

REF. ANT-HG-1010 (10 ml)

Polyspecific for Direct and Indirect Antiglobulin Tests
(Blended Rabbit Anti-Human IgG / Murine Monoclonal Anti-Human C3d)

PRINCIPLE:

Polyspecific Anti-Human Globulin reacts with human immunoglobulins and / or complement attached to the red cell surface, resulting in agglutination of adjacent sensitized cells. During the antiglobulin test the red cells are washed thoroughly to remove globulins not bound to the red cell. Washing prevents neutralization of the Polyspecific Anti-Human Globulin by unbound protein. The addition of Polyspecific Anti-Human Globulin (AHG) to washed cells causes agglutination if the cells are sensitized with immunoglobulin or complement. There will be no agglutination if the cells are unsensitized.

SUMMARY:

The Polyspecific Anti-Human Globulin has been prepared from a blend of rabbit anti-IgG and monoclonal anti-C3d diluted in A buffered solution containing bovine albumin. Sodium Azide (0.1%) is used as a preservative.

SAMPLES:

Direct Antiglobulin Testing (D.A.T.): Samples should be drawn into EDTA and tested within 24 hours. EDTA prevents complement binding invitro. Ideally unclotted samples are preferable. If only clotted samples are available they should not be refrigerated prior to testing. Positive test results obtained from samples that have been refrigerated should be interpreted with caution. Indirect Antiglobulin Testing (I.A.T.): Fresh serum from fully clotted samples should be used.

The use of plasma is not recommended since anticoagulants and fibrin clots may interfere with the detection of complement binding antibodies. Sera should be tested as soon as possible to minimize the occurrence of false results due to contamination or improper storage. Sera that cannot be tested immediately should be stored at 2°C – 8°C for no more than 48 hours. Serum should be separated from red cells prior to storage.

TEST PROCEDURES:

Anti-Human Globulin has been standardized for use in tube

TECHNIQUE – DIRECT ANTIGLOBULIN TEST – D.A.T.

This test is used as an indicator of in vivo sensitization of cells. The washed red cells of a patient or blood donor are tested directly with A.H.G.

1. Add one volume of a 2-3% suspension of red cells to an appropriately labeled tube.
2. Wash the red cells at least 4 times using isotonic buffered saline (pH 6.9) removing all saline after each wash.
3. Add two volumes of AHG to the dry cell button.
4. Mix the contents thoroughly.
5. Centrifuge for 15 – 20 seconds at 1000g
6. Record the results.
7. Confirm validity of all negative or weak positive reactions with IgG Sensitized red cells.

TECHNIQUE – INDIRECT ANTIGLOBULIN TEST – I.A.T.

The I.A.T. is used in compatibility testing, antibody detection, antibody identification, the DU test or other tests for antigen detection. The test is used to indicate in vitro red cell sensitization. Routine testing should include a 37°C incubation phase followed by the antiglobulin phase. An autologous control which consists of cells and serum from the specimen being tested, should be tested in parallel when doing compatibility tests, Antibody detection or identification tests. Red blood cells which have a positive D.A.T. results should not be used in tests for antigen detection by the I.A.T. method.

N.I.S.S. Tube Test

1. Dispense into appropriately labeled tubes two to four volumes of testserum and one volume of 2 – 3 % red cell suspension. If bovine albumin or other potentiators are to be used, add and use in accordance with the manufacturer's directions.
2. Mix thoroughly.
3. Incubate at 37°C for 45 – 60 minutes. The incubation period may be shortened depending on the potentiating agent employed – check manufacturer's package insert for this information.
4. Wash the red cells at least 4 times using isotonic buffered saline (pH 6.9) removing all saline after each wash.
5. Add two volumes of Egy chem Anti-Human Globulin to the dry cell button.
6. Mix thoroughly and centrifuge at 1000g for 15 – 20 seconds.
7. Resuspend the cell button and examine for agglutination either with an optical aid or microscopically.
8. Record the results.
9. Confirm validity of all negative or weak positive reactions with IgG sensitized red cells.

L.I.S.S. Tube Test

1. Wash the test cells and the positive and negative control cells twice in isotonic buffered saline (pH6.9).
2. Wash cells once in L.I.S.S.
3. Resuspend washed cells to a 1.5% cell suspension.
4. Place in a prewarmed glass test tube 2 volumes of the test serum and 2 volumes of the 1.5% cell suspension.
5. Mix well and incubate at 37°C for 15 – 20 minutes.
6. Wash the red cells at least 4 times using isotonic buffered saline (pH6.9) removing all saline after each wash.
7. Add two volumes of Egy chem Anti-Human Globulin to the dry cell button.
8. Mix thoroughly and centrifuge at 1000g for 15 – 20 seconds.
9. Record the results.
10. Confirm validity of all negative or weak positive reactions with IgG sensitized red cells.

INTERPRETATION OF RESULTS

Positive Result - Agglutination of red blood cells in the presence of AHG indicates the presence of human IgG or components of complement on the red blood cells.

Negative Result – A negative direct antiglobulin test does not necessarily exclude HDN, especially if ABO incompatibility is suspected. □ Haemolysis is not expected after the addition of Polyspecific AHG.

STABILITY OF REACTIONS

All results should be read immediately and the results interpreted and recorded without delay.

Delays in reading or in the completion of washing steps, where appropriate, may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.

QUALITY CONTROL

To confirm the specificity and reactivity of the NS BIOTEC

Polyspecific AHG it is recommended that this reagent be tested each day with at least IgG sensitized red cells (Coombs Control Cells) and unsensitized cells. The reagent can only be considered satisfactory for use if it reacts suitably only with the sensitized cells. Anti-complement activity may be demonstrated by testing cells prepared by the two stage or low ionic strength sucrose techniques

LIMITATIONS

False results may occur due to improper technique or contaminated test materials as well as inadequate incubation time or temperature, improper or excessive centrifugation, inadequate washing of the red cells and the introduction of human serum or gamma globulins.

STABILITY DATA

Kit components are stable to expiry if stored at 2 C° to 8 C°.
Do not freeze or expose to elevated temperatures.

NOTE

False negative indirect antiglobulin reactions have resulted from the use, for cell washing, of isotonic saline preparations sterilised in plastic bags designed primarily for irrigation and intravenous use. This problem has also been observed when solutions intended for cell counting and sizing have been used instead of saline in the antiglobulin test.

Marked turbidity may indicate reagent deterioration or contamination.

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