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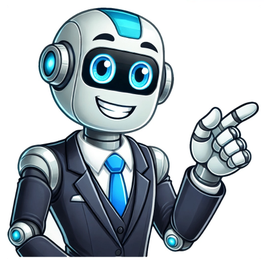

Updated by: Todd Gersten, MD, Hematology/Oncology, Florida Cancer Specialists & Research Institute, Wellington, FL. Review provided by VeriMed Healthcare Network. Also reviewed by David C. Dugdale, MD, Medical Director, Brenda Conaway, Editorial Director, and the A.D.A.M. Editorial team. Page 2Dean AJ, Lee DC. Bedside laboratory and microbiologic procedures. In: Roberts JR, Custalow CB, Thomsen TW, eds. Roberts and Hedges' Clinical Procedures in Emergency Medicine and Acute Care. 7th ed. Philadelphia, PA: Elsevier; 2019:chap 67.Feirstein J. Venipuncture. In: Dehn R, Asprey D, eds. Essential Clinical Procedures. 4th ed. Philadelphia, PA: Elsevier; 2021:chap 31.Patel K, Jones PM. Specimen collection and processing. In: Rifai N, Chiu RWK, Young I, Burnham Carey-Ann D, Wittwer CT, eds. Tietz Textbook of Laboratory Medicine. 7th ed. St Louis, MO: Elsevier; 2023:chap 4.Page 3Iancu-Rubin C, Cantor AB. Thrombocytopoiesis. In: Hoffman R, Benz EJ, Silberstein LE, et al, eds. Hematology: Basic Principles and Practice. 8th ed. Philadelphia, PA: Elsevier; 2023:chap 29.Schafer AI. Approach to the patient with bleeding and thrombosis. In: Goldman L, Schafer AI, eds. Goldman-Cecil Medicine. 26th ed. Philadelphia, PA: Elsevier; 2020:chap 162.Page 4HOW CHEMOTHERAPY IS GIVENDepending on the type of cancer and where it is found, chemotherapy medicines may be given different ways, including:Injections or shots into the musclesInjections or shots under the skinInto an arteryInto a vein (intravenous, or IV)Pills taken by mouthShots into the fluid around the spinal cord or brain When chemotherapy is given over a longer period, a thin catheter can be placed into a large vein near the heart. This is called a central line. The catheter is placed during a minor surgery.There are many types of catheters, including:A central line can stay in the body over a long period of time. It will need to be flushed on a periodic basis to prevent blood clots from forming inside the central line.Different chemotherapy medicines may be given at the same time or after each other. Radiation therapy may be received before, after, or during chemotherapy. Chemotherapy is most often given in cycles. These cycles may last 1 day, several days, or a few weeks or more. There will usually be a rest period when no chemotherapy is given between each cycle. A rest period may last for days, weeks, or months. This allows the body and blood counts to recover before the next dose.Often, chemotherapy is given at a special clinic or at the hospital. DIFFERENT TYPES OF CHEMOTHERAPYThe different types of chemotherapy include:Standard chemotherapy, which works by killing cancer cells and some normal cells.Targeted therapy, which targets a specific part of cancer cells.Immunotherapy, which uses the immune system to attack cancer cells.SIDE EFFECTS OF CHEMOTHERAPYBecause these medicines travel through the blood to the entire body, chemotherapy is described as a bodywide treatment.As a result, chemotherapy may damage or kill some normal cells. These include bone marrow cells, hair follicles, and cells in the lining of the mouth and the digestive tract.When this damage occurs, there can be side effects. Some people who receive chemotherapy:Are more likely to have infectionsBecome tired more easilyFeel pain or numbness from nerve damageHave a dry mouth, mouth sores, or swelling in the mouthHave a poor appetite or lose weightHave an upset stomach, vomiting, or diarrheaLose their hairHave problems with thinking and memory ("chemo brain") Side effects of chemotherapy depend on many things, including the type of cancer and which drugs are being used. Each person reacts differently to these drugs. Some newer chemotherapy drugs that better target cancer cells may cause fewer or different side effects.Your health care provider will explain what you can do at home to prevent or treat side effects. These measures include:Being careful with pets and other animals to avoid catching infections from themEating enough calories and protein to keep your weight upPreventing bleeding, and what to do if bleeding occursEating and drinking safelyWashing your hands often with soap and water You will need to have follow-up visits with your provider during and after chemotherapy. Blood tests and imaging tests, such as x-rays, MRI, CT, or PET scans will be done to:Monitor how well the chemotherapy is workingWatch for damage to the heart, lungs, kidneys, blood, and other parts of the body In most cases, you’ll find out the results of your test within 24 hours.What does a positive Coombs test mean?A positive (abnormal) Coombs test means that you have antibodies that can attack your red blood cells. This can be caused by several conditions, including:Autoimmune hemolytic anemia.Infectious mononucleosis.Chronic lymphocytic leukemia.Syphilis.Mycoplasma infection, a type of respiratory illness.Lupus.A negative reaction to a blood transfusion.What does a positive test before a blood transfusion mean?If you test positive before a blood transfusion, it means that your healthcare provider needs to use caution when choosing a donor. People who receive a lot of blood transfusions usually develop several different antibodies. As a result, they may have trouble finding blood that will work.What does a positive test during pregnancy mean?If you test positive during your pregnancy, it means you’ll need to take steps to protect the fetus from Rh sensitization.What does Coombs positive mean for a baby?Generally, a positive Coombs test result indicates anemia or jaundice. If the fetus tests positive, then your healthcare provider will perform an examination and recommend appropriate treatment.What does a negative Coombs test result mean?A negative (normal) test result is good news. It means that no antibodies attached to red blood cells were found. Depending on your situation, it means that you:Can safely receive blood from a donor during a transfusion.Don’t have to worry about Rh sensitization affecting the fetus.When should I call my healthcare provider?If you develop symptoms of hemolytic anemia, such as jaundice, weakness, dizziness or confusion, call your healthcare provider right away. They can perform an examination and run tests to determine the appropriate treatment.Many common warning signs can overlap with symptoms of other diseases and conditions. If you notice that something isn’t quite right, schedule an appointment with your provider. Generally clotted or anti-coagulated blood samples may use in Direct Coombs Test and can be tested up to 3 days from the date of collection. Principle of Coombs Test: Direct Coombs Test is used to demonstrate in-vivo coating (in-vivo sensitization) of red cells with antibodies or complement in particular, IgG and C3d. Washed red cells from a patient or donor are tested directly with antihuman globulin reagent (AHG). Agglutination that occurs when AHG (antihuman globulin) is added indicates that the specific antibodies are attached (in-vivo) on the red cell antigen. Direct Coombs Test Procedure: Label a test tube according to the lab number and patient’s name. Add 1-2 drops of 3 to 5% red cell suspension of patient. Add 1-2 drops of antihuman globulin reagent (AHG). Mix gently the contents of the tube. Centrifuge the tube at 2500 rpm for 20 sec. Gently re-suspend the tube. Carefully read, interpret and record the result. Interpretation: Agglutination in the test indicates the positive test result, thus the patient’s DAT (direct antiglobulin test) is positive. No agglutination in the test tube indicates the negative test result thus patient’s DAT (direct antiglobulin test) is negative. Important Note: DAT (direct antiglobulin test) is used in the identification of in-vivo sensitization of red cells. In contrast, for the identification of in-vitro