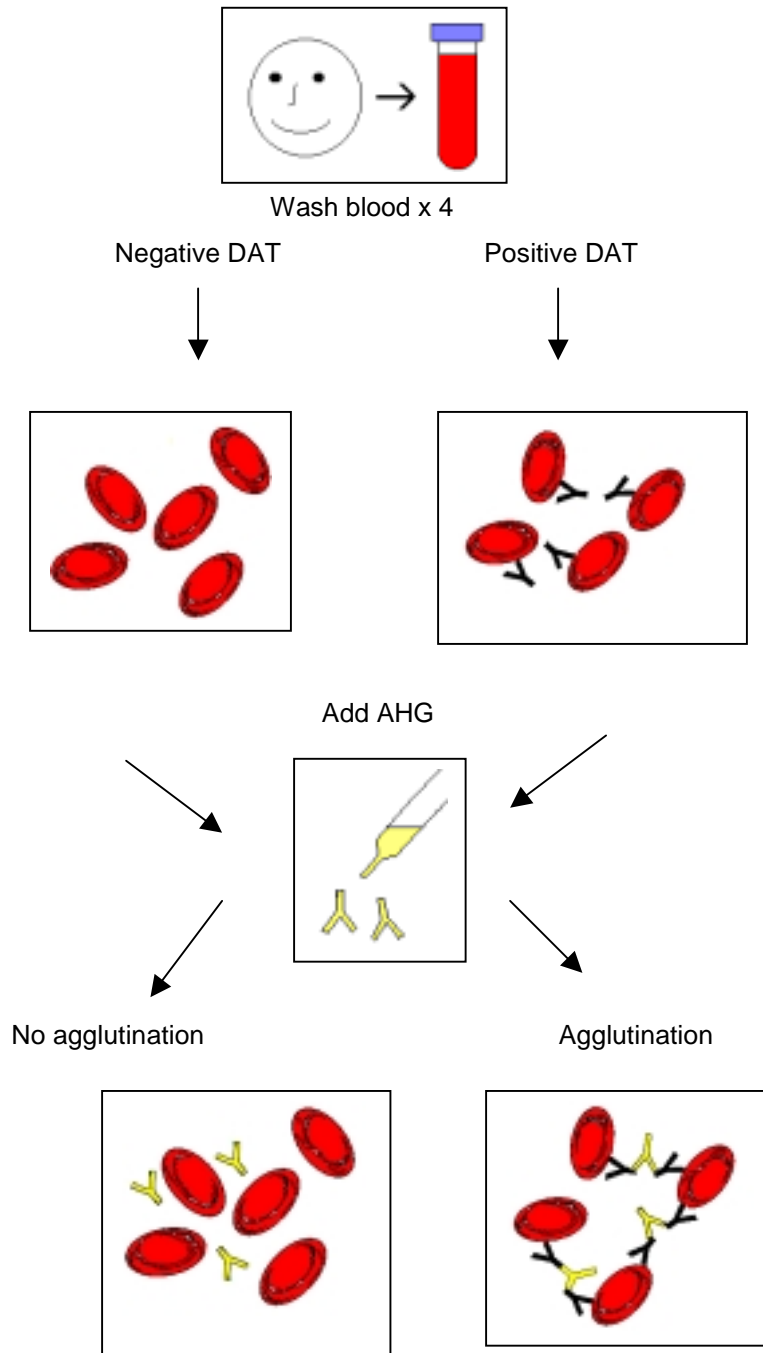
Upon completion of this exercise, the participant will be able to:

- List the conditions for which direct antiglobulin testing (DAT) is used as a diagnostic tool.
- Discuss the principle of DAT.
- Describe the uses of polyspecific and monospecific antihuman globulin reagents.

The direct antiglobulin test is widely used in the blood bank laboratory to detect antibodies that are attached in vivo (in the body) to the patient's red blood cells (RBCs). These antibodies are usually IgG or complement components (C3b, C3d, C4b, C4d). Direct antiglobulin testing (DAT) is useful in the diagnosis of hemolytic disease of the newborn, autoimmune hemolytic anemia (warm, cold, mixed type, and paroxysmal cold hemoglobinuria), and transfusion reactions. DAT is sometimes positive in drug-induced hemolytic anemia. A positive DAT is not diagnostic in itself. Other factors must also be considered when diagnosing these diseases.

IgG antibodies and complement components that have coated RBCs cannot be detected by direct agglutination procedures. Agglutination requires the formation of an antigen-antibody lattice between RBCs. IgG antibodies and complement components are too small to form a lattice. DAT utilizes a reagent which consists of an antibody that will react with any human globulin. Antihuman globulin (AHG) attaches to the antibody and/or complement that coats the RBCs and forms bridges between IgG antibodies. A lattice is formed and results in agglutination. The patient's RBCs must be washed prior to testing to remove plasma antibodies not attached to the RBCs that would interfere with the procedure. The DAT reaction is depicted in the diagram below.

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Antihuman globulin (AHG) reagent may be polyspecific or monospecific. Polyspecific AHG reagents are used to screen for IgG and complement components. A positive reaction indicates that there is either IgG and/or complement present on the patient's RBCs. If the test is positive, it must be repeated using individual monospecific AHG reagents to determine specifically what is coating the RBCs (IgG, complement, or both).

To rule out false-negative tests, all negative reactions must be confirmed with check cells. Reagent check cells are RBCs which have been coated with IgG or complement. The check cells are added to the negative tube. In a true negative test, AHG reagent that is not attached to antibody or complement on the patient's RBCs will be available to attach to the check cells. After centrifugation, the tube will be positive for agglutination. This procedure ensures that the patient's cells were properly washed before the addition of AHG to remove all antibodies not attached to the red cells and also verifies that AHG was added.

Detection of complement degradation products, such as C3d, indicates that an antigen-antibody combination occurred and resulted in activation of the complement pathway. If only complement is detected, the coating antibody is probably IgM that was removed during the preliminary washing procedure.

In conclusion, it is important to properly interpret direct antiglobulin testing. A positive polyspecific AHG test does not specify the antibody or complement component coating the RBCs. Always perform monospecific AHG testing to identify the specific RBC coating protein, ie, IgG or complement.

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