ODAK Blood Grouping Gel Systems

OBG Comp Test

REF No: OBGCT-12, OBGCT-48

INTENDED USE
This test allows the detection of ABO, Rh incompatibility and unexpected blood group antibodies in patient’s serum against antigens on donor cells or in donor’s serum against antigens on patient’s cells in gel technique.

INTRODUCTION
The compatibility test includes an ABO and Rh grouping performed on the donor and recipient samples, screening of the donor’s and patient’s sera for unexpected antibodies. The cross-match became part of a series of pre-transfusion test known as compatibility testing. The crossmatch procedure determines whether donor blood is compatible (or incompatible) with recipient blood. The major cross-match is used to detect unexpected blood group antibodies in patient’s serum against antigens on donor cells. The minor cross-match is used to detect unexpected blood group antibodies in donor’s serum against antigens on patient’s cells.

PRINCIPLE OF TEST
OBG Comp Test allows the detection of ABO, Rh incompatibility and unexpected blood group antibodies in patient’s serum against antigens on donor cells on a single gel card.

The test contains 8 microtubes gel matrix. First three microtubes contain monoclonal antibodies for ABO Rh, and the others AHG (2X), Neutral gel (3x).

The red blood cells will agglutinate in the presence of antibody directed towards the antigen. Agglutinated cells forming a red button on the surface of the gel or agglutinates dispersed in the gel is a positive result and indicates the presence of the corresponding antibody.

KIT CONTENTS
12 or 48 OBG Comp Test gel cards. 1 gel card contains 8x1 microtubes

A B D Ct Enz Major Enz Minor AHG AHG Ct

Ready to use. Contains Sodium azide as preservative. Each microtube of the card contains buffered gel medium with preservative and mixed different reagents.

- microtube A : monoclonal anti-A IgM (clone A-11H5 )
- microtube B: monoclonal anti-B IgM (clone B-6F9 )
- microtube D: mixture of IgG and IgM antibodies (clone BS 225, LDMM3, ES01 )
- microtube Ct: Neutral gel for Major Crossmatch
- microtube Enz Major: Neutral gel for Major Crossmatch
- microtube Enz Minor: Neutral gel for Minor Crossmatch
- microtube AHG Ct: AHG control
- microtube Ct: Neutral gel without antibodies (control microtube)

MATERIAL REQUIRED BUT NOT PROVIDED
- 10 µL, 50 µL and 1 ml automatic pipettes.
- Disposable pipette tips.
- Test tubes.
- OBG LSS Diluent
- Centrifuge for Blood Grouping Gel System.
- Papain or Bromelin solution.
- Incubator

STORAGE CONDITIONS AND STABILITY
- Store the gel cards at 2-8 °C in upright position.
- Do not freeze.
- Avoid exposure of the cards to any heat source, direct air-conditioning sources or direct sunlight.
- Do not use reagents over the expiration date.
- All the components of the kit are stable until the expiration date on the label when stored 2-8 °C.
- Do not remove foil seal until ready to use. Once opened, the gel may begin to dry out which could affect test results.
- Use the card 2 hours after removing the aluminum foil. Otherwise, don’t use the card.
- Stability of the opened gel card is 2 hours.

PRECAUTIONS AND WARNINGS
- This reagent kit is for in vitro diagnosis only.
- This reagent kit is professional use only.
- All human sourced material (monoclonal antibodies) are tested free from HBsAg, Anti-HCV and Anti-HIV. However, all materials of human origin should be considered as potentially infectious and it is recommended that all kit materials and test specimens to be handled using established good laboratory practice.

- All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory’s safety procedures.

SAMPLE COLLECTION
Fresh red blood cells are preferred for testing. Use the red blood cells collected with anticoagulants for determination of the antigens of the ABO/Rh system. If necessary, samples stored at 2-8 °C can be used up to 48 hours.

Red blood cells collected in ACD, CDP, SAGM and PAGGSM can be used also until the expiry date indicated on the label of the bag at 2-8 °C. Plasma and serum must be clear. Fibrin residues may interfere with the reaction pattern.