sensitization of red cells, IAT (in-direct antiglobulin test) is used. Confirmation of Direct Coombs Positive Test: Introduction: With the positive result of DAT (Coombs Test), the question is, if the agglutination occurred due to AHG (antihuman globulin) reacting with sensitizing IgG antibodies or it was due to spontaneous agglutination by sensitizing IgM antibodies. To check for this phenomenon, another tube is run in parallel with DAT test tube; Procedure: Label a control tube according to the lab number and patient’s name. Add 1-2 drops of 3 to 5% red cell suspension of patient. Add 1-2 drops of 6% albumin instead of antihuman globulin reagent (AHG). Mix gently the contents of the tube. Centrifuge the tube at 2500 rpm for 20 sec. Gently re-suspend the tube. Carefully read, interpret and record the result. Interpretation: Agglutination in the control indicates the positive result thus the patient’s blood is not positive for DAT (Coombs Test) and the patient’s RBC are in-vivo sensitized No agglutination indicates the negative control result thus patient’s RBC are not sensitized in-vivo, and if DAT (Coombs Test) test tube showed agglutination, it is true positive. Important Note: Both, DAT test and control tubes are run and read in parallel. DAT results are valid only when DAT (6%albumin) control tube is negative; Confirmation of Direct Coombs Negative Test: Introduction: In the case of DAT (Coombs Test) negative test, a positive control is used to identify the validity of the DAT negative test performed. In true negative case, the added AHG (anti human globulin) antibodies are free and not consumed. When known sensitized cells (check cells) are added to the same tube at the end of the test, free AHG will react with the sensitized cells and facilitate the agglutination. Procedure: Label a test tube according to the lab number and patient’s name. Add 1-2 drops of 3 to 5% red cell suspension of patient. Add 1-2 drops of antihuman globulin reagent (AHG). Mix gently the contents of the tube. Centrifuge the tube at 2500 rpm for 20 sec. Gently re-suspend the tube. Carefully read, interpret and record the result. If agglutination does not occur, add 1 to 2 drops of 5% sensitized cells (check cells) Mix gently and centrifuge the test tube for the calibrated spin time, gently re-suspend and observe for the agglutination. Interpretation: Agglutination in the tube after addition of sensitized cells (check cells) proves that entire testing of the negative DAT (Coombs Test) was performed correctly. If the agglutination does not occur after addition of known sensitized cells, shows the negative DAT is not valid and the entire test must be repeated. Important Note: Known sensitized red cells should be added in the same test tube at the end of the test when result shows negative, since this procedure is used to validate negative test results. Reference: DiaMed AG Package Insert, 1785 Cressier s/Morat, Switzerland. Technical Manual, American Association of Blood Banks, 14th Edition, 2002. The two types of Coombs test are as follows:The direct Coombs test involves looking directly at red blood cells found in a sample of blood. The direct Coombs test is sometimes called the direct antiglobulin test (DAT).The indirect Coombs test looks at the liquid part of the blood (the plasma). The indirect Coombs test is sometimes called the indirect antiglobulin test (IAT).Both types of Coombs test are looking for antibodies which may attack red blood cells and lead to them being destroyed.The direct Coombs test may be used if doctors suspect a person is affected by a type of anaemia called haemolytic anaemia. Haemolytic anaemia is a condition where there are not enough red blood cells in the body because something in the body is destroying them. The Coombs test is done to see if it is the immune system that could be causing the red blood cells to be destroyed.The indirect Coombs test is used to make sure that blood that has been donated is compatible with the patient who is going to receive it. It is also used to check that a pregnant mother's blood does not contain antibodies that might cause her baby harm. See the separate leaflet called Blood Tests.Direct Coombs testIn a direct Coombs test a special antibody is added to a sample of blood. This test checks whether there are antibodies that have already attached themselves to the surface of the red blood cell. If the antibodies that are added bind to the antibodies on the surface of the cell the test is positive. This causes the red blood cells to clump together in the test tube. A positive test shows that part of the immune system is causing red cells to be destroyed.Indirect Coombs testThe indirect Coombs test is done on a sample of the liquid part of the blood. It looks for antibodies in the bloodstream that aren't attached to the red blood cells but could bind to certain red blood cells and destroy them.Antibody responseYour red blood cells have certain proteins on their surface, called antigens. Also, your plasma contains a special type of protein called antibodies, which will attack certain antigens if they are present.Antigens are like flags to our immune system. They usually identify a substance that is not meant to be in the body (foreign). They can be found on the surface of germs (bacteria) but they can also be found on substances which don't cause disease. For example, they can be found in pollen, blood, or transplanted organs.The presence of an antigen which is not made by your body causes the immune system to act. This is called an antibody response. This is one of the ways our body protects us from illness. It recognises bacteria and viruses by their antigens and destroys them using antibodies. However, in some conditions, known as autoimmune diseases, your own body can destroy your own red blood cells.Transfusion reactionHuman blood is grouped by the different types of antigens that are on the surface of red blood cells. If you receive a blood transfusion, the transfused blood must be the same blood group (type) as yours. It must have the same antigens as those of your red blood cells.If you receive a transfusion of blood with antigens that are different from yours (incompatible blood), your immune system destroys the transfused blood cells. This is called a transfusion reaction and can cause serious illness or even death. This is why blood group matching is so important.A Coombs test involves taking a sample of blood. The blood sample is then sent to the laboratory where the Coombs test is carried out. Antibodies are a part of your immune system. They fight germs, but sometimes they make a mistake and target your body's healthy cells instead. The Coombs test checks your blood for antibodies that attack red blood cells. You might also hear it called an antiglobulin test or red blood cell antibody screening.Not everyone's red blood cells are alike. Your immune system will make antibodies if it finds ones that don't match yours. They're keyed to specific areas on the outside of the cell. Some of these antibodies are related to your blood type.There are two types of Coombs tests. The direct test looks for antibodies that are stuck to red blood cells. The indirect test looks for antibodies floating in the liquid part of your blood, called serum.Doctors use the indirect Coombs test, also called IAT, to prevent problems.They'll check your blood before you get a transfusion to make sure it doesn't have antibodies that would react badly to the donated blood. It's part of the "type and screen" process.Pregnant women get a prenatal antibody screening with an indirect Coombs test. It checks the mother's blood to see if there are antibodies that could pass to and harm their unborn baby.A direct Coombs test, or DAT, may help explain why you're not feeling great or have symptoms that suggest trouble related to your blood.You might get sick