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Standard Operating Procedures and Regulatory Guidelines

Blood Banking



GP Saluja • GL Singal

2nd Edition

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JAYPEE

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CHAPTER 33

Direct Coombs Test/Direct Antiglobulin Test

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SCOPE AND APPLICATION

Antihuman globulin (AHG) serum recognizes immunoglobulin G (IgG) and/or C3d detects the presence of immune antibody (IgG) or the complement component (generally C3d) coated onto red cells. In the direct antiglobulin test, such sensitization of red blood cell (RBC) is tested when washed RBCs are incubated with AHG. Polyspecific AHG is most sensitive for detecting RBC sensitization as this is directed against both human IgG and complement. Monospecific antisera are used to detect only IgG or C3. When a direct antiglobulin test (DAT) is requested, an initial test using polyspecific AHG is performed. If this is positive, it is repeated using both monospecific reagents (anti-IgG and anti-C3) as a "reflex" test. The presence of human serum in the cell suspension will neutralize the AHG, leading to false-negative reactions. Therefore, RBCs must be washed repeatedly with saline to remove all serum before the addition of AHG. Verification of proper and complete washing is done by the addition of IgG-coated Coombs control cells ("check cells") to all negative AHG reactions.

RESPONSIBILITY

A laboratory technician in red cell serology laboratory is responsible for performing and documenting Coombs test result.

MATERIALS REQUIRED

- *Equipment:*
  - Tabletop centrifuge
  - Incubator
  - Microscope

- *Sample*:
  - Clotted blood sample of the patient
  - Ethylenediaminetetraacetic acid (EDTA)/citrate-phosphate-dextrose solution with adenine (CPDA) blood sample of the patient
- *Reagent*:
  - Low-ionic-strength saline (LISS) solution
  - Control cells (IgG-coated cells)
  - 2% suspension of reagent O cells
  - AHG reagent (Coombs serum)
- *Glassware*:
  - Glass test tubes
  - Glass slide

PROCEDURE

1. Set the table and label the test tubes. Prepare record books.
2. Add two drops of AHG reagent (Coombs serum) to a small tube after labeling it.
3. Check the identity of the specimen.
4. Make a 2% suspension of the red cells of the specimen in LISS after washing the cells three times.
5. Add one drop of the red cells to be tested to it. Mix by shaking.
6. Spin at 1,000 rpm for 1 minute.
7. Observe for agglutination with the naked eye. If no agglutination is seen, read the contents under a microscope (low power).
8. If the test is negative, add one drop of control cells (IgG-coated cells).
9. Mix and spin at 1,000 rpm for 1 minute and observe for agglutination.
10. Observe for agglutination with the naked eye. If no agglutination is seen, the result is invalid. Repeat the test.

Repeat the test with monospecific reagents as when DAT is positive with polyspecific AHG, for all but cord blood workups.

Method using Monospecific Anti-IgG and Anti-C3

1. Label test tubes appropriately with patient ID and "IgG" and "C3."
2. Add one drop of the RBC suspension saline washed four times to each tube.
3. Add one or two drops of anti-IgG to the tube marked "IgG" and one or two drops of anti-C3 to the tube marked "C3" according to the manufacturer's instructions.

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4. Mix and centrifuge at the appropriate speed and time according to manufacturer's instructions.
5. Observe the tubes for agglutination. If a reaction is equivocal or mixed field macroscopically, examine it under a microscope.
6. Record the results.
7. Add one drop of check cells as appropriate to any tube(s) with a negative reaction. Centrifuge and examine for agglutination

INTERPRETATION

- No agglutination means *negative direct Coombs test* (*DCT*)
- Presence of agglutination means *positive DCT*

DOCUMENTATION

Enter the results in investigation register.

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Standard Operating Procedures
and Regulatory Guidelines
Blood Banking

Salient Features

- All SOPs updated and revised as per latest NACO guidelines and DGHS manual
- Incorporated amendments up to December 31, 2022 (2014 in the Drugs and Cosmetics Act 1940 and Rules 1945)
- Automation in immunohematology
- Automation in testing of transfusion transmitted infections
- Latest judicial verdicts on blood transfusion incorporated
- Alternative method of sterility testing of blood units
- Amended formats for documentation included.

GP Saluja after doing MBBS from Medical College, Rohtak, Haryana, joined Haryana State Health Department as a Medical Officer in 1975. He completed his postgraduation (MD) in Pathology from Dayanand Maharishi University, Rohtak, followed by fellowship of 6 months in "Blood Bank Technology" from Northern Ireland Blood Transfusion Services (NIBTS), Belfast, UK, under "TCTP Colombo Plan" from March to September 1991; WHO fellowship in Laboratory methods for HIV and STD Diseases in Community Health Surveillance and Laboratories Administration, Baltimore from 6th April, 1992 to 1st May, 1992; and JICA fellowship in "Country Focused Program on HIV/AIDS" in Osaka, Japan in October, 2000. He is a life member of the Indian Society of Blood Transfusion and Immunohaematology (ISBTI) and also Chairman of ISBTI State Chapter Haryana.

He is a nominated expert by the Government of India for the joint inspection for grant/renewal of license for the blood centers. He retired from the post of Civil Surgeon, Panchkula, Haryana, in 2007, after serving in various capacities in Health Department. He is life member of ISBTI.

Thereafter, he joined as Senior Consultant, Blood Centre, Alchemist Hospitals Ltd., Panchkula, Haryana and is presently working in the same capacity. He has established the state of art Blood Centre facilities in Alchemist Hospitals Ltd., Panchkula, Alchemist Institute of Medical Sciences, Gurugram; Gold Field Medical College, Faridabad; and M/s Malhotra Super Specialty Hospital (a unit of Malhotra Clinics Pvt Ltd) Blood Bank, Solan, Himachal Pradesh. Besides, he is also providing consultation services for establishment of the blood centers.

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