The Direct Antiglobulin Test

Indications, Interpretation, and Pitfalls

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• **The direct antiglobulin test (DAT; sometimes referred to as the "Coombs" test) continues to be one of the most widely used assays in laboratory medicine. First described about 70 years ago, it is elegantly simple in design, yet it is widely complex in its applications and interpretations, and it is prone to false-positive and false-negative results. The overall objective of our review is to provide practicing pathologists with a guide to identify situations when the DAT is useful and to highlight disease-specific shortcomings as well as general pitfalls of the test. To accomplish these goals, this review will discuss the following: (1) the history of the DAT, (2) how the test is performed in the clinical laboratory, (3) clinical situations for its use, (4) its interpretation, and (5) the pitfalls associated with DAT assays, including causes of false positivity.**

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INTRODUCTION AND BRIEF HISTORY OF THE DIRECT ANTIGLOBULIN TEST

The direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT) are both forms of the antiglobulin test. The antiglobulin test is a method of demonstrating the presence of antibody or complement bound to red blood cell (RBC) membranes by the use of anti-human globulin to form a visible agglutination reaction. The IAT tests for antibodies circulating in the patient's plasma, while the DAT tests for antibodies or complement bound directly to the patient's RBCs, indicating in vivo sensitization.

The principle of using anti-human globulins was first described by Moreschi in 1908,[1] but it was not until 1945 that Robin Coombs introduced it to clinical medicine initially as a method to demonstrate RBC agglutination in the presence of what was then thought to be an "incomplete" or "blocking" antibody as seen within the context of the IAT.[2] When testing patient plasma for the presence of Rh antibody with the use of Rh antigen–positive RBCs, it was observed that sometimes the RBCs appeared to be sensitized by the respective Rh antibody, but did not result in agglutination. Furthermore, the RBCs were no longer able to bind with their respective "complete" or agglutinating antibody.[3] Questioning how to measure these "incomplete" antibodies, Coombs realized the answer during a long delayed wartime train trip from London to Cambridge. He suddenly recognized he could use a second antibody formed to the RBC coating antibody that would "bridge" and cause visible RBC agglutination.[4] That antibody became the now commonly used anti-human globulin that uses the 2 Fab sites of the reagent antibody to bind the Fc portion of the antibody that is coating target RBCs. It was 1 year later that he published the use of the DAT as a method of demonstrating in vivo sensitization of RBCs in hemolytic disease of the fetus and newborn (HDFN).[3] Since this discovery, the DAT has become a critical tool in the assessment of both immune and drug-induced hemolytic anemias, HDFN, as well as a fundamental step in the evaluation of patients experiencing transfusion reactions.

BASICS OF DAT PERFORMANCE AND AVAILABLE TESTING PLATFORMS

The DAT is a method used to detect immunoglobulin or complement bound in vivo to RBCs, using anti-human reagents. There are 3 different reagents that may be used: monospecific reagents to detect either bound immunoglobulin G (IgG) or bound complement (C3) and polyspecific reagents that can simultaneously detect IgG and/or C3. In screening of RBCs for the presence of bound globulins and/or C3, many laboratories may choose to use the polyspecific reagent initially (ie, as a screen for any bound IgG and/or complement) and, if positive, subsequently use monospecific reagents to specifically distinguish what is bound to the RBC surface.

The appropriate specimen for the DAT is one that is anticoagulated with ethylenediaminetetraacetic acid (EDTA). EDTA is necessary to chelate calcium (a necessary component of C3 activation) so that in vitro C3 fixation will not occur.[5] In the classic tube testing version of the DAT, the RBCs to be tested should be washed with saline to remove excess, unbound IgG or complement present in the serum that could preferentially bind and inhibit the reactivity of the added reagent. After washing, it is important to proceed as soon as possible to testing before any bound antibody has a

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Table 1. Typical Results in Different Types of Immune Hemolytic Anemia[a]

	DAT Results	Ig Class of Antibody	Antibody Specificity
Autoimmune hemolytic anemia			
WAIHA	IgG, C3, or both	IgG	Variable, often Rh
CAS	C3	IgM	Anti-I or i
Mixed-type	IgG + C3	IgG warm + IgM cold	See WAIHA and CAS
PCH	C3	IgG	Anti-P
Drug-induced immune hemolytic anemia			
Drug-dependent antibody	IgG, C3, or both	IgG, IgM, or both	Variable
Drug-independent antibody	IgG ± C3	IgG	Variable
Alloimmune hemolytic anemia			
Transfusion reaction	IgG ± C3	IgG, IgM, or both	Variable
HDFN	IgG	IgG	Commonly anti-D, anti-K, and anti-c

Abbreviations: CAS, cold agglutinin syndrome; C3, complement component 3; DAT, direct antiglobulin test; HDFN, hemolytic disease of the fetus and newborn; Ig, immunoglobulin; PCH, paroxysmal cold hemoglobinuria; WAIHA, warm autoimmune hemolytic anemia.

