



REF 43200

**Principle:**  
The test is based on the principle of haemagglutination. Antibodies in the serum/plasma bound to the corresponding antigens of papain treated Red Blood Cells (RBC), and perform agglutination.

**Intended purpose:**  
These RBC suspensions are intended for the detection of IgG and IgM antibodies reacting in enzyme substance, mainly the antibodies of Rh-factor. Also the antibodies of Kell-system react in this way occasionally. The method is suitable to show the presence of more cold type IgM antibodies, too (anti-I-H, -P1, -Lea, -Leb). Do not use any additive Low Ion Strength Solution (LISS) together with papainized panel cells, in this case the enzyme pre-treated cells may cause aspecific reactions.

**Composition:**  
The panel cells of 11 members for antibody identification are produced from human red blood cells of known group "0" and known antigen composed of individual donor's blood and is supplied in vials with droppers. Dropper volume is 50 µl.

The current antigen chart of the panel cells is always included in the packaging.  
ReaSol diluent (REF 11114) is an isotonic saline solution, used to preserve the reactivity of antigens and to prevent haemolysis. ReaSol diluent solution contains: 1 mmol/l chloramphenicol and 0,4 mmol/l neomycin-sulphate.

The panel cell suspension is ready to use. Do not use if the supernatant is haemolytic. Shake gently the vials in order to homogenize the suspension before use.

**Storage and transporting conditions:**  
These panel cells should be stored and delivered between +2°C and +8°C. The cells can be used until the indicated expiry date after the first opening. Do not freeze.

**Samples and controls:**  
Serum/plasma of a blood sample not older than 48 hours stored between +2°C and +8°C. (If the sample is to be tested later, the serum/plasma should be stored frozen between -20°C and -30°C.) It is recommended the use of internal control **Rea IQC Total Blood Kit** (REF 44100) which contains 4 vials with 25-30% concentration human whole blood with well-known ABO Rh(D) and Rh(K) characteristics. Should be used as the patient samples at the beginning and at the end of the daily work, ensuring / validating the testing results between the two applications of the Rea IQC Total Blood Kit.

**Materials and reagents required:**  
Manual tube technique:  
- tubes  
- pipettes (30 µl, 50 µl, 100 µl)  
- water bath, 37°C  
- laboratory centrifuge  
- isotonic saline solution, pH 7.2 (REF 15015)  
- RBC diluent (REF 11114)  
- Coombs serum (AHG)  
- papain

Automated tube technique:  
- ACT-24 automat  
- special tubes for the automat  
- pipettes (30 µl, 50 µl, 100 µl)  
- water bath, 37°C-os  
- isotonic saline solution, pH 7.2 (REF 15015)  
- RBC diluent (REF 11114)  
- Coombs serum (AHG)  
- papain

**Procedures:**  
**Manual tube technique:**  
1. Number 12 tubes. The 12th is for autocontrol. Mark the identification number of the blood sample to be tested on each tube. **Preparation of autocontrol:**  
- wash the red blood cells of the sample to be tested in isotonic saline solution (pH=7.2) twice  
- prepare an appr. 3% suspension with ReaSol solution (e.g. add 30 µl papain-treated, washed RBC residue to 1 ml ReaSol diluent).

**Direct method**  
2. Add 50-50 µl serum/plasma of the blood samples to be tested into each tube.  
3. Add 50-50 µl of each panel cell suspension into the adequate tubes, except the 12th one that is for autocontrol.  
4. Shake gently the contents of the tubes and incubate them at 37°C for 15 minutes.  
5. Centrifuge the tubes for 20 seconds at 1000 g, or for 60 seconds at 150 g.  
6. Observe the supernatant, whether there is haemolysis. Record the result in the corresponding column of the antigen chart.  
7. Shake gently the tube, above of a white background, and observe the agglutination with a desk lamp magnifier. Record the result in the corresponding column of the antigen chart. Mark the strength of agglutination with crosses.  
8. Evaluate the agglutination scheme with the help of the antigen chart.  
9. After evaluation if is necessary, continue the testing with Coombs test.

