

Direct and Indirect Antiglobulin Testing**Product-Identification : 50549****INTRODUCTION**

The most important function of the anti-human globulin (AHG) reagent is to detect the presence of IgG antibodies. The importance of anticomplement in the AHG reagent is debatable since clinically significant antibodies detectable only by their ability to bind complement are rare. Some workers therefore prefer to use anti-IgG AHG serum because there is no interference from complement components which may be non-specifically bound to the red cell.

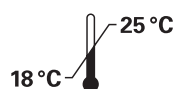
For the indirect antiglobulin test (IAT) with the ID-Card "Coombs Anti-IgG", labor-intensive washing procedures are eliminated, due to the fact that the red cell suspension is added first to the microtube, creating a barrier over the gel suspension, thus avoiding neutralisation of the AHG by immunoglobulins in the patient's plasma/serum.

The anti-human globulin IgG used in the ID-Card "Coombs Anti-IgG" is not heavy chain specific and thus may also be capable of reacting with the Kappa (κ) and Lambda (λ) light chains of IgA and IgM molecules.

The ID-Card "Coombs Anti-IgG" allows the testing of 6 samples, for direct and indirect antiglobulin test (DAT/ IAT), antibody screening, antibody identification and crossmatch.

REAGENTS

ID-Card "Coombs Anti-IgG" with 6 microtubes containing anti-human globulin anti-IgG (rabbit), within the gel matrix.
Preservative: < 0.1% NaN₃



Do not store near any heat, air conditioning sources or ventilation outlets.

Stability: see expiry date on label.

ADDITIONAL REAGENTS REQUIRED

- ID-Diluent 2: modified LISS for red cell suspensions.
- ID-DiaCell I-II, I-II-III; ID-DiaScreen; ID-DiaPanel.

(see related package insert)

FURTHER MATERIALS REQUIRED

- ID-Dispenser
- ID-Pipetor
- ID-Tips (pipetor tips)
- Suspension Tubes
- ID-Working table
- ID-Incubator 37 °C
- ID-Centrifuge 6, 12 or 24

SAMPLE MATERIAL

For optimal results, the determination should be performed using a freshly drawn sample, or in accordance with local laboratory procedures for sample acceptance criteria. Preferably, blood samples should be drawn into citrate, EDTA or CPD-A anticoagulant. Samples drawn into plain tubes (no anticoagulant) may also be used.

When the use of serum instead of plasma is required, the serum must be well cleared, by centrifugation at 1500 g for 10 minutes, before use avoid fibrin residues, which may interfere with the reaction pattern (see Limitations).

PREPARATION OF BLOOD SAMPLE**a) Red cells**

For crossmatch, autocontrol and direct antiglobulin test prepare a 0.8% red cell suspension in ID-Diluent 2 as follows:

Allow the diluent to reach room temperature before use.

1. Dispense 1.0 ml of ID-Diluent 2 into a clean tube.
2. Pipette 10 μ l of packed red cells; mix gently. Where whole blood directly from a segment of the blood bag is used, add 20 μ l of blood to the 1.0 ml of ID-Diluent 2.

The cell suspension may be used immediately.

b) Plasma or serum for indirect antiglobulin test (IAT)

Where samples are not for immediate testing they should be stored at 2–8 °C after separation (see also under "Sample material") for a maximum of 48 hours, thereafter at -20 °C or in accordance with local/national policies/guidelines.

TEST PROCEDURE**A) Direct antiglobulin test (DAT)**

Do not use ID-Cards which show signs of drying, have bubbles, damaged seals, drops of gel or supernatant in the upper part of the microtubes or on the underside of the aluminium foil.

Allow the test cell reagent to reach room temperature before use.

1. Identify the ID-Card with the unique patient or donor number/details as appropriate.
2. Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
3. Pipette 50 μ l of the red cell suspension to the appropriate microtube.
4. Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
5. Read and record the results.

B) Antibody Screening (IAT)

Using the ready-to-use test cell reagents "ID-DiaCell I-II", "ID-DiaCell I-II-III" or "ID-DiaScreen I-II-III-IV".

Allow reagents and plasma or serum samples to reach room temperature before use.

1. Identify the ID-Card with the unique patient or donor number/details as appropriate.
2. Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
3. Pipette 50 μ l of each test cell reagent to the appropriate microtubes.
4. If an autocontrol is to be included, pipette 50 μ l of the patient's own red cell suspension to the appropriate microtube.
5. Add 25 μ l of the patient's plasma or serum to each microtube.
6. Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.
7. Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
8. Read and record the results.

C) Antibody Identification (IAT)

Using the ready-to-use test cell reagent "ID-DiaPanel".

1. Identify 2 ID-Cards with the unique patient or donor number/details as appropriate.
2. Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
3. Pipette 50 µl of each "ID-DiaPanel" test cell to the appropriate microtubes (1–11).
4. Add 50 µl of the patient's own red cell suspension to the microtube number 12 (autocontrol).
5. Add 25 µl of the patient's serum or plasma to all 12 microtubes.
6. Incubate the ID-Cards for 15 minutes at 37 °C in the ID-Incubator.
7. Centrifuge the ID-Cards for 10 minutes in the ID-Centrifuge.
8. Read and record the results.