[a] Novel table created by the authors, but derived from text in Fung et al,[5] Petz,[23] and Zantek et al.[45]

chance to disassociate (or elute) off the patient's RBCs. Both inadequate RBC washing and/or a delay in test performance can lead to a false-negative result.[5] After addition of the polyspecific reagent, the RBCs are centrifuged. If a complement reagent is used, and if the reagent manufacturer's package insert permits, there may be a second incubation to allow enhancement of weak anti-complement reactions. The test tubes can then be examined visually for agglutination and graded from 0 to 4+, with 4+ representing a solid button of agglutination.

Some advancements above and beyond this tube method have been introduced in immunohematologic testing, and these platforms have been applied to the DAT. Microcolumn and solid phase are 2 commonly used alternatives to tube testing. In the United States, microcolumn technology is often referred to as the "gel" method. The gel method (examples include the ID-MicroTyping System by Ortho Diagnostics, Raritan, New Jersey; and Diamed by BioRad, Hercules, California) uses a dextran acrylamide column that is impregnated with anti-human globulin reagent, trapping antibody-bound RBCs.[6] The patient's RBCs are placed at the top of the column in a reaction chamber and then centrifuged. The cells from the reaction chamber are pulled into the column during centrifugation with unbound RBCs passing through the pores of the column creating a button at the bottom, whereas sensitized RBCs are trapped in the gel. The scoring is 0 to 4+ with 4+ positivity represented by a band of RBCs at the top of the column and a negative reaction represented by a pellet at the bottom. Advantages include a smaller volume of test sample and longevity of the result, allowing it to be read up to 24 hours after it is run.[7]

Solid phase testing for the DAT is available under different manufacturers (examples include the Capture-R assay by Immucor, Norcross, Georgia; and Solidscreen II by BioRad). The solid phase assays offered by Immucor and BioRad use different approaches to reach the same endpoint. Immucor binds the patient's RBCs to the microtiter plate and then anti-IgG–coated indicator cells are added, whereas BioRad adds the patient's RBCs to a microtiter plate coated with Protein A then anti-IgG is added. After centrifugation a button indicates a negative reaction whereas a "carpet" of RBCs over the well indicates a positive reaction. Grading is again via the classic 0 to 4+ scale.[6]

Gel and solid phase methods offer several advantages over the tube platform—they are both potentially automatable and both may be technically less difficult to perform than the tube method.[8] However, they have greater sensitivity than conventional tube testing[6,9] and this increased sensitivity may lead to the detection of clinically insignificant but weakly positive DAT results that sometimes demonstrate negative eluates.[9]

CLINICAL APPLICATIONS OF THE DAT

There are 3 basic clinical scenarios for which the DAT is used: (1) investigation of suspected alloimmune-mediated hemolytic transfusion reactions, (2) evaluation for HDFN, and (3) investigation of autoimmune-mediated hemolytic anemia. The basic interpretation of results in each of these clinical scenarios is presented in Table 1. Of course, a positive DAT result must be interpreted in the context of the clinical situation and this will guide the extent of additional antibody investigation.