**Indirect method**  
10. Wash the tube content for 3 times with isotonic saline solution  
11. Add to each tube 100-100 µl Coombs serum (AHG)  
12. Shake the tubes and centrifuge them for 20 second with 1000g or for 60 seconds with 150g  
13. After resuspension read the result above of a white background with a desk lamp magnifier and record the result in the corresponding column of the antigen chart. Mark the strength of the agglutination with crosses.  
14. Evaluate the agglutination scheme with the help of the antigen chart.

**Automated tube technique (ACT-24):**  
Follow the manual tube technique method from point 1 to 4. Place the tubes in the ACT-24 automat, enter the parameters of the test in the computer and start F3 program.

Interpretation of result and printing of the test result is done by the automat.  
Evaluate the agglutination-scheme with the help of the antigen chart.  
After evaluation if is necessary, continue the testing with Coombs test.

**Coombs serum (AHG) test on ACT-24:**  
Start F2 program. Adding 50-50 µl of serum/plasma and panel cells, 50 µl Coombs serum (AHG) should be added.

All the steps of Coombs test, interpretation of results and printing of the test is done by the automat.  
Evaluate the agglutination-scheme with the help of the antigen chart.

Positive and negative and Coombs (washing) control reagents must be used for every testing (see AABB Technical Manual 17<sup>th</sup> Edition, Chapter 16<sup>th</sup>-Autologous control (autocontrol).

**Evaluation of results:**  
**Positive result:**  
• if in the reaction mixture shows agglutination one + to ++++ crosses.  
• if haemolysis is observed.  
**Negative result:**  
• if the reaction mixture remain a homogeneous suspension.

The test results should be compared with the antigen chart. The antigen chart is unique and specific for each batch produced. It is included in the packaging.

Autocontrol\* test should be performed strictly in the same conditions (substance, method). The positive result of a control should be carefully cleared up (certain illness or medication may be the cause). Occasionally other test cells may be needed for antibody identification. \*Autocontrol: red blood cells of the sample are tested against it's own plasma, under the same conditions eg. the antibody identification results can be confirmed with the patient's RBC antigen identification.

\*Autocontrol: red blood cells of the sample are tested against it's own plasma, under the same condition.

**Source of possible errors:**  
Reason of false negative results may occurs if:  
• the test sample and/or the reagents were not stored correctly and they lost their reactivity  
• the incubation duration and/or temperature was not adequate  
• the RBC were centrifuged inadequately  
• the over resuspension extinguish the weak reactions  
• the AHG reagent is neutralized (i.e.: inadequate cell washing)

Reason of false positive results:  
• bacterial contamination or other impurity of test cells  
• inadequate centrifugation and resuspension  
• rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspension solutions component  
• In LISS the enzymatized cells results in aspecific reactions, due to this reason do not use reaction accelerator additives together with papainized cells

**Limitations of the method:**  
• The preserved RBC, generally the strength of reaction of the product may decrease during the shelf life. Its degree is depending on the individual characteristics of donors, which cannot be controlled nor foreseen by the producer.  
• In case of positive autocontrol result the tested serum/plasma may contain autoantibodies, which needs to be investigated.  
• If the test result is negative, this not excludes that very rare antibody may not be present in the serum/plasma. In case of very high frequency antibodies or multiple antibody presence may need adequate, rare cells or other capable methods for differentiating the antibodies.  
• Enzyme treatment destroys M, N, S, Fya, Fyb antigens, so the corresponding antibodies will not react to the enzyme treated RBC.

**Precautions:**  
All reagents of human origin shall be considered as potentially infectious products.  
All human blood preparations, from which test cells are produced, were found non-reactive for Lues, HIV1,2, HbsAg and HCV by procedures recommended by the European Council, however, none of the methods currently known can absolutely guarantee that the products do not contain any transmissible pathogen.

It is advisable to wear protective gloves and safety spectacles. All materials getting into contact with the samples shall be considered as potentially infected. Upon destruction of the residues, the good laboratory procedure (GLP) shall be followed.