after a blood transfusion if the donor's blood wasn't a good match. Your body may recognize those other blood cells as foreign and make antibodies to get rid of them, even though they're meant to help.A blood disease called autoimmune hemolytic anemia happens when antibodies destroy your own red blood cells faster than your body can make them. You can get it because of:Babies with yellowish skin and eyes may have hemolytic disease of the newborn (HDN). Some antibodies from their mother could be attacking their red blood cells. This happens most often when the part of the baby's blood type inherited from the father doesn't mix well with the mother's.A technician uses a needle to take a small sample of blood from a vein in your hand or arm. You may feel a small skin prick and have a little bleeding or bruising where the needle goes in. Then they'll send your blood to a lab. Both the direct and indirect tests can look for simply the presence of antibodies in general or for a specific antibody.Before a blood transfusion, each package of donated blood also needs to be tested.Cross-matching is a special kind of IAT that may be done before a blood transfusion. The lab mixes your serum (where the antibodies are) with red blood cells from the donor.A negative indirect Coombs test is good news. It usually means you don't have antibodies in your serum, so you:Can safely get blood from that donorDon't need to worry about trouble with your unborn babyA positive result before a blood transfusion is a warning that the doctor will have to be careful when choosing donor blood. People who need a lot of blood transfusions may develop a lot of different antibodies and have a harder time finding blood that will work.A positive indirect Coombs test during pregnancy means you may need to take steps to protect your baby. Not all antibodies the test finds are harmful, so depending on what the test was looking for, you may need more tests to narrow down which ones you have so your doctor will know what to do next.A positive direct Coombs test shows you have antibodies attached to your red blood cells, but it doesn't necessarily tell you which ones or why.Regardless of the result of a direct Coombs test, you may need other tests to find the right diagnosis and treatment. Coombs test is also known as antiglobulin test. The Coombs test tests for antibodies that may stick to the red blood cells and cause red blood cells to die too early. It was discovered by Coombs, Mourant and Race in 1945. Coombs reagent is antihuman globulin. It is made by injecting human globulin into animals, which produce polyclonal antibodies specific for human immunoglobulins and human complement system factors.Red cells coated with complement or IgG antibodies do not agglutinate directly when centrifuged. These cells are said to be sensitized with IgG or complement. In order for agglutination to occur an additional antibody, which reacts with the Fc portion of the IgG antibody, or with the C3b or C3d component of complement, must be added to the system. This will form a “bridge” between the antibodies or complement coating the red cells, causing agglutination.The direct Coombs test is used to detect antibodies (IgG or C3) that are stuck to the surface of red blood cells. Many diseases and drugs can cause this. These antibodies sometimes destroy red blood cells and cause anemia.This is the test that is done on the newborn’s blood sample, usually in the setting of a newborn with jaundice. The two most commonly recognized forms of antibody-mediated hemolysis in newborns are Rh incompatibility and ABO incompatibility.Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested.With clean pipette add one drop of the prepared cell suspension to a small tube.Wash three times with normal saline to remove all the traces of serum.Decant completely after the last washing.Add two drops of Anti-human serum.Mix well and centrifuge for one minute at 1500 RPM.Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.The indirect Coombs test looks for free-flowing antibodies against certain red blood cells. It is most often done to determine if you may have a reaction to a blood transfusion.This is the test that is done on the mother’s blood sample as part of her prenatal labs. Frequently referred to as the “antibody screen”, this test identifies a long list of minor antigens that could either cause problems in the newborns or cause problems in the mother if transfusion is necessary.Approximately 5% of patients have a positive IAT due to IgG antibodies, IgM antibodies, or both.Label three test tubes as T (test serum) PC (Positive control) and NC (negative control).In the tube labeled as T (Test), take 2 drops of test serum.In the test tube labeled as PC (Positive control), take 1 drop of anti D serum.In the test tube labeled as NC (Negative control), take 1 drop of normal saline.Add one drop of 5 % saline suspension of the pooled ‘O’ Rho (D) positive cells in each tube.Incubate all the three tubes for one hour at 37°C.Wash the cells three times in normal saline to remove excess serum with no free antibodies, (in the case of inadequate washings of the red cells, negative results may be obtained).Add two drops of Coombs serum (anti human serum) to each tube.Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.Resuspend the cells and examine macroscopically as well as microscopically.Negative Result:No clumping of cells (no agglutination). This means you have no antibodies to red blood cells.Positive Result:Clumping (agglutination) of the blood cells during a direct Coombs test means that you have antibodies on the red blood cells and that you may have a condition that causes the destruction of red blood cells by your immune system (hemolysis). This may be due toHemolytic anemia,Chronic lymphocytic leukemia or similar disorder,Erythroblastosis fetalis (hemolytic disease of the newborn),Infectious mononucleosis,Mycoplasmal infection,Syphilis,Systemic lupus erythematosus andTransfusion reaction, such as one due to improperly matched units of blood.Stanford School of MedicineInstitute For Transfusion MedicineJohns Hopkins Lupus CenterUniversity of AlbertaMadison CollegeHealthlineMedline PlusWeb MDLab Test OnlinePatientCignaMedscapeHealthlink BCLaboratory InfoWikipediaSimilar Posts:Normal Laboratory Values of Blood, Plasma, Serum, Urine, CSF and StoolWidal Test- Introduction, Principle, Procedure, Interpretation and LimitationRapid Plasma Reagin (RPR) Test for the diagnosis of SyphilisC-Reactive Protein (CRP) Test- Principle, Uses, Procedure and Result Interpretation The direct antiglobulin test (DAT), also known as the Coombs test, is a crucial tool in diagnosing immune-mediated red blood cell (RBC) destruction (autoimmune hemolytic anemia). This test detects the presence of antibodies or complement proteins bound to the surface of RBCs, indicating a potential autoimmune reaction or incompatibility with an external antigen. Red blood cells (RBCs) carry a net negative charge due to sialic acid residues on their surface. This negative charge creates a repulsive force, preventing them from directly sticking together. However, the presence of antibodies and complement proteins can bridge this gap and cause RBCs to clump together, a process called agglutination. There are two main types of antibodies involved in agglutination: Agglutinating antibodies: These antibodies have two antigen-binding sites, allowing them to bind to two different RBCs simultaneously. This strong binding overcomes the repulsive force and leads to visible clumping. Non-agglutinating antibodies: These antibodies typically have only one antigen-binding site and are weaker than agglutinating antibodies. While they can bind to RBCs, they cannot overcome the repulsive force and form visible clumps. These non-agglutinating antibodies are mostly of the IgG class, although some IgM antibodies