Alloantibodies Associated With Transfusion or Transplant

A DAT can detect immunotargeting of transfused RBCs before hemolysis is clinically evident, or it can confirm the presence of an alloantibody in suspected hemolytic transfusion reactions. In blood banking, one of the most common uses of the DAT is in investigating alloimmune-mediated delayed serologic transfusion reaction (DSTR) and delayed hemolytic transfusion reactions (DHTRs) in the setting of transfusion-associated incompatibility. A DSTR is present when a posttransfusion specimen has a positive DAT result with the presence of a newly found alloantibody specificity, while a DHTR is a DSTR that includes clinical or laboratory evidence of hemolysis.[10] In fact, for patients with a history of transfusion, a positive DAT result may be the first indication that the patient has developed an alloantibody. Alloantibodies can be detected as quickly as 2 to 3 days in a secondary or anamnestic response following RBC transfusion.[11] While DSTR can be clinically benign, a DHTR may have serious sequelae, especially if it is unrecognized and more incompatible blood is transfused to compensate for that which is lost to hemolysis.[12] In some cases a DHTR may cause a loss of circulating RBCs beyond that which would be expected if only the incompatible transfused RBCs were immunologically targeted. This phenomenon is known as *bystander hemolysis* and can cause immunotargeting of the autologous RBCs with a DAT result that will be positive with C3.[12]

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For each of these situations, the immune response to RBC transfusion can be measured by performing a DAT to see whether IgG, C3, or both are bound to the recently transfused, allogeneic RBCs (or in the case of bystander hemolysis the DAT may have C3 positivity with autologous RBCs).[12] In the situation of hemolysis due to an incompatible transfused product, the percentage of "coated" cells in circulation may be very small, and the DAT result could be negative (even in the gel or solid phase platforms). Thus, if there is concern for the presence of an alloantibody, but the DAT result is still negative, one can perform an elution to concentrate the antibody. Elution studies are performed by removing the antibody from the patient's RBCs, by using various methodologies (eg, chemical, heat, or pH changes), and subsequently testing the RBC eluate against a standard panel of reagent cells. Literature suggests that antibody detection by elution is as efficacious as performing flow cytometry for the detection of antibody from a small population of opsonized cells.[13] When a DAT result that was previously negative is positive for IgG in a recently transfused patient, an eluate can demonstrate antibodies that are not yet detectable (have low titer) in the plasma. Likewise, an eluate may help elucidate the presence of a new warm autoantibody if a panagglutinin is detected, or if negative, may point to the possibility of drug-induced reactivity.[14]

A relatively unique and less commonly encountered form of alloimmune hemolysis can be seen in the setting of hematopoietic stem cell transplants. In this clinical setting, hemolysis may be due to a major mismatch (ie, a recipient harboring antibodies against donor cells) or a minor mismatch (ie, a donor graft harboring antibodies against recipient cells). Both ABO and non-ABO antibodies have been associated with alloimmune hemolysis after transplant, with ABO-unmatched hematopoietic stem cells often leading to a positive DAT result, and in 10% to 15% of cases, also immune-mediated hemolysis.[15] While some steps can be taken to limit this hemolysis (eg, depleting a stem cell product of plasma harboring ABO or non-ABO antibodies), a particularly troubling form of alloimmune hemolysis results from a phenomenon called *passenger lymphocyte syndrome*. In these cases, antibody production by "passenger" lymphocytes from the donor graft can mediate sometimes severe forms of hemolysis, associated with IgG- or C3-positive DAT results. Typically, in these settings, positive DAT findings and associated hemolysis abate as recipient cells are cleared and donor RBCs engraft and populate the circulatory system.[15]

Hemolytic Disease of the Fetus and Newborn

HDFN can occur whenever maternal IgG is formed against fetal antigens. The most clinically significant of these are the Rh antigens (predominantly D, c, and E) and Kell antigens. HDFN can also be mediated by antibodies against the Kidd and MNS families of antigens, but this is relatively rare. Overall, the most common type of HDFN is due to ABO incompatibility between mother and fetus, which occurs in 15% to 25% of pregnancies, yet only 1% of these will develop a positive DAT result (anti-IgG only) on cord blood sampling.[16] ABO HDFN tends to be clinically less severe because ABO antigens are only weakly expressed in fetal development and also because of the presence of ABO antigens in secretions of some fetuses that will neutralize maternal antibodies. Even in the 1% with a positive DAT result, only a fraction of those (about 23%) will go on to have clinically significant jaundice,[16] making the DAT a poor positive predictor of newborns that will require treatment.[16,17]

Table 2. Potential Causes of False-Positive or False-Negative Direct Antiglobulin Test (DAT) Results Commonly Seen in Clinical Practice[a]

Potential causes of false-positive results and/or positive results due to presence of nonspecific immunoglobulins

- Clotted specimen causing in vitro complement binding
- High serum immunoglobulin levels or proteins/rouleaux
- Intravenous immune globulin administration
- Antiphospholipid syndrome
- Infections: HIV, malaria
- Wharton jelly (in the setting of pregnancy and cord blood testing)
- Inadequate serologic technique or technical error

Potential causes of false-negative results

- Severe hemolysis
- Hemolysis mediated by IgA or IgM
- RBC-bound IgG below detectable limits of DAT
- Low-affinity antibodies
- Inadequate serologic technique or technical error

Abbreviations: HIV, human immunodeficiency virus; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; RBC, red blood cell.