**Packaging:**  
REF 43200 11 x 5 ml

**Bibliography:**  
1.) Transzfúziós szabályzat – Az OVSZ módszertani levele 2. kiadás, OVSZ, Bp. 2008. (Transfusion Guideline – 2nd Edition of Methodology Letters HNBS, Hungary)  
2.) ABB Technical Manual, 17th Edition, AABB, Bethesda, Maryland, USA  
3.) Guidelines of Transfusion Services in the United Kingdom 7th Edition 2005  
4.) Decree 2/2005 (II. 10.) of EüM regulation of quality and safety for collecting, testing, processing, storing and distribution of human blood and blood components, and their individual technical requirements (localization of Directive 2002/98/EC and Directive 2004/33/EC)

REAGENS Kft.  
Wysocki u. 1.  
1155 Budapest,  
Hungary



REF 43800

**Principle:**  
The test is based on the principle of haemagglutination. Antibodies in the serum/plasma bound to the corresponding antigens of papain treated Red Blood Cells (RBC), and perform agglutination.

**Intended purpose:**  
These RBC suspensions are intended for the detection of IgG and IgM antibodies reacting in enzyme substance, mainly the antibodies of Rh-factor. Also the antibodies of Kell-system react in this way occasionally. The method is suitable to show the presence of more cold type IgM antibodies, too (anti-I-H, -P1, -Lea, -Leb). Do not use any additive Low Ion Strength Solution (LISS) together with papainized panel cells, in this case the enzyme pre-treated cells may cause aspecific reactions.

**Composition:**  
The panel cells of 11 members for antibody identification are produced from human red blood cell of known group "0" and known antigen composed of individual donor's blood and is supplied in vials with droppers. Dropper volume is 50 µl.  
The current antigen chart of the panel cells is always included in the packaging.  
ReaSol diluent (REF 11114) is an isotonic saline solution, used to preserve the reactivity of antigens and to prevent haemolysis. ReaSol diluent solution contains: 1 mmol/l chloramphenicol, and 0,4 mmol/l neomycin-sulphate.

The panel cell suspension is ready to use. Do not use if the supernatant is haemolytic. Gently shake the vials in order to homogenize the suspension before use.

**Storage and transporting conditions:**  
These panel cells should be stored and delivered between +2°C and +8°C. The cells can be used until the indicated expiry date after the first opening. Do not freeze.

**Samples and controls:**  
Serum/plasma of a blood sample not older than 48 hours stored between +2°C and +8°C. (If the sample is to be tested later, the serum/plasma should be stored frozen between -20°C and -30°C.) It is recommended the use of internal control **Rea IQC Total Blood Kit** (REF 44100) which contains 4 vials with 25-30% concentration human whole blood with well-known ABO Rh(D) and Rh(K) characteristics. Should be used as the patient samples at the beginning and at the end of the daily work, ensuring / validating the testing results between the two applications of the Rea IQC Total Blood Kit.

**Materials and reagents required:**  
Microplate method:  
- microplates,  
- pipettes (10 µl, 50 µl)  
- isotonic saline solution, pH 7.2 (REF 15015),  
- RBC diluent (REF 11114),  
- Papain,  
- incubator of 37 °C  
- microplate centrifuge  
- microplate shaker  
- mirror reader / automate reader

Gel column method:  
- Coombs and Neutral cards  
- pipettes (10 µl 25 µl, 50 µl)  
- isotonic saline solution, pH 7.2 (REF 15015)  
- RBC diluent  
- papain  
- incubator of 37 °C  
- card centrifuge

**Procedures:**  
**Microplate method:**  
Before starting the test let the devices, reagents and blood samples come to room temperature.  
1. The identification number of the blood sample is to be marked on the microplates.  
2. Add 50-50 µl serum/plasma from the blood sample to be tested into 12 wells.  
3. Add 50-50 µl of each panel cell suspension in the adequate wells.  
4. The 12th column of the gel card is for autocontrol.

