

Original Research Article

ROLE OF ANTIHUMAN IMMUNOGLOBULIN TESTS IN A TERTIARY CARE TEACHING HOSPITAL, NAVI-MUMBAI: RETROSPECTIVE ANALYSIS.

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Received : 01/02/2025
Received in revised form : 02/04/2025
Accepted : 18/04/2025

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DOI: 10.70034/ijmedph.2025.2.102

Source of Support: Nil,

Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (2); 569-572

ABSTRACT

Background: The Coombs' test or otherwise known as Antiglobulin Testing detects compatibility, alloantibodies or autoantibodies in patients. This testing allows antigen-antibody reactions with patients' RBCs and donors' sera in cases of Direct Antiglobulin Test or patients' sera and donors' RBCs in Indirect Antiglobulin Test. These testings help us in identifying blood transfusion compatibility; pregnancy-related hemolysis; drug-induced hemolysis; Hemolytic Disease of the Fetus and Newborn; Autoimmune Hemolytic Anemia. **Materials and Methods:** In this study we have analyzed data collected retrospectively for a period of 12 months i.e. from January 2023 to December 2023 at the Department of Immunohematology and Blood Transfusion, D. Y. Patil Medical College, Hospital and Research Center, Navi Mumbai. **The Aims and Objectives** - To study the indications for Antihuman Globulin Test, various methods (tube Vs gel card) and types of tests (direct Vs indirect).

Results: Findings revealed 131 cases requested for DAT, 147 cases requested for IAT, 81 cases requested for both IAT and DAT, 28 cases requested Antihuman Globulin testing as part of Post-Transfusion Reaction workup.

Conclusion: Antigen testing is a vital diagnostic tool in hemolytic anemias, particularly AIHA. However, interpreting the results requires a comprehensive evaluation of clinical data and patient history to ensure accurate diagnosis and appropriate management.

Keywords: Direct Antihuman Globulin Test (DAT), Indirect Antihuman Globulin Test (IAT), Hemolytic Disease of Fetus and New-born (HDFN), Autoimmune Hemolytic Anemia (AIHA).

INTRODUCTION

Anti-human Globulin (AHG) Test, also known as Coombs test, is a diagnostic method used for identifying autoimmune diseases based on the principle that antihuman globulins obtained from immunized nonhuman species bind to human globulins such as IgG or Complement C3d, either free in serum or attached to antigens on Red Blood Cells. This test was discovered by Coombs, Mourant,

and Race in 1945 and it was used for the detection of weak and non-agglutinating Rh antibodies in serum.^[1]

AHG is a diagnostic test routinely performed in a tertiary health care to identify the in-vivo sensitivity of the red cells by antibodies; known as Direct Antiglobulin Test (DAT) and to detect red cell antibodies present in plasma i.e. in the vitro sensitized red cells; known as Indirect Antiglobulin Test (IAT). By doing these tests we differentiate

immune causes of hemolysis from non-immune causes, drug induced/ autoimmune hemolytic anemia, compatibility testing, identifying clinically significant red cell alloantibodies etc.^[2]

AHG testing reagents can be of polyclonal origin or monoclonal origin. Polyclonal origin being sensitized to IgG and C3d and Monoclonal being sensitized to either anti- IgG or antibody or to specific components of complement (C3b or C3d).^[3]

In cases of Hemolytic diseases of the newborn (Rh Incompatible pregnancy); by simply performing an IAT Test one can identify/ diagnose the compatibility, alloantibodies or autoantibodies. Also, in cases of Transfusion Reactions by doing IAT and DAT Tests, we can identify the incompatibility or the alloantibodies causing the reactions.^[4]

These tests can be done by two different techniques conventional tube method and Gel card. amongst these, a Gel card method is considered to be more sensitive to detect minimum number of antibodies even the failure of tube method to detect.^[5] The purpose of this study was to analyze the practices conducted in our hospital for use antihuman immunoglobulin test, methods and their techniques.

Aims and Objectives: To study the indications for Antihuman immunoglobulin Test, various methods (tube Vs gel card) and types of test (direct Vs indirect).