[a] Novel table using data derived from text in Snyder and Falast.[46]

While the DAT is not recommended as a screening test for HDFN owing to its poor positive predictive value,[16,17] a critical situation that requires a DAT is when there is suspicion of HDFN due to clinically evident hemolysis or hyperbilirubinemia in the neonate.[16] In these cases, even with a negative maternal antibody screen, the DAT should be checked in the fetus owing to the possibility of a low-frequency antigen that might not be present on the screening cells. Some hospitals will also use the DAT as a screening test for HDFN under selective conditions such as blood group O mothers, positive maternal antibody screens due to HDFN-mediating antibodies, prior pregnancies with HDFN, and in cases where knowledge is lacking owing to inadequate prenatal screening. Routine cord blood DAT performance is no longer recommended in Rh(D)-negative mothers owing to the large number of positive DAT results that would be attributable to the routine use of antenatal anti-RhD prophylaxis.[18]

While the positive predictive value of the DAT is low, the negative predictive value is high and thus even in the case of ABO-incompatible newborns with significant jaundice, a negative DAT result should lead to a search for non-isoimmunization causes.[19] It is important to note that cord blood contaminated by Wharton jelly may cause nonspecific agglutination and can lead to clinically false-positive DAT results (Table 2).[16]

Autoimmune Hemolytic Anemia

In a patient with clinical and/or laboratory evidence of hemolysis and with the absence of an obvious inciting event (eg, transfusion), the presence of an autoimmune hemolytic anemia (AIHA) should be considered. It is in this setting that the results of the DAT can be extremely helpful not only in establishing the diagnosis, but also in helping to classify the type of immune hemolytic anemia (IHA; see Table 1). Autoimmune hemolytic anemias are classified as warm, cold, and mixed-type on the basis of thermal amplitude of the associated antibody. Drug-induced RBC hemolysis is yet

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another cause of AIHA that may present with a positive DAT result. While an in-depth discussion of drug-associated AIHA is beyond the scope of this article, elution studies in this setting can be of great help in examining for drug-associated hemolysis because a DAT finding that is positive for the presence of IgG with a negative eluate can be due to drugs.[14] For additional discussion, the reader may find other useful reviews on the subject of drug-induced hemolytic anemia in the articles by Johnson,[20] Garratty,[21] and Garratty and Arndt.[22]

Warm AIHA is generally IgG dependent but can also (rarely) be immunoglobulin M (IgM) or immunoglobulin A (IgA) dependent, or there could also be more than 1 immunoglobulin class present simultaneously (eg, IgM/C3 together with IgG on the RBC membrane).[23] The DAT result in most cases of warm AIHA, however, is positive for IgG only.[23]

The presence of an IHA with a DAT result that is negative for IgG and positive for C3 should prompt the consideration for a cold AIHA. The category of cold hemolysins includes 2 distinct disease entities: cold agglutinin syndrome and paroxysmal cold hemoglobinuria, either of which should demonstrate a DAT positive only with C3 antisera.[23] Cold agglutinin syndrome antibodies tend to be IgM with specificity for I/i class of antigens, or much less commonly, an IgG or IgA with specificity for Pr antigens.[24] Cold agglutinin syndrome should also be considered when IHA is presenting in a patient with respiratory disorders, especially infections with *Mycoplasma pneumoniae*.[23,24] Because CAS is primarily IgM mediated, it may be associated with spontaneous room temperature agglutination in EDTA specimens.[25]

Paroxysmal cold hemoglobinuria tends to be a transient response to viral infections or vaccination, especially in children. Patients with paroxysmal cold hemoglobinuria have a biphasic hemolysin referred to as the Donath-Landsteiner antibody, which most often has anti-P specificity.[24] The Donath-Landsteiner antibody is an IgG class antibody that binds at low temperatures, where it fixes complement to the RBCs, then the cells are hemolyzed at warmer temperatures owing to the opsonization with complement.[24]

Mixed-type AIHA will have both IgG (or warm) and IgM (or cold) autoantibodies with the IgM exhibiting broader thermal amplitude ($\geq$30°C), hence this form of AIHA tends to present with a DAT finding positive for both IgG and C3.[23] Mixed-type AIHA may be overdiagnosed because a warm autoantibody can elevate the titer of clinically insignificant cold autoantibodies[23] (though in these cases the cold agglutinin would tend to be evident only at the lower thermal amplitudes such as $<$30°C).