**Preparation of autocontrol:**  
- wash the red blood cell residue of the blood sample to be tested, in isotonic saline solution (pH=7.2)  
- add 100 µl red blood cell residue and incubate it with 100 µl 1% papain solution for 5 minutes at 37 °C

5. Shake the contents of the microplate at maximum grade for 1-2 minutes.  
6. Incubate the microplate for 15 minutes at 37°C.  
7. Centrifuge them at 1000 rpm for 1 minute.  
8. Shake the plates at maximum grade for a few seconds, then at low grade for 2-3 minutes.  
9. Read the results in mirror reader, record the result in the corresponding column of the antigen chart. Mark the strength of agglutination with crosses.  
10. Evaluate the agglutination-scheme with the help of the antigen chart.

**Gel column method:**  
Before starting the test let the devices, reagents and blood samples come to room temperature. (The technical inserts of gel cards of different manufacturers may be variant. The original technical inserts must be read with attention before use.)

**Direct method**  
1. Neutral card shall be used. 2 cards (with 6 columns each) are necessary for the test of one blood sample. The identification number of the patient and the numbers of the certain panel cells are to be marked on both of them.  
2. Remove the film and add 50-50 µl of each panel cell suspension into the adequate column of the gel card.

REAGENS Kft.  
Wysocki u. 1.  
1155 Budapest,  
Hungary

3. The 12th column of the gel card is for autocontrol. **Preparation of autocontrol:** see above (previous section 4).  
4. Add 25-25 µl of the serum/plasma to be tested into each gel column  
5. Incubate the cards for 15 minutes at 37°C.  
6. Centrifuge them for 10 minutes.  
7. Read and record the reactions in the adequate cells of the antigen chart. Mark the strength of agglutination with crosses.  
8. Evaluate the agglutination-scheme with the help of the antigen chart.

**Note:** By the use of these 0,8% papainized panel cells on gel cards, IAT (indirect antiglobulin test) can be performed, as well, if Coombs card is used.

**Evaluation of results:**  
**Positive result:**  
• if agglutination or haemolysis is observed on the microplate  
• if agglutination is observed in one of the well  
**Negative result:**  
• if the reaction mixture remain a homogeneous suspension.  
• if the cells are on the bottom of the wells

The results of the testing should be compared with the antigen chart antigen configuration. The antigen chart is unique for each batch. The antigen chart is included in the product packaging.

Autocontrol\* test should be performed strictly in the same conditions (substance, method used). The positive result of a control should be carefully cleared up (certain illness or medication may be the cause). Occasionally other test cells may be needed for antibody identification. The antibody identification results can be confirmed with the patient's RBC antigen identification.

\*Autocontrol: red blood cells of the sample are tested against it's own plasma, under the same condition.

**Source of possible errors:**  
Reason of false negative results may occurs if:  
• the test sample and/or the reagents were not stored correctly and they lost their reactivity  
• the incubation duration and/or temperature was not adequate  
• the RBC were centrifuged inadequately  
• the over resuspension extinguish the weak reactions  
• the AHG reagent is neutralized (i.e.: inadequate cell washing)

Reason of false positive results:  
• bacterial contamination or other impurity of test cells  
• inadequate centrifugation and resuspension  
• rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspension solutions component  
• In LISS the enzymatized cells results in aspecific reactions, due to this reason do not use reaction accelerator additives together with papainized cells

**Limitations of the method:**  
• The preserved RBC, generally the strength of reaction of the product may decrease during the shelf life. Its degree is depending on the individual characteristics of donors, which cannot be controlled nor foreseen by the producer.  
• In case of positive autocontrol result the tested serum/plasma may contain autoantibodies, which needs to be investigated.  
• If the test result is negative, this not excludes that very rare antibody may not be present in the serum/plasma. In case of very high frequency antibodies or multiple antibody presence may need adequate, rare cells or other capable methods for differentiating the antibodies.  
• Enzyme treatment destroys M, N, S, Fy<sup>a</sup>, Fy<sup>b</sup> antigens, so the corresponding antibodies will not react to the enzyme treated RBC.

**Precautions:**  
All reagents of human origin shall be considered as potentially infectious products.  
All human blood preparations, from which test cells are produced, were found non-reactive for Lues, HIV1,2, HbsAg and HCV by procedures recommended by the European Council, however, none of the methods currently known can absolutely guarantee that the products do not contain any transmissible pathogen.

