# DIRECT ANTIGLOBULIN TEST (DAT)

## TERM DEFINITION

**Sometimes referred to as the direct Coombs test, the DAT is a serologic test performed in the Blood Bank. It is used to determine if red cells are coated (in vivo) with immunoglobulin, complement, or both. The DAT is typically ordered during a workup for hemolysis, as bound immunoglobulin and/or complement can lead to the destruction of red cells.**

## METHOD

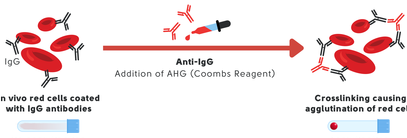
### PRINCIPLE OF THE TEST

- The DAT relies on the principle of agglutination. The addition of anti-human globulin (AHG) to a red cell sample will cause agglutination if the red cells are coated with antibodies or complement fragments.
- The anti-human globulin reagent can be polyspecific (includes both anti-IgG and anti-C3d antibodies) or can be monospecific (antibodies targeting either IgG or C3d).

### STEPS

1. Red blood cells are taken from a patient's sample; the cells are washed to remove any lingering, unbound antibodies.
2. These washed cells are suspended, and an anti-human globulin reagent (also called the Coombs reagent) is added to the solution.
3. The solution is centrifuged.
4. The sample is evaluated for agglutination. The agglutination can be graded based on strength (Negative to 4+).
5. Negative reactions should be validated using the addition of antibody-coated reagent cells.

### The Antiglobulin Reaction (Anti-IgG)




**In vivo red cells coated with IgG antibodies**

*No agglutination*

**Crosslinking causing agglutination of red cells**

*Agglutination*

## FACTOIDS

A positive DAT can be found in 1 in 1,000 to 1 in 14,000 healthy blood donors without hemolysis.

The DAT is positive in ~7 to 8% of all hospitalized patients; not all of these patients are hemolyzing.

97 to 99% of individuals with warm AIHA will have a positive result with anti-IgG, anti-C3d, or both.

A positive DAT does not tell us the antigenic target of a discovered bound antibody, only that one is present.

The strength of the DAT positivity generally correlates with the severity of hemolysis.

Anti-human globulin (Coombs reagent) is made by immunizing animals (commonly rabbits) with human gamma globulin. The animals respond by making anti-human globulin (i.e., antibodies against human gamma globulin and complement). The AHG is then purified before use.

## - RESULT

**FALSE NEGATIVE**

- Improper or under-washing
- Under-centrifuging the sample
- Failure to add AHG reagents
- Addition of inactive AHG reagents due to improper storage and handling
- Delay in the addition of AHG after washing

**TRUE NEGATIVE**

- No antibody or complement detected on the surface of the red cells by the AGH reagents
- DAT-negative hemolytic anemia (i.e., IgA mediated, drug mediated)

## + RESULT

**FALSE POSITIVE**

- Nonspecific binding of the DAT reagents
- Delay in the addition of AHG after washing
- Over-centrifugation

**TRUE POSITIVE**

- Autoimmune hemolytic anemia (HA)
  - Warm autoimmune HA
  - Cold autoimmune HA
  - Mixed autoimmune HA
  - Paroxysmal cold hemoglobinuria
- Drug-induced hemolytic anemia
- Alloimmune causes of HA
  - Transfusion related (e.g., acute hemolytic transfusion reaction, delayed hemolytic or serologic transfusion reaction)
  - Maternal alloantibodies that cross the placenta and bind to fetal cells (hemolytic disease of the fetus / newborn)
- Passive transfer of antibody in immunoglobulin preparations (e.g., IVIG)

## CLASSIC PATTERNS OF RESPONSE

**Positive polyspecific DAT**

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**Perform monospecific DAT**

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**What is positive?**

### Anti-IgG

- Warm Autoimmune Hemolytic Anemia (WAIHA)
- Alloimmune hemolytic anemia (i.e., Acute or delayed hemolytic transfusion reaction)
- Hemolytic disease of the fetus and newborn (HDFN)
- Drug induced hemolytic anemia
- Passive acquisition

### Anti-IgG & Anti-C3

- Warm Autoimmune Hemolytic Anemia (WAIHA)
- Mixed Autoimmune hemolytic anemia
- Drug induced hemolytic anemia

### Anti-C3

- Cold Agglutinin Disease
- Paroxysmal cold hemoglobinuria
- Drug induced hemolytic anemia

## PROXIMATE MECHANISMS

The DAT relies on the principle of agglutination.

When anti-human globulin (AHG) binds to antibodies or complement on one red cell's surface, and then to those on the surface of another, it can create a linking bridge between the red cells. As the process continues, aggregates (or clumps) of red cells are visible.

The type of bound antibody can be inferred from the reagent that caused the positive reaction; a positive reaction with anti-IgG implies an IgG antibody was bound to the red cells in vivo, and a positive reaction with anti-C3d implies an IgM antibody was bound to the red cells in vivo.

On tested red cells, the DAT can detect as low as:

- 100-500 molecules of IgG
- 400-1,100 molecules of C3

The agglutination can be graded based on strength (Negative to 4+), which generally correlates with degree of clinical severity of hemolysis.

Negative reactions should always be validated by adding antibody-coated reagent cells (check cells) to the non-reactive solution. Lack of agglutination with the addition of check cells should trigger concern for possible technical error.


## HISTORY OF MEDICINE

In 1945, Coombs, Mourant, and Race described the use of anti-human globulin serum for the detection of red cell-bound non-agglutinating antibodies. This test (the Coombs test) was rapidly applied and was helpful in numerous discoveries, including describing blood groups, hemolytic anemia caused by antibodies, and hemolytic disease of the fetus and newborn (HDFN).

Interestingly, it seems that this principle was described several decades earlier by Moreschi in 1908.

The DAT is still in use and remains one of the most valuable tools in the Blood Bank.

NOTES

*ATTRIBUTIONS*
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The Blood Project
ENCYCLOPEDIA OF BLOOD