can also fall into this category. Non-agglutinating antibodies are often called “sensitizing antibodies” because they mark the RBCs for destruction by the spleen or phagocytes. While they may not cause visible clumping, the bound RBCs are still vulnerable to clearance. This is why the direct antiglobulin (Coombs) test (DAT) is essential for detecting these sensitized RBCs, even if agglutination is not evident. “File:Coombs test schematic.png” by No machine-readable author provided. A. Rad~commonswiki assumed (based on copyright claims). is licensed under CC BY-SA 3.0. At the heart of the direct antiglobulin (Coombs) test (DAT) lies the unique property of RBCs – their negative surface charge. This charge acts like a built-in shield, repelling each other and preventing them from clumping together. However, when an antibody or complement protein binds to an RBC, it acts as a bridge, overcoming this repulsive force. This allows multiple RBCs coated with the same antibody or complement to clump together, a phenomenon called agglutination. Antihuman globulin (AHG) is a specially designed antibody that recognizes and binds to the Fc portion of human antibodies and complement proteins. Essentially, AHG acts like a “double agent,” recognizing the immune cloak worn by the sensitized RBCs. The direct antiglobulin (Coombs) test (DAT) protocol revolves around this interaction. The direct antiglobulin (Coombs) test (DAT) plays a crucial role in diagnosing conditions where in vivo coating of RBCs by antibodies or C3d occurs, leading to their premature destruction. Washing the cells eliminates freely circulating antibodies, allowing for the specific detection of those directly attached to the RBCs. Then, AHG is introduced. If the RBCs are coated with antibodies or complement, AHG binds to them, forming visible clumps. This agglutination signifies a positive direct antiglobulin (Coombs) test (DAT). This bound antibody-AHG complex formation reveals sensitized RBCs, providing valuable clues for the diagnosis of autoimmune hemolytic anemia, hemolytic disease of the newborn, drug-induced hemolysis, and other immune-mediated disorders. A negative direct antiglobulin (Coombs) test (DAT), on the other hand, suggests the absence of bound antibodies or complement. However, this doesn’t guarantee the absence of an immune reaction. Some antibodies, particularly weak ones, may not cause visible agglutination even though they are present. This is why the direct antiglobulin (Coombs) test (DAT) has different sensitivity levels, utilizing polyspecific AHG for broad detection and monospecific AHG for identifying specific antibody types. Patient’s EDTA-anticoagulated blood sample Normal saline (0.9% NaCl) Antihuman globulin (AHG) reagents: polyspecific, anti-IgG, and anti-C3d Glass tubes Centrifuge Coombs control red cells – IgG coated red cell (CCC) Prepare a red cell suspension by washing 5 mL patient RBCs with normal saline 3 – 4 times, discard the supernatant and resuspend RBCs to a 3-5% suspension in saline. Label four test tubes: Polyspecific, IgG, C3d, and Negative Control. Add one drop of RBC suspension to each tube. Add 2 drops of the respective AHG reagent to each tube (Polyspecific, IgG, C3d). Add 2 drops of saline to the Negative Control tube. Mix the suspension by flicking the tubes gently to thoroughly resuspend the cells. Centrifuge the tubes for 15-20 seconds at 1000-1500 RPM. Incubate Polyspecific and C3d tubes at 37°C for 5 minutes (optional). Gently resuspend cell buttons and observe for agglutination macroscopically and microscopically. Grade agglutination strength (0 to 4+) if present. In the absence of agglutination after initial centrifugation, incubate the mixture at room temperature for 5 minutes (if recommended by the manufacturer). Recentrifuge, resuspend, and re-assess for agglutination. If still negative, add Coombs control cells (CCC) and centrifuge as per manufacturer instructions. Observe and record any agglutination with CCC, confirming test validity. This image showcases the red cell agglutination test score. The distinct agglutination patterns observed in the test tubes provide definitive clues to presence of antibodies in direct antiglobulin (Coombs) test (DAT). Agglutination of red blood cells (RBCs) in the test indicates the presence of antibodies or complement proteins bound to the RBC surface in vivo. Strength of agglutination (graded 0 to 4+) can provide clues about the amount of bound antibody or complement. Specific AHG reagent used (polyspecific, anti-IgG, anti-C3d) can help narrow down the type of immune involvement. No agglutination suggests the absence of significant RBC-bound antibodies or complement. Does not completely rule out immune hemolysis, as weakly bound antibodies or complement may not be detected. Clinical correlation with patient symptoms and other laboratory findings is essential. Assessment of red cell agglutination test grading SymbolAgglutination scoreDescription4+ / Complete (C)12Macroscopically visible cell button with a clear supernatant.3+10Macroscopically visible large clumps of cell button with a clear supernatant.2+8Macroscopically visible small clumps of cell button with a clear supernatant. 1+5Just macroscopically visible fine granular clumps of the cell button and the supernatant is turbid and reddish. or weak (w)3Only microscopically visible fine granules of the cell button and the supernatant is turbid.00No agglutination seen. The supernatant is clear and reddish in color.MF (mixed field)MFA mixture of agglutinated and unagglutinated red cells seen. HComplete hemolysis of the patient sample. The supernatant is grossly red with no evidence of red cells. Quality control measure to ensure the validity of the DAT reagents and technique. Consists of RBCs pre-coated with antibodies in the laboratory. Should agglutinate when mixed with AHG reagent, demonstrating its ability to detect antibody-coated RBCs. Negative CCC reaction with AHG suggests a technical error or reagent issue for example AHG was not added or inadequate, the reagent has expired or the AHG has been neutralized by free antibodies due to insufficient washing of the red cells. Algorithm for the main causes in positive direct antiglobulin test (DAT, or direct Coombs test). “Algorithm in positive direct antiglobulin test (DAT, or direct Coombs test)” by Mikael Häggström, M.D. Author info – Reusing images- Conflicts of interest: None Mikael Häggström, M.D. is marked with CC0 1.0. Autoimmune hemolytic anemia (AIHA): Immune system attacks and destroys its own RBCs. Hemolytic disease of the newborn (HDN): Maternal antibodies attack fetal RBCs, causing hemolysis. Drug-induced immune hemolytic anemia (DIIHA): Certain drugs can trigger antibody formation against RBCs. Transfusion reactions: Incompatible blood transfusions can lead to antibody-mediated hemolysis. Paroxysmal cold hemoglobinuria (PCH): Cold-induced destruction of RBCs by antibodies. Myelofibrosis: Bone marrow disorder sometimes associated with positive direct antiglobulin (Coombs) test (DAT). Evans syndrome: Combination of AIHA and immune thrombocytopenia (low platelets). Infections: Some infections (e.g., cytomegalovirus, Epstein-Barr virus) can cause transient positive direct antiglobulin (Coombs) test (DAT). Solid tumors and lymphomas: Rarely associated with positive direct antiglobulin (Coombs) test (DAT). Allogeneic bone marrow transplantation: Immune reactions against donor RBCs can cause a positive direct antiglobulin (Coombs) test (DAT). The DAT (Direct Antiglobulin (Coombs) Test) is a laboratory test used to diagnose conditions related to the destruction of red blood cells (RBCs) by the immune system. Causes of positive direct antiglobulin (Coombs) test (DAT) has been listed in the article above. The key difference between a direct antiglobulin (Coombs) test (DAT) and an antibody test lies in what they