MATERIALS AND METHODS

Study procedure: The present retrospective study was conducted in the Department of Immunohematology and blood transfusion department (IHBT) of D. Y. Patil School of Medicine, Hospital and Research Center, Navi Mumbai a tertiary care hospital. Data was collected from the departmental records from January 2023 to December 2023. The records were screened for inclusion and exclusion criteria. Data from patients file who has undergone ICT and /DCT for diagnostic purpose was collected irrespective of gender, age & indications. Incomplete report of test was excluded from study. Both indoor and outdoor patients were included. The data pertaining to demography, indication for ICT and /DCT, method and type of test was collected and entered the Microsoft excel sheet. It was analyzed by descriptive statistics using SPSS version 28.

RESULTS

1. Total number of patients undergone ICT and/ DCT – 278

As per record 278 patients were undergone AHG test, amongst them both type of AHG (DAT and IAT) was

done in 81(29.13%) and 197(70.87%) had undergone either DAT or IAT testing. Amongst the 70.87% more number of patients 104(52.79%) had undergone IAT while almost equal patients 93(47.04%) with DAT [Figure 1A and 1B]

2. Type of Antihuman immunoglobulin test

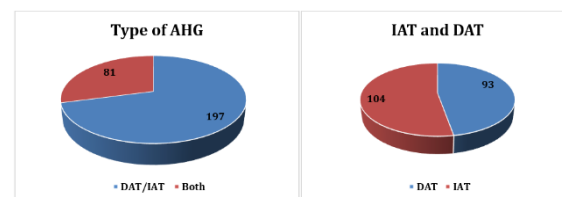


Figure 1: A and B AHG TYPES

As shown in fig 2, majority of the patients has undergone indirect AHG testing (50.35%) followed by direct AHG (33.45%) and both the types of AHG was used in (29.13%). Most common indication for AHG test was testing in Rh negative pregnancy 104(50.35%) followed by autoimmune hemolytic anemia 93(33.45%), Pancytopenia under evaluation 73(26.25%) and least common indication was hip arthrosis with RA 8(2.87%)

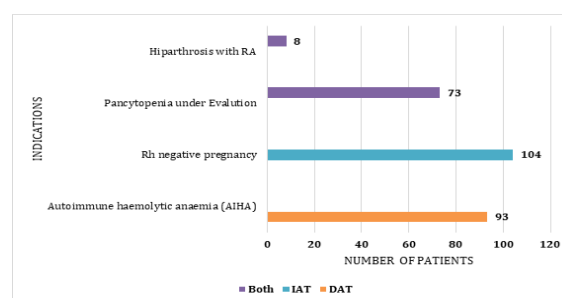


Figure 2: indications for AHG

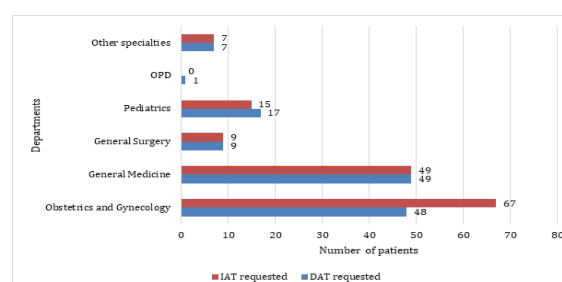


Figure 3: Department wise distribution of AHG request

As shown in [Figure 3], a greater number of patients were from OBGY department 115(41.4%) followed by general medicine 98(35.25%), pediatrics 32 (11.51%), General surgery 18(6.5%), other specialties 14(5.03%) and least from outpatient department1 (0.03%)

**Some patients have had requested both DAT and IAT together.

Table 1: Demographic Profile of Patients that reported positive for Combs' Testing

| Age Class Interval (Years) | Female | Male |
|----------------------------|--------|------|
| Newborn | 2 | 1 |
| 10 - 30 yrs | 3 | Nil |
| 31 - 40 yrs | 3 | Nil |

As shown in [Table 1], more number of patients with positive coombs test were from newborn, youth and young adults 3 (27.3%) in each group, while least in elderly 2 (18.2%).