In 5% to 10% of patients with IHA the DAT result may be negative.[26] There are 3 potential mechanisms believed to be involved to produce DAT-negative AIHAs: (1) IgG molecules bound to the RBC membrane at a density that is below the threshold of the DAT, (2) hemolysis mediated by IgA or warm IgM autoantibodies, or (3) low-affinity autoantibodies.[26] Methods to improve sensitivity in such circumstances are discussed below.

ALTERNATIVES TO THE TRADITIONAL DAT AND FUTURE DIRECTIONS

Low-affinity antibodies may be eluted from RBCs by routine washes with room temperature saline, causing the DAT result to be misleadingly negative. In some cases this may be overcome by washing with cold saline or washing with low ionic strength saline.[26] Since using cold reagents and washes (0°C–4°C) may evoke clinically insignificant cold autoantibodies, it is useful to run these tests with a 10% albumin negative control, in parallel, to exclude these cold autoagglutinins as a potential source of a clinically insignificant positive DAT result.[26]

More sensitive methods other than the routine DAT have been used to detect low levels of RBC-bound IgG. Such methods include Polybrene (hexamethrine bromide [1,5-dimethyl-1,5-diazaundecamethylene polymethobromide], Abbott Laboratories Corp, Chicago, Illinois) and polyethylene glycol.[26] The Polybrene method works because unsensitized RBCs, with the addition of sodium citrate, will disassociate. However, antibody-coated RBCs will be resistant to dispersal by sodium citrate in the presence of the hexadimethrine bromide.[26]

Flow cytometry is also a readily available tool in many laboratories and can be calibrated to detect low-density anti-human IgG molecules that otherwise would be missed by a traditional DAT test.[25] Alternatively, flow cytometry may be useful when there are a small number of circulating, sensitized cells such as in some IHAs.[11] In addition to these uses, flow cytometry has been applied in transfusion medicine for detecting subsets of cell populations (eg, quantification of fetomaternal hemorrhage, chimerism, and residual white blood cells in blood components) and, therefore, could have clinical applications for DAT-negative IHAs if suitable cutoffs could be determined.[27] Garratty has also pointed out that flow cytometry can be useful if the negative DAT result is due to a lack of antibody cross-linking.[27] Cross-linking (ie, when 2 immunoglobulins are bound across 2 different cells) is the necessary endpoint for the agglutination reaction, but flow cytometry only requires the RBC membrane to be coated with immunoglobulins.[27] Flow cytometry methodologies for white blood cell cytometry, however, are not directly translatable for use in RBC cytometry because of issues such as agglutination, and it is advisable to become familiar with the optimal parameters for performance of RBC flow cytometry lest quantification be underestimated.[28]

Other methods are available but are less likely to lend themselves to clinical use owing to their complexity and time-consuming nature. These more sensitive methods include enzyme-linked antiglobulin test, which provides a quantification by measurement of optical density,[25] and the complement-fixing antiglobulin consumption tests.[25] The mitogen-stimulated DAT is both a functional and quantitative method for looking at low-level antibody production by first stimulating the cells with a mitogen to increase antibody production.[29] In a review by Barcellini et al[29] of 16 cases, the authors found mitogen-stimulated DAT to be useful in 6 of the cases where all other test results were negative (including tube, microcolumn, and solid phase while using both IgM and IgA antisera). One of the difficulties in all of the more sensitive techniques is distinguishing a pathologic positive result from the "background" normal level of bound IgG present in healthy populations. One study using immunoradiometric assays used a statistical cutoff of 78.5 IgG molecules per RBC for differentiating pathologic levels of IgG from normal.[25]

Warm IgM antibodies can cause severe intravascular hemolysis[30] and RBC-bound IgM can be difficult to detect serologically. The dual DAT, which involves a second