It is advisable to wear protective gloves and safety spectacles. All materials getting into contact with the samples shall be considered as potentially infected. Upon destruction of the residues, the good laboratory procedure (GLP) shall be followed

**Packaging:**  
REF 43800 11 x 5 ml

**Bibliography:**  
1.) Transzfúziós szabályzat – Az OVSZ módszertani levele 2. kiadás, OVSZ, Bp. 2008. (Transfusion Guideline – 2nd Edition of Methodology Letters HNBS, Hungary)  
2.) ABB Technical Manual, 17th Edition, AABB, Bethesda, Maryland, USA  
3.) Handbook of Transfusion Medicine 4th html Edition - United Kingdom Blood Services  
4.) Decree 2/2005 (II. 10.) of EüM regulation of quality and safety for collecting, testing, processing, storing and distribution of human blood and blood components, and their individual technical requirements (localization of Directive 2002/98/EC and Directive 2004/33/EC)

REAGENS Kft.  
Wysocki u. 1.  
1155 Budapest,  
Hungary

Red Blood Cell Suspension: 3±0,5%



REF 43100

**Principle:**

In the first step of the Indirect Antiglobulin Test (IAT, Coombs test) IgG antibodies in the serum/plasma bound to the corresponding antigens of red blood cells (RBC). In the second step additional anti-human-globulin (AHG) induce the agglutination.

**Intended purpose:**

These RBC suspensions are intended for the identification of IgG (Rh-, Kell-, Duffy-, Kidd-, etc.) and IgM (mainly anti-M, anti-N) irregular RBC antibodies with DAT and IAT methods. By direct agglutination IgM antibodies of the serum/plasma react to the corresponding RBC antigens and perform agglutination. The sensibility of the method can be increased and the reaction time can be shortened with Low Ionic Strength Solution (LISS) at the same time. The additive, LISS have to be used in compliance with the manufacturer's instructions for use.

**Composition:**

The panel cells of 11 members for antibody identification are produced from human RBCs of known group "0" and known antigen composed of individual donor's blood and is supplied in vials with droppers. Dropper volume is 50 µl.

The current antigen chart of the panel cells is always included in the packaging.

ReaSol diluent (REF 11114) is an isotonic saline solution, used to preserve the reactivity of antigens and to prevent haemolysis. ReaSol diluent solution contains: 1 mmol/l chloramphenicol and 0,4 mmol/l neomycin-sulphate.

The panel cell suspension is ready to use.

Do not use the reagent if the supernatant is haemolytic. Gently shake the vials in order to homogenize the suspension before use.

**Storage conditions:**

These panel cells should be stored and delivered between +2°C and +8°C. The cells can be used until the indicated expiry date after the first opening. Do not freeze.

**Samples and control:**

Serum/plasma of a blood sample not older than 48 hours stored between +2°C and +8°C can be used. (If the sample is to be tested later, the serum/plasma should be stored frozen between -20°C and -30°C.)

It is recommended the use of internal control Rea IQC Total Blood Kit (REF 44100) which contains 4 vials with 25-30% concentration human whole blood with well-known AB0 Rh(D) and Rh(K) characteristics. Should be used as the patient samples at the beginning and at the end of the daily work, ensuring / validating the testing results between the two applications of the Rea IQC Total Blood Kit.

**Materials and reagents required:**

**Manual tube technique**

- tubes
- pipettes (30 µl, 50 µl, 100 µl)
- water bath, 37°C
- laboratory centrifuge / cell washing equipment
- isotonic saline solution, pH 7,2 (REF 15015)
- Coombs serum (AHG)
- LISS (AGGI-LISS, REF 13110)
- ReaSol diluent (REF 11114)

**Automated tube technique:**

- ACT-24 automat
- Special tubes for the automat
- pipettes (30 µl, 50 µl, 100 µl)
- water bath, 37°C-os,
- isotonic saline solution, pH=7,2 (REF 15015)
- Coombs serum (AHG)
- LISS (AGGI-LISS, REF 13110)
- ReaSol diluent (REF 11114)

**Procedures:**

**Manual tube technique (Indirect Coombs, antiglobulin test):**

1. Number 12 tubes. The 12th tube is for autocontrol. Mark the identification number of the blood sample to be tested on each tube.