DISCUSSION

This was a retrospective survey conducted in tertiary care teaching hospital, Navi Mumbai to the practices of AHG testing and indication for AHG testing. It is common practice to subject all the samples for Coomb's test, both Direct and Indirect and implemented by both Gel card and Conventional tube method simultaneously. As we all know, Coomb's test is a laboratory investigation done for the detection of either in-vivo (Direct Coomb's test) or in-vitro (Indirect Coomb's test) antibodies directed towards RBC's.^[2] For the detection of antibodies present on the surface of the red cells, Direct Coomb's test is the diagnostic tool used.^[6]

The Gel technique has been proven to be more efficacious and simplified technique as well as the interpretation of results along with a better reliability, reproducibility, stability and increased sensitivity.^[6] Initially introduced in Thailand, 1993, and has become popular and used worldwide in several blood banks.^[6] For Gel Card test we use specific microtubes which are being prepared using standard reagents. This method has been widely used for cross matching, the detection of antigen, alloantibody screening/identification.^[3] Since the tube method was the first technique used, some of the blood bank personnels still prefer the Conventional Tube method despite aving many drawbacks like skilled technical expertise especially in the cell washing step (which may lead to false positive results) and also the intervariability complex. On the other hand, the benefits of Gel technology have surpassed the Conventional Tube method – quick, safe, elimination of cell washing step, technician friendly, less handling of samples, lesser interpretive errors, lesser protocol errors does not require special skills for performance.^[7]

Before the antiglobulin test was developed, only IgM antibodies were detected by direct testing methods. The introduction of the AHG test permitted the detection of non-agglutinating IgG antibodies and led to the discovery and characterization of many new blood group systems.^[8]

The antiglobulin test can be used to detect RBCs sensitized with IgG alloantibodies, IgG autoantibodies, and complement components. Sensitization can occur either in vivo or in vitro. The use of AHG to detect in vitro sensitization of RBCs is a two-stage technique referred to as the Indirect Antiglobulin Test (IAT). In vivo sensitization is detected by a one-stage procedure called the Direct Antiglobulin Test (DAT). IAT and DAT remain the

most common procedures performed in blood group serology.

DAT is used to detect in-vivo sensitization of red cells where the addition of AHG directly into a suspension of washed red cells of the patient will result in visible agglutination. It thus helps in differentiating immune causes of red cell destruction from the non-immune causes.

A positive DAT results in a patient with hemolytic anemia indicates that the most likely diagnosis is one of the immune hemolytic anemias. However, positive DAT does not always indicate the presence of hemolytic anemia. Therefore, an interpretation of the positive DAT requires knowledge about the patient's diagnosis; drug history, pregnancy, history of the previous transfusion along with the other laboratory parameters indicative of hemolytic anemia (falling Hemoglobin/hematocrit, increased indirect bilirubin, increased reticulocytes, increased serum Lactate Dehydrogenase, decreased haptoglobin, peripheral smear showing the presence of agglutinates or schistocytes).

IAT is used to detect the presence of red cell antibodies in patient's plasma by sensitizing the red cells in-vitro and then detecting the in-vitro sensitized red cells using antiglobulin reagent. It helps to identify red cell alloantibodies in response to previous exposure of foreign antigens by transfusion or pregnancy.

A positive IAT result will indicate the presence of red cell allo/autoantibodies in the test serum/plasma and should prompt further investigation in the form of antibody identification. Positive agglutination / IAT during compatibility testing would indicate that the donor cells are incompatible with antibodies in the patient's plasma.

In cases of HTR or HDFN, specific antibody (or antibodies) is usually detected in the eluate that may or may not be detectable in the serum. For transfusion reactions, newly developed anti bodies that are initially detectable only in the eluate are usually detectable in the serum after about 14 to 21 days (about 3 weeks). In our study 4 cases reported positive for IAT and 3 cases reported positive for DAT.

In another study, out of 15 cases that tested positive for DAT only 12 were AIHA, 5 negative cases of DAT were AIHA and 1 case of AIHA was IAT positive. In our study, out of 15 cases that were reported 3 tested positives for IAT and 3 for DAT.^[9] Drugs rarely cause immune hemolytic anemia; the estimated incidence is 1 in 1 million people. Inadequate sample collection or handling can also lead to inaccurate results. A sample that is not properly collected, stored, or transported may lead to hemolysis or other changes in the red blood cells that could affect the test results.

CONCLUSION

DAT is the first line of investigations in patients with hemolysis, post transfusion non febrile reactions and in patients with history of previous transfusions to ensure safer transfusion practices. IAT is very useful to detect alloantibodies in the patient's serum. Post Transfusion reaction work ups showed no positivity in the Antihuman Globulin testing noted.

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