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incubation with a different anti-human immunoglobulin after first sensitizing RBCs to anti-IgM, is useful for revealing these weak IgM antibodies and was able to demonstrate agglutination in 4 cases in a study by Bartolmas and Salama.[31] Immunoglobulin M– and IgA-specific reagents are not readily available, owing in part to their tendency to interact with heterophile antibodies, but are under development and likely to become commercially available in the future. Flow cytometry may also be used for IgM and IgA deposition on RBCs.[32]

DAT TESTING PITFALLS

Reported Causes of Positive DAT Results Due To Nonspecific and Confounding Factors

Studies using DAT results from healthy blood donors have found that up to 0.1% of healthy people have a positive DAT finding without evidence of hemolysis.[33] Of these, about two-thirds are positive with IgG and one-third, with complement. Garratty[34] states that all individuals have some IgG molecules on their RBC surfaces, but mostly below the limit of detection, probably fewer than 50 IgG molecules per cell (limit of detection = 100 IgG molecules per cell).

In acute illnesses the percentage of individuals with a positive DAT result is higher, with studies showing a 1% to 15% incidence of a positive DAT finding in hospitalized patients who do not have overt signs of hemolysis or clearly evident etiology.[35] These studies show variable degrees of both IgG and C3 sensitization. Specific disease states that demonstrate elevated levels of bound IgG on RBC membranes include thalassemia syndromes and sickle cell disease including sickle cell trait. In these circumstances, increased immunoglobulin coating may correlate with increased sequestration.[36] Hospitalized patients are also more likely to have other factors associated with positive DAT results, including elevated serum immunoglobulin levels, rouleaux due to high protein concentration, cardiolipin antibodies, infection, reticulocytosis, and the use of medications.[37,38] Patients with hypergammaglobulinemia may also have nonspecific passive adsorption of IgG onto their RBCs.[25,39] Administration of commercial preparations of intravenous immune globulin may also cause a positive DAT result owing to the presence of contaminant RBC-specific antibodies.[40] Antiphospholipid antibodies are known to cross-react with RBC membrane phospholipid epitopes and were found to produce positive DAT findings in 16% of patients with antiphospholipid syndrome and 4.3% of patients with lupus erythematosus; these positive results were also observed in healthy donors with elevated antiphospholipid antibodies.[41] Positive DAT results have been reported in patients with some infectious diseases including acute malaria[42,43] and human immunodeficiency virus.[44]

The positive predictive value of the DAT for IHA is actually very high so long as there is clinical suspicion for autoimmune hemolysis or it is being used to rule out an alloantibody reaction, highlighting the need to evaluate the DAT result from the perspective of the patient's full clinical scenario. Table 2 summarizes many of the reported causes of misleadingly positive DAT findings discussed above.

Reported Causes of False Negatives

Falsely negative DAT results will occur in the aforementioned cases of DAT-negative AIHA and can also be due to technical errors of inadequate cell washing or a delay in addition of the reagent. False-negative DAT results may also occur in instances of severe hemolysis where RBCs are cleared so rapidly, and in such great numbers, that there are few circulating sensitized RBCs left for detection. In cases of strong clinical concern for immune-mediated RBC destruction, but a negative DAT finding, the use of modified techniques and/or referral to an immunohematology reference laboratory can be considered (as discussed above).

CONCLUSION

When hemolysis is clinically suspected, the DAT is a critical step to distinguish immune-mediated hemolysis from other, nonimmune causes. The DAT is best interpreted with regard to background levels of RBC-bound immunoglobulins and the various clinical situations that can lead to a false-positive result. A positive DAT finding deserves further investigation whenever there is evidence of hemolysis, recent transfusion or transplant, or the administration of drugs known to produce IHAs. Alternatives and modifications to the DAT have been developed and can aid in establishing the basis of hemolysis in difficult cases. Overall, this knowledge should help pathologists to become competent consultants when tasked with interpreting DAT results in the various clinical settings in which they may be performed.