D) Crossmatch (IAT)

1. Identify the ID-Card with the unique patient or donor number/details as appropriate.
2. Remove the aluminium foil from as many microtubes as required by holding the ID-Card in the upright position.
3. Pipette 50 µl of the donor's red cell suspensions to the appropriate microtubes.
4. If an autocontrol is to be included, add 50 µl of the patient's own red cell suspension to the appropriate microtube.
5. Add 25 µl of the patient's plasma or serum to the microtubes.
6. Incubate the ID-Card for 15 minutes at 37 °C in the ID-Incubator.
7. Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge.
8. Read and record the results.

INTERPRETATION OF RESULTS**A) Principle**

Positive: Agglutinated cells forming a red line on the surface of the gel or agglutinates dispersed in the gel.

Negative: Compact button of cells on the bottom of the microtube.

B) Reaction for:**I. Direct antiglobulin test: (DAT)**

- A positive reaction ($\pm$ to +++) indicates that the patient's red cells are IgG sensitised.
- A negative reaction indicates absence of detectable IgG antibodies on the red cells.

II. Antibody Screening

- A negative reaction indicates the absence of detectable irregular antibodies in the patient's serum/plasma.
- A positive reaction indicates the presence of irregular antibodies. Enter the reactions observed on the antigen table. Verify that the lot number of the test cell reagents "ID-DiaCell I-II", "ID-DiaCell I-II-III" or "ID-DiaScreen I-II-III-IV" corresponds to the lot number indicated on the antigen table. Following the reaction pattern and the antigen configuration, the type of antibody present may be indicated. Perform the usual further tests to identify the antibody.
- A positive reaction with some screening cells and a negative autocontrol indicate presence of a specific antibody.
- A positive reaction with all screening cells and a positive autocontrol are probably due to autoantibody or non-specific reactions.
- Where there is a positive reaction with all screening cells and a positive autocontrol but with one or several screening cells showing a stronger positive reaction than the autocontrol, it should be assumed that, besides the nonspecific reaction, a specific antibody may be present and the patients sample should be submitted to further testing.

III. Antibody Identification

- A positive reaction indicates the presence of irregular antibodies. Enter the reactions observed on the antigen table. Verify that the lot number of the test cell reagents "ID-DiaPanel" corresponds to the lot number indicated on the antigen table. Following the reaction pattern and the antigen configuration, the specificity of the antibody present can, in most cases, be identified (autocontrol must be negative).
- Positive reactions with all "ID-DiaPanel" test cells and a negative autocontrol are probably due to nonspecific reactions or may indicate the presence of an alloantibody directed against a high frequency antigen.
- If the autocontrol is also positive, this may be due to unbound autoantibodies or non-specific reaction.

IV. Crossmatch

- A negative reaction indicates compatibility of the donor blood with the recipient.
- A positive reaction indicates incompatibility of the donor blood with the recipient. ABO and RhD blood groups of both recipient and donor must be verified and antibody screening/identification be performed. For complete ABO and RhD blood grouping procedure see related instruction sheets.

LIMITATIONS

- a) ID-Cards which show air bubbles in the gel or drops in the upper part of the microtubes and/or the seal, must be centrifuged before use.
- b) Certain drugs are known to cause positive reactions in anti-human globulin procedures.
- c) Some pathological conditions are also reported as causing positive reactions in anti-human globulin procedures.
- d) Bacterial or other contamination of materials used can cause false positive or false negative results.
- e) Fibrin residues in the red cell suspension may trap non-agglutinated cells presenting a fine pink line on top of the gel while most of the cells are on the bottom of the microtube after centrifugation.
- f) Strict adherence to the procedures and recommended equipment is essential. The equipment should be checked regularly according to GLP procedures.
- g) Use of suspension solutions for red cells other than ID-Diluent 2 may modify the reactions.
- h) Too heavy or too weak red cell suspensions can cause aberrant reactions.

BIBLIOGRAPHY

1. Coombs, R.R.A., Mourant, A.E. and Race, R.R.: Lancet 1945; 2: 15.
2. Coombs, R.R.A., Mourant, A.E. and Race, R.R.: Brit. J. Exp. Path 1945; 26: 255.
3. Lapiere, Y., Rigal, D., Adam, J. et al.: The gel test; A new way to detect red cell antigen-antibody reactions. Transfusion 1990; 30: 109–113.
4. Technical Manual; R.H. Walker (ed.); American Association of Blood Banks; 12th ed. 1996.

PRODUCTS

ID-Card "Coombs Anti-IgG"

4 x 12 REF 004044
112 x 12 REF 004045

These products are guaranteed to perform as described on the label and in the instruction sheet. The manufacturer declines all responsibility arising out of the use or sale of these products in any way or for any purpose other than those described therein.

Changes to the version 08.12 are shaded grey.