are looking for and how they approach detection. Direct antiglobulin (Coombs) test (DAT): This test specifically focuses on red blood cells (RBCs). It checks for antibodies that are already attached to the surface of RBCs. A positive direct antiglobulin (Coombs) test (DAT) indicates that these antibodies are binding to and potentially destroying the RBCs. Antibody Test (Broader Term): This is a broader term encompassing various tests that can detect different types of antibodies in the blood, not just those targeting RBCs. It can include tests like: Indirect Antiglobulin Test (IAT): This test, also known as the indirect Coombs test, looks for free antibodies in the blood plasma that have the potential to bind to RBCs. Unlike the direct antiglobulin (Coombs) test (DAT), these antibodies aren’t attached yet. A positive IAT indicates a risk of problems during a blood transfusion or in a newborn with hemolytic disease. Infectious Disease Antibody Tests: These tests detect antibodies produced by the immune system in response to a specific infection, such as HIV or hepatitis. Autoimmune Antibody Tests: These tests identify antibodies directed against the body’s own tissues, which can be helpful in diagnosing autoimmune diseases like lupus or rheumatoid arthritis. A Coombs positive test result for a baby, also known as a positive direct antiglobulin test (DAT), indicates that antibodies are attached to the baby’s red blood cells (RBCs). This doesn’t necessarily mean there’s a serious problem, but it does require further investigation to determine the cause and potential risks. Possible Reasons for Positive Coombs Test Rh Incompatibility: This is a major concern when a Rh-negative mother carries an Rh-positive baby. The mother’s immune system might develop antibodies against the baby’s Rh-positive RBCs, which can lead to hemolytic disease of the fetus and newborn (HDFN). HDFN can cause anemia and other complications in the baby. ABO Incompatibility: This is a less concerning scenario where the mother’s blood type (A, B, or AB) is incompatible with the baby’s blood type (usually O). However, unlike Rh incompatibility, ABO incompatibility rarely causes severe problems for the baby. Maternal Antibodies: In some cases, the mother might have pre-existing antibodies from a previous pregnancy or blood transfusion that can target the baby’s RBCs. What Happens Next Further Testing: Doctors will likely perform additional tests to determine the cause of the positive direct antiglobulin (Coombs) test (DAT) and assess potential risks. This might include: Antibody Identification: This test pinpoints the specific antigens targeted by the antibodies in the mother’s blood. Evaluation of Mom’s Blood Type and Rh Status: This confirms the mother’s blood type and Rh factor. Fetal Blood Typing (if possible): This test, depending on the pregnancy stage and medical technology available, can determine the baby’s Rh factor and blood type, if possible. Amniocentesis (in some cases): This prenatal diagnostic test involves collecting a small amount of amniotic fluid to analyze fetal cells and potentially determine the baby’s Rh status. However, this is usually a later-stage option due to its invasive nature. Bilirubin Levels: Monitoring the baby’s bilirubin levels can help assess the severity of any potential breakdown of red blood cells. Potential Outcomes Close Monitoring: If the cause is ABO incompatibility or the baby shows no signs of anemia, close monitoring of the baby’s bilirubin levels and health might be sufficient. Rh Incompatibility Management: If Rh incompatibility is confirmed and the baby is at risk for HDFN, doctors can take steps to prevent complications. This might involve administering Rh immunoglobulin (RhoGam) to the mother at specific intervals during pregnancy. RhoGam helps suppress her immune response and protect the baby’s red blood cells. Treatment for Anemia: If the baby develops anemia due to RBC destruction, they might require phototherapy treatment to help break down bilirubin or even blood transfusions in severe cases. Disclaimer: This protocol is intended for informational purposes only and may need to be modified depending on the specific laboratory procedures and patient circumstances. Always consult with a qualified healthcare professional for guidance. See additional information. Direct Coomb Test (DCT) is a type of Coomb test also called the anti-globulin test. It is of two types-Direct Coomb’s test (DCT) and the Indirect Coomb’s test (ICT). DCT uses to detect sensitized red blood cells (RBCs) while ICT detects the presence of ‘incomplete’ Rh antibodies i.e. IgG antibodies. IgG antibodies are capable of sensitizing RBCs but incapable of causing agglutination of RBCs (hemagglutination). Coomb’s test was introduced by Cambridge immunologists Robin Coomb and his et. al. (Arthur Mourant and Rob Race) in 1945. Fig. Coomb Test Reagent Direct anti-human globulin test is used to detect the sensitized red blood cells in particular Immunoglobulin gamma (IgG) and Immunoglobulin meu (IgM) that is present in patient red blood cells. Following equipment and reagents are needed to proceed direct Coomb’s test. Refrigerator Tabletop centrifuge View box/Microscope Water bath Anti-human globulin (AHG) Normal saline Glass test tubes (10 × 75 mm) Test tube rack Pasteur pipette or micro pipette Marker Container for waste disposal Tissue paper Specimen-Blood-Particularly EDTA is preferred but oxalate, or clotted, citrated whole blood may be used (the specimen needs to be a fasting sample). Take 3 test tubes and label them as T, N, and P for the test sample, negative control, and positive control respectively. Add 2/2 drops of washed patient cells, washed O red cells, and sensitized red blood cells in T, N, and P test tubes respectively. Add 1/1 drop of anti-human globulin to all test tubes. Incubate at room temperature for 5 minutes. Now, mix it and centrifuge at 1000 rpm for 1 minute. Gently re-suspend the red cell bottom and examine for agglutination or clumping. Observe macroscopically. If it is doubtful in macroscopic observation, proceed microscopically. Fig. Direct Coomb Test (DCT) Observation Negative control: No agglutination Positive control: Agglutination Test: Agglutination or no agglutination Presence of agglutination or clumping: Direct Coomb Test (DCT) Positive Absence of agglutination or clumping: Direct Coomb Test (DCT) Negative Fig. Direct Coomb Test – Negative Fig. Direct Coomb Test – Positive This test is performed to detect sensitized RBCs or anti-D antibodies or other antibodies attached to the red blood cell surface within the bloodstream. This occurs in the following conditions: Erythroblastosis fetalis or hemolytic disease of the newborn (HDN): When there is an Rh-positive baby in the womb of a sensitized Rh-negative woman, the antibodies produced in the mother’s serum cross the placenta, and after entering the baby’s bloodstream, these antibodies will attach to the bloodstream, these antibodies will attach to the baby’s Rh-positive red blood cells. These coated (or sensitized) cells are clumped and removed from the circulations causing hemolytic anemia. When the baby is born, the baby’s blood (cord blood collected from the umbilical cord) is collected and tested for the Direct Coomb test. Transfusion reaction Drug-induced red cell sensitization Autoimmune hemolytic anemia Anti-Rh antibodies are of IgG type that normally do not agglutinate Rh-positive RBCs so anti-Rh antibodies are also known as incomplete antibodies as resistant to ‘complete’ IgM antibodies, which do agglutinate red cells. Anti-human globulin (AHG) is also called Coomb’s reagent The sensitivity of the test can be increased by incubation at room temperature for 5 to 10 minutes and by re-centrifugation. In a simple setup, DCT is performed in test tubes while in the advanced laboratory, it is commonly