References

1. Moreschi C. Neue Tatsachen über die Blutkörperchenagglutination. *Zentralbl Bakt*. 1908;46:49–51.
2. Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and 'incomplete' Rh agglutinins. *British J Exp Pathol*. 1945;26(4):255–266.
3. Pamphilon DH, Scott ML. Robin Coombs: his life and contribution to haematology and transfusion medicine. *British J Haematol*. 2007;137(5):401–408.
4. Coombs RA. Historical note: past, present and future of the antiglobulin test. *Vox Sang*. 1998;74(2):67–73.
5. Fung M, Grossman BJ, Hillyer CD, Westhoff CM. *The Technical Manual*. 18th ed. Bethesda, MD: AABB; 2014:427.
6. Rumsey DH, Ciesielski DJ. New protocols in serolgic testing: a review of techniques to meet today's challenges. 2000;16(4):131–137.
7. Immucor [package insert]. Norcross, GA: Immucor Inc; 2010.
8. Alwar V, Shantahla Devi AM, Sitalakshmi S, Karuna RK. Evaluation of the use of gel card system for assessment of direct Coombs test: weighing the pros and cons. *Indian J Hematol Blood Transfus*. 2012;28(1):15–18.
9. Moulds JM, Diekman L, Wells TD. Evaluation of column technology for direct antiglobulin testing. *Immunohematology*. 1998;14(4):146–148.
10. Ness PM, Shirey RS, Thoman SK, Buck SA. The differentiation of delayed serologic and delayed hemolytic transfusion reactions: incidence, long-term serologic findings, and clinical significance. *Transfusion*. 1990;30(8):688–693.
11. Dolatkhah R, Esfahani A, Torabi SE, et al. Delayed hemolytic transfusion reaction with multiple alloantibody (anti S, N, K) and a monospecific autoanti-JKb in intermediate beta-thalassemia patient in Tabriz. *Asian J Transfus Sci*. 2013;7(2):149–150.
12. Popovsky M. *Transfusion Reactions*. 2nd ed. Bethesda, MD: AABB; 2001:6–7.
13. Alvarez A, Rives S, Montoto S, Sanz C, Pereira A. Relative sensitivity of direct antiglobulin test, antibody's elution and flow cytometry in the serologic diagnosis of immune hemolytic transfusion reactions. *Haematologica*. 2000;85(2):186–188.
14. Veeraputhiran M, Pesek G, Blackall D. Strict vs lenient criteria for elution testing: comparison of yields between two tertiary care medical centers. *Am J Clin Pathol*. 2011;136(3):359–363.
15. Laurencet FM, Samii K, Bressoud A, et al. Massive delayed hemolysis following peripheral blood stem cell transplantation with minor ABO incompatibility. *Hematol Cell Ther*. 1997;39(3):159–162.
16. Keir A, Agpalo M, Lieberman L, Callum J. How to use: the direct antiglobulin test in newborns. *Arch Dis Child Educ Pract Ed*. 2015;100(4):198–203.
17. Dinesh D. Review of positive direct antiglobulin tests found on cord blood sampling. *J Paediatr Child Health*. 2005;41(9–10):504–507.
18. Judd W, Scientific Section Coordinating Committee of the AABB. Practice guidelines on prenatal and perinatal immunohematology, revisited. *Transfusion*. 2001;41(11):1445–1452.
19. Herschel M, Karrison T, Wen M, Caldarelli L, Baron B. Isoimmunization is unlikely to be the cause of hemolysis in ABO-incompatible but direct antiglobulin test-negative neonates. *Pediatrics*. 2002;110(1, pt 1):127–130.
20. Johnson S. Drug-induced immune hemolytic anemia. *Transfus Apher Sci*. 2009;40(3):205–207.