**Preparation of autocontrol:**

- wash the red blood cells of the sample to be tested in isotonic saline solution (pH=7,2) twice
- prepare an appr. 3% suspension with ReaSol solution (e.g.: add 30 µl washed RBC residue to 1 ml ReaSol diluent)

**Direct method**

2. Add 100-100 µl serum/plasma of the blood samples to be tested into each tube.
3. Add 50-50 µl of each panel cell suspension into the adequate tubes.
4. Shake gently the contents of the tubes and incubate them at 37°C for 60 minutes.
5. Centrifuge the tubes for 20 seconds at 1000 g, or for 60 seconds at 150 g
6. Observe the supernatant, whether there is haemolysis. Record the result in the antigen chart.
7. White shaking gently, above of a white background, with a desk lamp magnifier read and record the result in the correct column of the antigen chart. Mark the strength of agglutination with crosses.
8. Evaluate the agglutination scheme-with help of the antigen chart.

**Indirect method**

9. Wash the tubes three times in isotonic saline solution.
10. Add 100-100 µl Coombs serum (AHG) to each tube.
11. Shake gently and centrifuge for 20 seconds at 1000 g or for 60 seconds at 150 g.
12. After resuspension read the result, possibly on a white background, with a desk lamp magnifier and record the result in the corresponding column of the antigen-chart. The force of agglutination is to be marked with crosses.
13. Evaluate the agglutination scheme with help of the antigen chart.

**Automated tube technique (ACT-24):**

1. Follow the manual tube technique method from point 1 to 4. Half quantities of reagents can be used, add 25 µl of the panel cells to 50 µl serum. In this case 50 µl Coombs serum (AHG) is necessary.
2. Place the tubes in the ACT-24 automat, enter the parameters of the test in the computer and start F2 program.
3. Interpretation of results and printing of the test results is done by the ACT automat.
4. Evaluate the agglutination scheme-with help of the antigen chart.

Low ion strength solution (LISS) can be used for both methods, but technical insert of the manufacturer has to be followed strictly and make the necessary steps accordingly.

Positive and negative and Coombs (washing) control reagents must be used for every testing (see AABB Technical Manual 17<sup>th</sup> Edition, Chapter 16<sup>th</sup>. Autologous control (autocontrol).

**Evaluation of results:**

**Positive result:**

- If in the reaction mixture shows agglutination one + to ++++ crosses.
- If haemolysis is observed.

**Negative result:**

- If the reaction mixture remain a homogeneous suspension.

The test results should be compared with the antigen chart. The antigen chart is unique and specific for each batch produced. It is included in the packaging.

Autocontrol\* test should be performed strictly in the same conditions (substance, method). The positive result of a control should be carefully cleared up (certain illness or medication may be the cause). Occasionally other test cells may be needed for antibody identification. \*Autocontrol: red blood cells of the sample are tested against it's own plasma, under the same conditions eg. the antibody identification results can be confirmed with the patient's RBC antigen identification.

**Source of possible errors:**

Reason of false negative results may occur if:

- the test sample and/or the reagents were not stored correctly and they lost their reactivity
- the incubation duration and/or temperature was not adequate
- inadequate centrifugation of cells
- the over resuspension extinguish the weak reactions
- the AHG reagent is neutralized (i.e.: inadequate cell washing)

**Reason of false positive results:**

- bacterial contamination or impurity of test cells
- inadequate centrifugation and resuspension
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspension solutions component

**Limitations of the method:**

- The preserved RBC, generally the strength of reaction of the product may decrease during the shelf life. Its degree is depending on the individual characteristics of donors, which cannot be controlled nor foreseen by the producer.
- In case of positive autocontrol result the tested serum/plasma may contain antibodies, which needs to be investigated.
- If the test result is negative, this not excludes that very rare antibody may not be present in the serum/plasma.
- In case of very high frequency antibodies or multiple antibody presence may need adequate, rare calls or other capable methods for differentiating the antibodies.