done using micro-array and gel technology. Coomb’s reagent contains antibodies against all four classes of IgG and components of complement (usually C3 and C4). Technical Manual of the American Association of Blood Banks-13th Edition, 1999 Introduction to Transfusion Medicine –Zarin Bharucha & D.D. Chouhan,1st Edition, 1990 In most cases, you’ll find out the results of your test within 24 hours.What does a positive Coombs test mean?A positive (abnormal) Coombs test means that you have antibodies that can attack your red blood cells. This can be caused by several conditions, including:Autoimmune hemolytic anemia.Infectious mononucleosis.Chronic lymphocytic leukemia.Syphilis.Mycoplasma infection, a type of respiratory illness.Lupus.A negative reaction to a blood transfusion.What does a positive test before a blood transfusion mean?If you test positive before a blood transfusion, it means that your healthcare provider needs to use caution when choosing a donor. People who receive a lot of blood transfusions usually develop several different antibodies. As a result, they may have trouble finding blood that will work.What does a positive test during pregnancy mean?If you test positive during your pregnancy, it means you’ll need to take steps to protect the fetus from Rh sensitization.What does Coombs positive mean for a baby?Generally, a positive Coombs test result indicates anemia or jaundice. If the fetus tests positive, then your healthcare provider will perform an examination and recommend appropriate treatment.What does a negative Coombs test result mean?A negative (normal) test result is good news. It means that no antibodies attached to red blood cells were found. Depending on your situation, it means that you:Can safely receive blood from a donor during a transfusion.Don’t have to worry about Rh sensitization affecting the fetus.When should I call my healthcare provider?If you develop symptoms of hemolytic anemia, such as jaundice, weakness, dizziness or confusion, call your healthcare provider right away. They can perform an examination and run tests to determine the appropriate treatment.Many common warning signs can overlap with symptoms of other diseases and conditions. If you notice that something isn’t quite right, schedule an appointment with your provider. MeSH Heading Coombs Test Tree Number(s) E01.370.225.812.735.050.375.150 E05.200.812.735.050.375.150 E05.478.594.760.050.375.150 Unique IDD003298 RDF Unique Identifier Annotationfor erythrocytes only: for lymphocytes consider HISTOCOMPATIBILITY TESTING or CYTOTOXICITY TESTS, IMMUNOLOGIC Scope NoteA test to detect non-agglutinating ANTIBODIES against ERYTHROCYTES by use of anti-antibodies (the Coombs' reagent.) The direct test is applied to freshly drawn blood to detect antibody bound to circulating red cells. The indirect test is applied to serum to detect the presence of antibodies that can bind to red blood cells. Entry Term(s) Anti-Human Globulin Consumption Test Antiglobulin Consumption Test Antiglobulin Test Antihuman Globulin Consumption Test Coombs' Test Direct Antiglobulin Test Direct Coombs Test Indirect Antiglobulin Test Indirect Coombs Test Public MeSH Note2013; see COOMBS' TEST 1966-2012 History Note2013 (1966) Date Established 2013/01/01 Date of Entry 1999/01/01 Revision Date 2012/07/03 Coombs Test Preferred Concept UIM0005148 Scope NoteA test to detect non-agglutinating ANTIBODIES against ERYTHROCYTES by use of anti-antibodies (the Coombs' reagent.) The direct test is applied to freshly drawn blood to detect antibody bound to circulating red cells. The indirect test is applied to serum to detect the presence of antibodies that can bind to red blood cells. Terms Coombs Test Preferred Term Term UI T821564 Date04/30/2012 LexicalTag EPO ThesaurusID NLM (2013) Antiglobulin Test Term UI T009654 Date03/29/1974 LexicalTag NON ThesaurusID UNK (19XX) Antiglobulin Consumption Test Term UI T009653 Date01/01/1975 LexicalTag NON ThesaurusID UNK (19XX) Antihuman Globulin Consumption Test Term UI T009656 Date10/15/1990 LexicalTag NON ThesaurusID NLM (1992) Coombs' Test Term UI T009655 Date01/01/1999 LexicalTag EPO ThesaurusID NLM (1966) Antiglobulin testing, also known as the Coombs test, is an immunology laboratory procedure used to detect the presence of antibodies against circulating red blood cells (RBCs) in the body, which induce hemolysis. The destruction of these red blood cells (RBCs) by antibodies directed against them is described diagnostically as autoimmune hemolytic anemia (AIHA). Many etiologies fall under this classification.Antiglobulin testing can be either direct antiglobulin testing (DAT) or indirect antiglobulin testing (IAT). The principle of DAT is to detect the presence of antibodies attached directly to the RBCs, which takes place by washing a collected blood sample in saline to isolate the patient’s RBCs; this procedure removes unbound antibodies that may otherwise confound the result. IAT, by contrast, is used to detect unbound antibodies to RBCs, which may be present in the patient’s serum. Direct antiglobulin testing adds a monospecific or polyspecific reagent to the washed RBCs to detect bound IgG and/or complement C3. In practice, many laboratories first use the polyspecific reagent that can detect both IgG and C3; a positive result is followed by monospecific testing to characterize the antibody further.[1] For indirect antiglobulin testing, serum from a blood sample gets isolated, and native RBCs are removed. The isolated serum sample then gets incubated with foreign RBCs of known antigenicity. Antiglobulin reagent is then added, and the presence of agglutination indicates a positive result.Collecting a blood sample for antiglobulin testing requires an anticoagulated tube with ethylenediaminetetraacetic acid (EDTA); in standard practice, this collection tube traditionally has a lavender, red, or pink top. EDTA is used to chelate serum calcium to prevent in vitro fixation of complement factor C3, which would otherwise lead to a false negative result.[1]Various modifications have been reported to improve the Coombs test, including polyethylene glycol (PEG) and the antiglobulin gel test (AGT).[2][3] Some advantages of the AGT compared to the standard Coombs test include better reproducibility and easy testing. The AGT is the most sensitive test for detecting anti-RBC antibodies in the serum. It is essential in pre-transfusion testing and the diagnosis of hemolytic disease in the newborn.[4] The AGT was released in Europe in 1988. It became available in the USA in 1995. Lapierre and collaborators developed this technology.The AGT is routinely used to screen human sera for anti-human RBC antibodies. The procedure is as follows:Use a gel microtube (it contains anti-IgG)Add 25 microliters of serum to the microtubeAdd 50 microliters of low ionic strength solution (LISS) - suspended red blood cells at a 0.8% concentration to the reaction chamber of the microtubeIncubate at 37 degrees Celcius for 15 minutesSpin for 10 minutes in a centrifuge at approximately 70 x gAfter centrifugation, the positive reaction gets graded from 0 to 4+Negative reactions have RBC pellets on the bottom of the microtube with no agglutinationOne + reaction is indicated by erythrocyte agglutination at the lower half of the gel columnTwo + reaction has erythrocytes dispersed throughout the microtubeThree + reaction contains erythrocytes displayed in the upper half of the gel columnFour + reaction is indicated by a solid band of erythrocyte on the top of the microtube chamber [5] Coombs test is necessary when autoimmunity to red blood cells is a consideration in the differential diagnosis, including warm and cold hemolytic anemia. Following are some indications where antiglobulin testing becomes useful:Autoimmune hemolytic anemiaDrug-induced immune hemolytic anemiaAlloantibodies-mediated hemolytic transfusion reactionsHemolytic disease of the newbornSystemic lupus erythematosus (without hemolytic anemia)The potential diagnosis of the Coombs test includes pre-transfusion testing, hemolytic transfusion reaction, and autoimmune or drug-induced hemolytic anemias.