PREPARATION OF BLOOD SAMPLE
0.8% red blood cell suspension
Do not use haemolysed, cloudy or contaminated samples or those containing clots.

Collect known A1 and B cells. Wash the cells twice with 0.9% saline. Discard the supernatant and prepare 0.8% cell suspension:
1. Bring OBG LSS Diluent to reach room temperature before use
2. Disperse 1 ml of OBG LSS Diluent into a clean tube.
3. Add 20 µL of whole blood or 10 µL of packed cells to the diluent and mix gently.

Plasma or Serum:
If necessary, samples stored at 2-8 °C can be used up to 48 hours after their extraction; or frozen samples (from -20 °C to -70 °C) up to the time of performing the test.

TEST PROCEDURE
Allow the test card and cell reagent to reach room temperature before use.

A. Confirmation of the ABO/Rh groups and Crossmatch
1. Label the appropriate gel card with patient’s name or identification number. Remove the aluminum foil carefully.
2. Pipette 50 µl of the patient’s red cell suspension to microtubes 1,2,3,5,7 and 8 (A,B,Enz Cl,AHG, ct,Ctl).
3. Pipette 50 µl of the donor’s red cell suspension to microtubes 4 and 6 (Enz,AHG) (Crossmatch test).
4. Add 25 µl of the patient’s plasma or serum to microtubes 4 and 6 (Enz Major,AHG).
5. Add 25 µl of the donor’s plasma or serum to microtube 5 (Enz Minor ) .
6. Add 25 µl of papain or bromelin solution to microtube 4 and 5 (Enz Major,Enz Minor ).
7. Incubate the gel card for 15 minutes at 37° C in the incubator.
8. Centrifuge the gel cards for 10 minutes in the gel card centrifuge.
9. Read the results.

B. ABO/Rh Compatibility tests and Crossmatch
1. Label the appropriate gel card with patient’s name or identification number. Remove the aluminum foil carefully.
2. Pipette 50 µl of the patient’s red cell suspension to microtubes 1,2,3,5,7 and 8 (A,B,Enz Cl,AHG, ct,Ctl).
3. Pipette 50 µl of the donor’s red cell suspension to microtubes 1,2,3,4 and 6 (A,B,D,Enz,AHG) (Crossmatch test).
4. Add 25 µl of the patient’s plasma or serum to microtubes 4 and 6 (Enz Major,AHG).
5. Add 25 µl of the donor’s plasma or serum to microtube 5 (Enz Minor ).
6. Add 25 µl of papain or bromelin solution to microtube 4 and 5 (Enz Major,Enz Minor ).
7. Incubate the gel card for 15 minutes at 37° C in the incubator.
8. Centrifuge the gel cards for 10 minutes in the gel card centrifuge.
9. Read the results.

Performance Characteristics
Diagnostic sensitivity and specificity of ABO/Rh system:

The diagnostic sensitivity and specificity of the antibodies present in OBG Comp Test for the determination of the antigens of the ABO and Rh systems have been studied in a representative number of positive and negative samples. In the study using 3092 blood samples the sensitivity and specificity of ODAK Blood Grouping Gel System was as follows.
Stability of the results: it is recommended an immediate reading of the results after centrifuging the cards. Do not leave processed cards in horizontal position. If necessary, a delayed reading can be made up until 24 hours after processing the cards if kept in vertical position, refrigerated (2-8°C) and sealed with paraffin or similar material to avoid evaporation of the supernatant.

Interpretation of the results:

A.Confirmation of the ABO/Rh groups

<table>
<thead>
<tr>
<th>Anti A</th>
<th>Anti B</th>
<th>Anti AB</th>
<th>Ctl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>+1 to +4</td>
<td>Negative</td>
<td>+1 to +4</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>+1 to +4</td>
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<td>+1 to +4</td>
<td>Negative</td>
</tr>
</tbody>
</table>

The microtube Ctl must be negative. If it is positive, invalidate the test. The reactions between +1 and +3 may indicate A or B subgroups and further investigations should be performed.