**Precautions:**

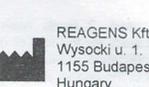
All reagents of human origin shall be considered as potentially infectious products. All human blood preparations, from which test cells are produced, were found non-reactive for Lues, HIV1,2, HbsAg and HCV by procedures recommended by the European Council, however, none of the methods currently known can absolutely guarantee that the products do not contain any transmissible pathogen. It is advisable to wear protective gloves and safety spectacles. All materials getting into contact with the samples shall be considered as potentially infected. Upon destruction of the residues, the good laboratory procedure (GLP) shall be followed.

**Packaging:**

REF 43100 11 x 5 ml

**Bibliography:**

- 1.) Transzfúziós szabályzat – Az OVSZ módszertani levele 2. kiadás, OVSZ, Bp. 2008. (Transfusion Guideline – 2nd Edition of Methodology Letters HNBTS, Hungary)
- 2.) AABB Technical Manual, 17th Edition, AABB, Bethesda, Maryland, USA
- 3.) Guidelines of Transfusion Services in the United Kingdom 7th Edition 2005
- 4.) Decree 2/2005 (II. 10.) of EüM regulation of quality and safety for collecting, testing, processing, storing and distribution of human blood and blood components, and their individual technical requirements (localization of Directive 2002/98/EC and Directive 2004/33/EC)



REF 43500

**Principle:**

In the first step of the Indirect Antiglobulin Test (IAT, Coombs test) IgG antibodies in the serum/plasma bound to the corresponding antigens of red blood cells (RBC). In the second step additional anti-human-globulin (AHG) induce the agglutination.

**Intended purpose:**

These RBC suspensions are intended for the identification of RBC against antibody IgG (Rh-, Kell-, Duffy-, Kidd-, etc.) irregular RBC antibodies by IAT (indirect Coombs test), and some IgM (mainly MN system) antibodies identification with direct agglutination (DAT) methods on gel cards.

**Composition:**

The panel cells of 11 for antibody identification are produced from human red blood cells of known group "0" and known antigen composed of individual donor's blood and is supplied in vials with droppers. They are a panel of red blood cell suspensions. Dropper volume is 50 µl.

The current antigen chart of the panel cells is always included in the packaging.

ReaSol diluent (REF 11114) is an isotonic saline solution, used to preserve the reactivity of antigens and to prevent haemolysis. ReaSol diluent solution contains: 1 mmol/l chloramphenicol, and 0,4 mmol/l neomycin-sulphate.

The panel cell suspension is ready to use

Do not use the reagent if the supernatant is haemolytic. Gently shake the vials in order to homogenize the suspension before use.

**Storage and transporting conditions:**

These panel cells should be stored and delivered between +2°C and +8°C. The cells can be used until the indicated expiry date after the first opening. Do not freeze!

**Samples and control:**

Serum/plasma of a blood sample not older than 48 hours stored between +2°C and +8°C can be used. (If the sample is to be tested later, the serum/plasma should be stored frozen between -20°C and -30°C.)

It is recommended the use of internal control Rea IQC Total Blood Kit (REF 44100) which contains 4 vials with 25-30% concentration human whole blood with well-known AB0 Rh(D) and Rh(K) characteristics. Should be used as the patient samples at the beginning and at the end of the daily work, ensuring / validating the testing results between the two applications of the Rea IQC Total Blood Kit.

**Materials and reagents required:**

**Gel column method:**

- LISS/Coombs cards or Neutral cards
- Card centrifuge
- Incubator of 37°C
- pipettes (10 µl, 25 µl, 50 µl),
- physiologic buffered solution or isotonic saline solution, pH 7,2 (REF 15015)
- ReaSol diluent (REF 11114), or any other solutions recommended by the card manufacturer

**Procedure:**

**Gel column method:**

Before starting the test let the devices, reagents and blood samples come to room temperature. (The technical inserts of gel cards of different manufacturers may be variant. The gel card technical inserts must be read with attention before use.)