[6][7] There are several causes of a positive Coombs test, such as:Hemolytic transfusion reactionsAutoantibodies to intrinsic RBC antigensHemolytic disease of the newbornDrug-induced antibodiesPassively acquired alloantibodies, such as from donor plasma or immunoglobulinNonspecifically adsorbed proteinsComplement activation because of bacterial infection, alloantibodies, or autoantibodiesAntibodies produced by passenger lymphocytesReporting of antiglobulin agglutination test results can be on a qualitative or quantitative basis. For qualitative methods, the interpreter examines the test tube and assigns a score based on a graded scale:M: Mixed field – any degree of agglutination in a sea of non-agglutinated cellsW: Siny aggregates, turbid reddish background1: Small aggregates, turbid reddish background2: Small to medium-sized aggregates, clear background3: Several large aggregates, clear background4: Aggregate or clump of cellsIn patients with autoimmune hemolytic anemia, the degree of agglutination typically correlates with the severity of hemolysis. If no macroscopic agglutination appears, the sample is examined microscopically to ensure no aggregates. A sample exposed to a reagent demonstrating aggregates of at least 3 to 5 cells under microscopic examination is considered a positive result. Agglutination typically takes 5 to 10 minutes after adding the reagent. Direct antiglobulin testing may also be measured quantitatively using enzyme-linked immunosorbent assay (ELISA), flow cytometry, or other immunoassay techniques.[8][9][10][11] Quantitative sample measurement may be necessary when isolation of a specific antibody is desired, such as in cases of autoimmune hemolysis due to antibodies other than IgG or C3.When performed correctly and utilized in the appropriate clinical context, direct antiglobulin testing has been shown to demonstrate a positive predictive value of 97% to 99%, although a more recent study involving hospitalized patients reported a false positive rate of up to 7% to 8% in patients without any evidence of hemolysis clinically or histologically. The majority of these false positives show a low grade of agglutination, though up to 1% of these results may demonstrate higher grades of agglutination.[12] In another study consisting of a cohort of healthy, non-hospitalized individuals, the incidence of a positive DAT result without evidence of hemolysis was found to be 0.1%; approximately two-thirds of this cohort expressed IgG positivity.[13]Several other confounding variables can affect the accuracy of DAT and IAT test results:Type of antibody - most commercial antiglobulin testing screens for antibodies to IgG, complement C3, or both. As such, false-negative results may occur in cases of AIHA caused by autoantibodies other than IgG or C3, such as IgM or IgA.[14] In these uncommon cases, quantitative DAT may aid in detection.Amount of antibody present - in some rare instances, autoimmune anemia may be induced by antibody levels below the detection limit of DAT, which is approximately 150 to 500 molecules of IgG per red cell.[15][16] For example, the study conducted by Zupanska et al demonstrated in vitro phagocytosis of RBCs by monocytes at levels of 150 to 640 molecules of IgG3 and 1230 to 4240 molecules of IgG1 per red cell.[17][18] Thus, RBCs may undergo phagocytosis when opsonized by an antibody level below the detection threshold, resulting in a falsely negative result.[12]High serum protein - certain diseases, such as myeloproliferative diseases, may cause a falsely positive agglutination study due to abnormally high protein levels unrelated to antibody-RBC agglutination. Exogenous sources of excess protein or immunoglobulin, such as cases in which a patient receives intravenous immune globulin (IVIG), may also result in a false positive study.Infection - the serum of individuals infected with certain microorganisms may create a false positive agglutination result. Examples include human immunodeficiency virus (HIV), malaria, hepatitis C virus (HCV), and in rare cases, the hepatitis E virus (HEV).[19]Antiphospholipid syndrome - cross-reactivity between antiphospholipid antibodies and RBC membranes can result in falsely positive DAT testing, as reported by Win et al [20]Wharton jelly - in neonatal umbilical cord blood samples, mucopolysaccharide-rich Wharton jelly has been shown to produce false-positive antiglobulin results.The patient should be aware that Coombs testing is a relatively safe procedure. The risks associated with testing are the same as that of standard blood sample collection. Patients do not need to fast before testing.Antiglobulin testing, particularly qualitative direct antiglobulin testing, is clinically useful in cases where there is clinical suspicion of autoantibody-induced RBC hemolysis. DAT testing typically involves using a polyspecific reagent consisting of IgG and complement C3. Indirect antiglobulin testing is clinically useful for the detection of circulating antibodies that have the potential to induce RBC hemolysis; this test is most commonly utilized for RBC phenotyping and in crossmatch screening for blood transfusion. A positive antiglobulin result requires analysis in the clinical context to make an accurate diagnosis. Healthcare costs and the burden of laboratory time can be minimized by screening with a polyspecific reagent before confirming the antibody with monospecific or quantitative analysis. Rarely, autoimmune hemolysis may be suspected even without positive DAT testing; in this instance, quantitative DAT testing may help identify less common antibody subtypes other than IgG or C3. In the absence of other confounding variables (see “Interfering Factors” above), positive antiglobulin testing indicates the presence of hemolysis by antibodies directed against native RBCs. There are several major areas of clinical significance:Autoimmune hemolytic anemia (AIHA): AIHA is traditionally the most recognized cause of positive antiglobulin testing, and has been the topic of extensive study. The classification “AIHA” serves as an overarching descriptor that unifies a large group of diagnoses with differing etiologies that cause hemolysis using antibodies against RBCs.[21] The classification can be dichotomized further by considering factors such as warm versus cold agglutination and primary versus secondary cause. AIHA may also be drug-induced or syndromic (see “Evans syndrome”). Further characterization of diseases that fall under this classification is beyond the scope of this entry.Alloimmune-mediated hemolytic transfusion reaction (AHTR): AHTR occurs when a post-transfusion specimen develops a newly found alloantibody. The formation of an alloantibody can occur as quickly as within 2 to 3 days.[22] The development of alloantibodies results in a positive DAT test but may or may not be associated with hemolysis.[1]ABO blood group typing: In blood transfusions and hematopoietic stem cell transplants, indirect antiglobulin testing can be used to identify the RBC phenotype to minimize the chances of donor incompatibility.Hemolytic disease of the fetus and the newborn (HDFN): HDFN occurs when maternal IgG forms against fetal antigens, notably the Rh or Kell antigen. The most common type of HDFN is due to ABO incompatibility, which occurs in approximately 15% to 25% of pregnancies and tends to be less severe.[23] The incidence of positive DAT testing in ABO HDFN is very low at around 1%, and of that group, only approximately 23% of newborns develop clinically significant jaundice; hence, DAT is a poor positive predictor of newborns that require treatment.