Rh System

<table>
<thead>
<tr>
<th>D</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>+1 to +4</td>
<td>Rhd Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Rhd Negative</td>
</tr>
</tbody>
</table>

The reactions weaker than +2 should be subject to further investigations to distinguish between weak and partial D types as appropriate for the category of sample being tested.

B. ABO/Rh Compatibility tests

ABO/D compatibility: If there is a single band, either in the upper zone or at the bottom of the column, indicates that the recipient’s and donor’s groups are the same and compatible. ABO/D incompatibility: If there is double cell population phenomenon (two different cell populations), indicates that the recipient’s and donor’s groups are different and incompatible.

If incompatibility is observed, ABO/D blood groups of both recipient and donors need to be retested.

Crossmatch; Screening of unexpected antibodies

- A positive reaction (+1 to +4) in one or both microtubes 4 and 6 (Enz, AHG) and a negative reaction in microtubes and 7 (AHG Ct) indicates the presence of antibodies in patient’s serum against donor cells.
- A positive reaction in microtube 5 (Enz Minor) indicates the presence of antibodies in donor’s serum against patient cells.
- A negative reaction in the microtubes 4, 5 and 6 (Enz, AHG) and a negative reaction in microtube 7
- AHG Ct indicates the absence of antibodies (Compatibility).
- The patient’s sample should be submitted to further testing if a positive reaction in one or both microtubes 4 and 6 and a positive autocontrol is observed (AHG Ct).
- A positive reaction in microtube 7 may indicate a positive direct antiglobulin test (DAT) of the patient’s cells. The patient’s sample should be submitted to further testing.

LIMITATIONS

- Do not remove foil seal until ready to use. Once opened, the gel may begin to dry out which could affect test results.
- The aluminum foil on the matrix gel card should be removed carefully and gently.
- Do not use matrix gel cards, which show signs of drying.
- The gel 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.
- Do not touch the pipet tip inside the gel card. Carrying problem may occur.
- Do not use hemolyzed, cloudy or contaminated samples or with clot presence.
- Use of suspension solutions other than OBG Diluent 1 may modify the reactions.
- Red blood cells from individuals with A or B variants may present a weak expression of the antigens.
- Abnormal concentrations of serum proteins, the presence of macromolecular solutions in the serum may cause the non-specific agglutination of the red blood cells. It is recommended to wash the red blood cells before performing the test.
- Transfused patients or those subjected to bone marrow transplant may present images of double population.
- Patients with high-potency cold antibodies may coat the red blood cells completely so spontaneous agglutination may occur.

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Sensitivity</th>
<th>Specificity</th>
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</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>3092</td>
<td>% 100</td>
</tr>
<tr>
<td>Anti-B</td>
<td>3092</td>
<td>% 100</td>
</tr>
<tr>
<td>Anti-D</td>
<td>3092</td>
<td>% 100</td>
</tr>
</tbody>
</table>

ABO/Rh compatibility

The diagnostic sensitivity and specificity of the antibodies present in OBG Comp Test, for the determination of compatibility of ABO and Rh systems, have been studied in 240 donor-recipient (experimental donor-recipient).

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Crossmatch tests:

There is no described procedure or technique capable of detecting all the possible unexpected antibodies present in a sample. We conducted our own performance study. Coombs and enzymatic technique have been studied in 240 donor-recipients (experimental donor-recipient) and obtained same results with other established products of equivalent intended use.

Precision:

Inter-assay and intra-assay reproducibility tests; no false positive or false negative results were obtained. Variability between agglutination views in positive samples were 1 agglutination rating or less in all assays.

Label symbols

- Batch code
- Expiry date
- Storage temperature
- Consult instructions for use
- In vitro diagnostic medical device
- Product code
- Manufacturer
- Harmful

BIBLIOGRAPHY


Products: OBG Comp Test

1x12 REF OBGCT-12
4x12 REF OBGCT-48

Manufacturer:
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