**Direct method**

1. Neutral card shall be used. 2 cards (with 6 columns each) are necessary for the test of one blood sample. The identification number of the patient and the numbers of the certain panel cells are to be marked on both of them.
2. Remove the film and put 50-50 µl of each panel cell suspension into the adequate column of the gel card.
3. Add 25-25 µl of the serum/plasma to be tested into each gel column
4. Incubate the cards for 15 minutes at 37°C.
5. Centrifuge the cards for 10 minutes.
6. Read and record the result in the adequate columns of the antigen chart. Mark the strength of agglutination with crosses.
7. Evaluate the agglutination scheme-with help of the antigen chart.

**Indirect method**

1. LISS/Coombs card shall be used. 2 cards (with 6 columns each) are necessary for the test of one blood sample. The identification number of the patient and the numbers of the certain panel cells are to be marked on both of them.
2. Remove the film and put 50-50 µl of each panel cell suspension into the adequate column of the gel card. Always pipette the RBC first so that the uncombined immunoglobulins in the serum/plasma could not reach the gel neutralizing the anti-human-globulin.
3. The 12<sup>th</sup> column of the gel card is for autocontrol.

Positive and negative and Coombs (washing) control reagents must be used for every testing (see AABB Technical Manual 17<sup>th</sup> Edition, Chapter 16<sup>th</sup>. Autologous control (autocontrol).

**Evaluation of results:**

**Positive results:**

- if reaction is observed in the gel column

**Negative results:**

- If cells are observed in the bottom of the gel column

The test results should be compared with the antigen chart. The antigen chart is unique and specific for each batch produced. It is included in the packaging.

Autocontrol\* test should be performed strictly in the same conditions (substance, method). The positive result of a control should be carefully cleared up (certain illness or medication may be the cause). Occasionally other test cells may be needed for antibody identification. \*Autocontrol: red blood cells of the sample are tested against it's own plasma, under the same conditions, eg. the antibody identification results can be confirmed with the patient's RBC antigen identification.

**Source of possible errors:**

Reason of false negative results:

- the test sample and/or the reagents were not stored correctly and they lost their reactivity
- the incubation duration and/or temperature was not adequate
- inadequate centrifugation of cells
- the over resuspension extinguish the weak reactions
- the AHG reagent is neutralized (i.e.: inadequate cell washing)

Reason of false positive results:

- bacterial contamination or impurity of test cells
- inadequate centrifugation and resuspension
- rarely the tested serum or plasma may contain antibodies which are against of some of the used resuspension solutions component

**Limitations of the method:**

- The preserved RBC, generally the strength of reaction of the product may decrease during the shelf life. Its degree is depending on the individual characteristics of donors, which cannot be controlled nor foreseen by the producer.
- In case of positive autocontrol result the tested serum/plasma may contain antibodies, which needs to be investigated.
- If the test result is negative, this not excludes that very rare antibody may not be present in the serum/plasma.
- In case of very high frequency antibodies or multiple antibody presence may need adequate, rare calls or other capable methods for differentiating the antibodies.

**Precautions:**

All reagents of human origin shall be considered as potentially infectious products. All human blood preparations, from which test cells are produced, were found non-reactive for Lues, HIV1,2, HbsAg and HCV by procedures recommended by the European Council, however, none of the methods currently known can absolutely guarantee that the products do not contain any transmissible pathogen.

It is advisable to wear protective gloves and safety spectacles. All materials getting into contact with the samples shall be considered as potentially infected.

Upon destruction of the residues, the good laboratory procedure (GLP) shall be followed.

**Packaging:**

REF 43500 11 x 5 ml

**Bibliography:**

- 1.) Transzfúziós szabályzat – Az OVSZ módszertani levele 2. kiadás, OVSZ, Bp. 2008. (Transfusion Guideline – 2nd Edition of Methodology Letters HNBTS, Hungary)
- 2.) AABB Technical Manual, 17th Edition, AABB, Bethesda, Maryland, USA
- 3.) Guidelines of Transfusion Services in the United Kingdom 7th Edition 2005
- 4.) Decree 2/2005 (II. 10.) of EüM regulation of quality and safety for collecting, testing, processing, storing and distribution of human blood and blood components, and their individual technical requirements (localization of Directive 2002/98/EC and Directive 2004/33/EC)