[1][23][24]Review Questions1.Parker V, Tormey CA. The Direct Antiglobulin Test: Indications, Interpretation, and Pitfalls. Arch Pathol Lab Med. 2017 Feb;141(2):305-310. [PubMed: 28134589]2.Wenz B, Apuzzo J. Polyethylene glycol improves the indirect antiglobulin test. Transfusion. 1989 Mar-Apr;29(3):218-20. [PubMed: 2922789]3.Reisner R, Butler G, Bundy K, Moore SB. Comparison of the polyethylene glycol antiglobulin test and the use of enzymes in antibody detection and identification. Transfusion. 1996 Jun;36(6):487-9. 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[PMC free article: PMC3757777] [PubMed: 24014947]23.Keir A, Agpalo M, Lieberman L, Callum J. How to use: the direct antiglobulin test in newborns. Arch Dis Child Educ Pract Ed. 2015 Aug;100(4):198-203. [PubMed: 25395493]24.Dinesh D. Review of positive direct antiglobulin tests found on cord blood sampling. J Paediatr Child Health. 2005 Sep-Oct;41(9-10):504-7. [PubMed: 16150068] Disclosure: Samuel Theis declares no relevant financial relationships with ineligible companies. Disclosure: Muhammad Hashmi declares no relevant financial relationships with ineligible companies. 25-09-2024 Team Medicover Hematology The Direct Coombs Test, also known as the Direct Antiglobulin Test (DAT), is an essential diagnostic tool in hematology and transfusion medicine. This guide aims to provide a comprehensive understanding of the Direct Coombs Test, its procedure, and its applications in diagnosing various medical conditions, particularly autoimmune hemolytic anemia and neonatal jaundice. Get a second opinion from trusted experts and makeconfident, informed decisions. Get Second Opinion What is the Direct Coombs Test? The Direct Coombs Test is a laboratory test that detects antibodies or complement proteins attached to the surface of red blood cells (RBCs). This test is instrumental in diagnosing conditions where the immune system mistakenly targets RBCs, leading to their destruction—a condition known as hemolysis. Purpose of the Direct Coombs Test The primary purposes of the Direct Coombs Test include: Diagnosing Autoimmune Hemolytic Anemia (AIHA): This condition occurs when the body's immune system attacks its own RBCs, causing hemolysis. Investigating Hemolytic Transfusion Reactions: The test helps identify antibodies formed against transfused blood cells. Assessing Neonatal Jaundice: The test is used to determine if jaundice in newborns is due to hemolytic disease of the newborn (HDN) caused by Rh or ABO incompatibility. Evaluating Drug-Induced Hemolysis: Some medications can lead to the formation of antibodies against RBCs, which the Direct Coombs Test can detect. Direct Coombs Test Procedure Sample Collection The test involves collecting a blood sample, typically from a vein in the arm, using a sterile needle and syringe. The sample is then sent to a laboratory for analysis. Laboratory Analysis Preparation of Red Blood Cells: The RBCs are separated from the plasma and washed to remove any unbound antibodies or proteins. Addition of Coombs Reagent: The Coombs reagent, which contains anti-human globulin, is added to the washed RBCs. Observation of Agglutination: The mixture is observed under a microscope for agglutination (clumping) of RBCs. Agglutination indicates a positive Direct Coombs Test, signifying the presence of antibodies or complement proteins on the RBC surface. Direct Coombs Test Results Interpretation Interpreting the results of a Direct Coombs Test requires a thorough understanding of the clinical context and the patient's medical history. Positive Direct Coombs Test A positive result indicates that antibodies or complement proteins are attached to the patient's RBCs. This finding is consistent with conditions such as: Autoimmune Hemolytic Anemia (AIHA): The immune system produces antibodies against its own RBCs. Hemolytic Disease of the Newborn (HDN): Maternal antibodies cross the placenta and attack fetal RBCs. Hemolytic Transfusion Reactions: Antibodies form against transfused blood cells. Drug-Induced Hemolysis: Certain medications can trigger the formation of antibodies against RBCs. Negative Direct Coombs Test A negative result indicates that no antibodies or complement proteins are attached to the RBCs. This finding can help rule out immune-mediated hemolysis. Applications of the Direct Coombs Test Autoimmune Hemolytic Anemia (AIHA) The Direct Coombs Test is crucial in diagnosing AIHA, a condition where the immune system attacks and destroys its own RBCs. AIHA can be classified into two types based on the temperature at which antibodies react: Warm AIHA: Antibodies react at body temperature (37°C). Cold AIHA: Antibodies react at colder temperatures (0-4°C). Hemolytic Disease of the Newborn (HDN) In cases of Rh or ABO incompatibility between the mother and fetus, maternal antibodies can cross the placenta and attack fetal RBCs, leading to HDN. The Direct Coombs Test helps diagnose this condition by detecting these antibodies on the newborn's RBCs. Drug-Induced Hemolysis Certain medications, such as penicillin and methyldopa, can induce the formation of antibodies against RBCs. The Direct Coombs Test can identify these drug-induced antibodies and aid in diagnosing hemolysis. Hemolytic Transfusion Reactions Hemolytic transfusion reactions occur when the recipient's immune system attacks transfused RBCs. The Direct Coombs Test can detect antibodies formed against transfused blood cells, helping diagnose and manage these reactions. Direct Coombs Test vs. Indirect Coombs Test It's essential to distinguish between the Direct and Indirect Coombs Tests, as they serve different purposes. Direct Coombs Test Purpose: Detects antibodies or complement proteins attached to RBCs in vivo. Applications: Diagnosing AIHA, HDN, hemolytic transfusion reactions, and drug-induced hemolysis. Indirect Coombs Test Purpose: Detects free antibodies in the serum that can bind to RBCs in vitro. Applications: Blood typing, crossmatching before transfusions, and screening for antibodies in pregnant women. Direct Coombs Test and Rh Factor The Rh factor, particularly the RhD antigen, plays a significant role in hemolytic disease of the newborn (HDN). If an Rh-negative mother carries an Rh-positive fetus, she may produce anti-Rh antibodies that can cross the placenta and attack fetal RBCs. The Direct Coombs Test can detect these antibodies on the newborn's RBCs, aiding in the diagnosis and management of HDN. Conclusion The Direct Coombs Test is a vital diagnostic tool in hematology and transfusion medicine. By detecting antibodies or complement proteins attached to RBCs, it helps diagnose various conditions, including autoimmune hemolytic anemia, hemolytic disease of the newborn, drug-induced hemolysis, and hemolytic transfusion reactions. Understanding the procedure, interpretation of results, and applications of the Direct Coombs Test is crucial for healthcare professionals in providing accurate diagnoses and appropriate patient care. Frequently Asked Questions The Direct Coombs Test, also known as the Direct Antiglobulin Test, is used to detect antibodies or complement proteins that are bound to the surface of red blood cells. It is primarily used to diagnose conditions where red blood cells are being destroyed by antibodies, such as autoimmune hemolytic anemia, hemolytic disease of the newborn, or transfusion reactions. A blood sample from the patient is mixed with Coombs reagent (antihuman globulin). If antibodies are bound to the surface of the red blood cells, agglutination (clumping) will occur. A positive result indicates that antibodies or complement proteins are bound to the red blood cells, suggesting an immune-mediated destruction of red blood cells. A negative result means that no antibodies or complement proteins are bound to the red blood cells, indicating that the destruction of red blood cells is not due to immune causes.