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21. Garratty G. Immune hemolytic anemia associated with drug therapy. *Blood Rev*. 2010;24(4–5):143–150.
22. Garratty G, Arndt PA. Drugs that have been shown to cause drug-induced immune hemolytic anemia or positive direct antiglobulin tests: some interesting findings since 2007. *Immunohematology*. 2014;30(2):66–79.
23. Petz L. Review: evaluation of patients with immune hemolysis. *Immunohematology*. 2004;20(3):167–176.
24. Gertz M. Management of cold haemolytic syndrome. *Br J Haematol*. 2007;138(4):422–429.
25. Segal GB, Lichtman MA. Direct antiglobulin ("Coombs") test-negative autoimmune hemolytic anemia: a review. *Blood Cells Mol Dis*. 2014;52(4):152–160.
26. Garratty G. Immune hemolytic anemia associated with negative routine serology. *Semin Hematol*. 2005;42(3):156–164.
27. Garratty G, Arndt P. Applications of flow cytofluorometry to transfusion science. *Transfusion*. 1995;35(2):157–178.
28. Arndt P, Garratty G. A critical review of published methods for analysis of red cell antigen-antibody reactions by flow cytometry, and approaches for resolving problems with red cell agglutination. *Transfus Med Rev*. 2010;24(3):172–194.
29. Barcellini W, Revelli N, Impariali FG, et al. Comparison of traditional methods and mitogen-stimulated direct antiglobulin test for detection of anti-red blood cell autoimmunity. *Int J Hematol*. 2010;91(5):762–769.
30. Arndt PA, Leger RM, Garratty G. Serologic findings in autoimmune hemolytic anemia associated with immunoglobulin M warm autoantibodies. *Transfusion*. 2009;49(2):235–242.
31. Bartolmas T, Salama A. A dual antiglobulin test for the detection of weak or nonagglutinating immunoglobulin M warm autoantibodies. *Transfusion*. 2010;50(5):1131–1134.
32. Alzate MA, Manrique LF, Bolanos NI, Duarte M, Ganzalez JM. Simultaneous detection of IgG, IgM, IgA complexes and C3d attachment to erythrocytes by flow cytometry. *Int J Lab Hematol*. 2015;37(3):382–389.
33. Hannon JL. Management of blood donors and blood donations from individuals found to have a positive a direct antiglobulin test. *Transfus Med Rev*. 2012;26(2):142–152.
34. Garratty G. The significance of IgG on the red cell surface. *Transfus Med Rev*. 1987;1(1):47–57.
35. Freedman J. False positive antiglobulin tests in healthy subjects and in hospital patients. *J Clin Pathol*. 1979;32(10):1014–1018.
36. Green GA. Autologous IgM, IgA, and complement binding to sickle erythrocytes in vivo: evidence for the existence of dense sickle cell subsets. *Blood*. 1993;82(3):985–992.
37. Nelken D. False positive anti human globulin tests caused by reticulocytes. *Vox Sang*. 1961;6:348–352.
38. Rottenberg Y, Yahalom V, Shinar E, Barchana M, Adler B, Paltiel O. Blood donors with positive direct antiglobulin tests are at increased risk for cancer. *Transfusion*. 2009;49(5):838–842.
39. Heddle NM, Kelton JG, Turchynan KL, Ali MAM. Hypergammaglobulinemia can be associated with a positive direct antiglobulin test, a nonreactive eluate, and no evidence of hemolysis. *Transfusion*. 1988;28(1):29–33.
40. Moscow JA, Casper AJ, Kodis C, Fricke WA. Positive direct antiglobulin test results after intravenous immune globulin administration. *Transfusion*. 1987;27(3):248–249.
41. Win N, Islam S, Peterkin MA, Walker ID. Positive direct antiglobulin test due to antiphospholipid antibodies in normal healthy blood donors. *Vox Sang*. 1997;72(3):182–184.
42. Ghosh K, Javeri KN, Mohanty D, Parmar BD, Surati RR, Joshi SH. False-positive serological tests in acute malaria. *Br J Biomed Sci*. 2001;58(1):20–23.
43. Facer CA, Bray RS, Brown J. Direct Coombs antiglobulin reactions in Gambian children with Plasmodium falciparum malaria, I: incidence and class specificity. *Clin Exp Immunol*. 1979;35(1):119–127.
44. DeAngelis V, Biasinutto C, Pradella P, Vaccher E, Spina M, Tirelli U. Clinical significance of positive direct antiglobulin test in patienst with HIV infection. *Infection*. 1994;22(2):92–95.
45. Zantek ND, Koepsell SA, Tharp DR Jr, Cohn CS. The direct antiglobulin test: a critical step in the evaluation of hemolysis. *Am J Hematol*. 2012;87(7):707–709.
46. Snyder EL, Falast GA. Significance of the direct antiglobulin test. *Lab Med*. 1985;16(2):89–96.

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Submissions Now Accepted for the CAP17 Abstract Program

Abstract and case study submissions to the College of American Pathologists (CAP) 2017 Abstract Program are now being accepted. Submissions will be accepted until 5 p.m. Central time Friday, March 10, 2017.

Accepted submissions will appear on the *Archives of Pathology & Laboratory Medicine* Web site as a Web-only supplement to the September 2017 issue. The CAP17 meeting will be held from October 8 to 11 in National Harbor, Maryland.

For a link to the submission site and detailed program information visit the CAP17 Web site (www.cap.org/cap17) and the *Archives* Web site (www.archivesofpathology.org).